REVIEW ARTICLE

Coenzyme A biosynthesis: an antimicrobial drug target

Christina Spry¹, Kiaran Kirk¹ & Kevin J. Saliba¹,²

¹School of Biochemistry and Molecular Biology, The Australian National University, Canberra, Australia; and ²Medical School, The Australian National University, Canberra, Australia

Correspondence: Kevin J. Saliba, School of Biochemistry and Molecular Biology, The Australian National University, Canberra ACT 0200, Australia. Tel.: +61 2 6125 7549; fax: +61 2 6125 0313; e-mail: kevin.saliba@anu.edu.au

Received 13 March 2007; revised 13 September 2007; accepted 14 October 2007.

First published online December 2007.

DOI:10.1111/j.1574-6976.2007.00093.x

Editor: Graham Coombs

Keywords
pantothenic acid; coenzyme A; pantothenate kinase; bacteria; fungi; malaria.

Abstract

Pantothenic acid, a precursor of coenzyme A (CoA), is essential for the growth of pathogenic microorganisms. Since the structure of pantothenic acid was determined, many analogues of this essential metabolite have been prepared. Several have been demonstrated to exert an antimicrobial effect against a range of microorganisms by inhibiting the utilization of pantothenic acid, validating pantothenic acid utilization as a potential novel antimicrobial drug target. This review commences with an overview of the mechanisms by which various microorganisms acquire the pantothenic acid they require for growth, and the universal CoA biosynthesis pathway by which pantothenic acid is converted into CoA. A detailed survey of studies that have investigated the inhibitory activity of analogues of pantothenic acid and other precursors of CoA follows. The potential of inhibitors of both pantothenic acid utilization and biosynthesis as novel antibacterial, antifungal and antimalarial agents is discussed, focusing on inhibitors and substrates of pantothenate kinase, the enzyme catalysing the rate-limiting step of CoA biosynthesis in many organisms. The best strategies are considered for identifying inhibitors of pantothenic acid utilization and biosynthesis that are potent and selective inhibitors of microbial growth and that may be suitable for use as chemotherapeutic agents in humans.

Introduction: in desperate need of novel antimicrobial agents

Infectious diseases remain a leading cause of morbidity and mortality worldwide, not only in the developing world where economic and social factors promote these diseases but also in the developed world. Drug-resistant pathogens are increasingly common, and social and environmental change has seen the emergence of previously unknown or uncommon diseases and the re-emergence of diseases previously considered to berelegated to history (reviewed in Vicente et al., 2006).

Many bacterial infections that were once controlled effectively with antibiotics are increasingly insensitive to treatment with multiple drugs, leading to treatment failure and in some cases death. Global examples of drug-resistant bacteria include multidrug-resistant Mycobacterium tuberculosis, methicillin-resistant and multidrug-resistant Staphylococcus aureus, penicillin- and macrolide-resistant pneumococci, vancomycin-resistant enterococci, as well as multidrug-resistant Gram-negative bacteria such as Pseudomonas aeruginosa (World Health Organization, 2002; Levy & Marshall, 2004).

Tuberculosis, the disease caused by the bacterium Mycobacterium tuberculosis, is a leading cause of death worldwide. In recent years, the incidence of tuberculosis has increased as a consequence of both the spread of HIV and the spread of multidrug-resistant Mycobacterium tuberculosis (World Health Organization, 2006). Multidrug resistance dramatically affects the course of tuberculosis chemotherapy, extending the already long-term courses of combination therapy of 6–9 months to up to 2 years, increasing the cost and toxicity of treatment, and impacting on the chemotherapeutic outcomes (Saltini, 2006; World Health Organization, 2006).

Infections caused by extraintestinal pathogenic Escherichia coli (ExPEC) are a major, yet relatively under-appreciated, health concern. ExPEC cause a range of extraintestinal infections such as urinary tract infections, neonatal meningitis, intra-abdominal and pelvic infections, pneumonia, bacteraemia and sepsis (Russo & Johnson, 2003). At particular risk of ExPEC infections are individuals with a compromised immune system, resulting from disease, age or...
chemotherapy. The frequency of ExPEC infections is increasing and drug-resistant strains of ExPEC have emerged (Russo & Johnson, 2003). New agents for the treatment and prevention of ExPEC infections are required to combat the morbidity and mortality associated with these infections.

Bacterial pathogens are not the only cause of infectious diseases. Fungi, while generally opportunistic (or the cause of only mild superficial infections in healthy individuals), cause potentially life-threatening illnesses in individuals with a compromised immune system, such as AIDS patients and cancer patients undergoing chemotherapy. Aspergillus species and Pneumocystis species are among the fungal pathogens that pose a serious threat. Aspergillus species frequently enter the lungs of humans. The body’s innate immune system is normally capable of destroying the fungus; however, under certain circumstances, for example when the integrity of the respiratory tract lining is compromised as a result of respiratory infection or surgery, the fungi invade the blood vessels, causing a serious and potentially life-threatening systemic infection. Pneumocystis species infect the lungs, and cause serious pneumonia-like symptoms, predominantly in immunocompromised populations. Pneumocystis infection is one of the most common causes of death among AIDS patients (Deacon, 2006). Although several new antifungal agents have emerged over the past few decades, the current lag in the development of new antifungal agents must be addressed to allow effective management of fungal diseases in the future (Bennett, 2006).

Infectious diseases caused by pathogenic parasites contribute significantly to morbidity and mortality in the world. The parasitic disease that we focus on in this review is malaria, currently the world’s most devastating human parasitic infection; however, several other parasitic diseases, such as amoebiasis and trypansomiasis (or African sleeping sickness), are also responsible for significant morbidity and mortality. The development of drugs to combat these parasitic diseases, many of which predominantly affect the poor, is hampered by the lack of economic incentives. As a result, there are few new drugs available to treat infections caused by parasites resistant to the pre-existing antiparasitic agents that have been in use for years.

The difficulties associated with producing effective vaccines for bacterial, fungal and parasitic infections has meant that for many important pathogens, there is, as yet, no vaccine. Hence, antimicrobial chemotherapies are often the single effective method for treating and preventing the transmission of infectious diseases. Furthermore, because vaccines generally do not provide a cure once an infection is established, antimicrobial agents remain important even for those diseases for which a vaccine is available. To ensure effective management of infectious diseases in the future, an increased focus on the development of antimicrobial agents that inhibit novel microbial targets is urgently required.

The current malaria situation

Malaria is a lethal infectious disease caused by a unicellular protozoan parasite of the genus Plasmodium. There are estimated to be between 300 and 500 million cases of malaria annually, resulting in 1–3 million deaths, the majority of which are of children under the age of five, and pregnant women (Trigg & Kondrachine, 1998). The malaria burden is concentrated largely in the tropics. Over 90% of cases occur in sub-Saharan Africa, an area where the majority of those affected cannot access or afford effective treatments.

Five species of Plasmodium – Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi – are infectious to humans (Singh et al., 2004). Plasmodium falciparum is the most virulent, and is responsible for the majority of malaria-related morbidity and mortality. Malaria is transmitted to humans by female Anopheles mosquitoes, which take up parasites during a blood meal from an infected person. During the course of a malaria infection, the malaria parasites invade and multiply within the host’s red blood cells. This intraerythrocytic stage of the parasite’s lifecycle is the cause of the clinical symptoms of malaria, which include (but are not limited to) fever and anaemia, and in severe cases, hypoglycaemia, organ failure and often death.

The two major strategies utilized in the control of malaria are: (1) reducing human contact with mosquitoes through the use of insecticides and bed nets in order to prevent transmission and (2) using antimalarial drugs to treat as well as prevent cases of the disease (Klausner & Alonso, 2004; Greenwood et al., 2005). The ultimate addition to the current control strategies would no doubt be the development and implementation of an effective vaccine. As yet, however, an effective vaccine is not available, and as other preventative measures are not routinely practiced in most parts of malaria-endemic areas, inexpensive chemotherapies are likely to remain the major tool for controlling malaria for some time (Greenwood et al., 2005).

The spread of resistance to chloroquine and other first-line antimalarial agents has rendered these drugs largely ineffective as monotherapies and has left us in desperate need of new strategies for treating infections. The future of malaria chemotherapy and prophylaxis relies heavily on the development of new antimalarial agents that are potent, nontoxic and cheap, for use in combination chemotherapies.

The development of antimicrobial agents: targeting nutrient utilization

Structural analogues of para-aminobenzoic acid (PABA) were the first effective antimicrobial agents used systemically for the prevention and cure of human bacterial infections (Petri, 2006). The PABA analogues, collectively called the
sulphonamides, inhibit competitively the utilization of PABA by bacteria. Many bacteria require PABA for the synthesis of folic acid, as they are unable to utilize exogenous folic acid. Today, sulphonamides are still used as antibacterial agents, predominantly in combination therapies.

Sulphonamides are also well established as antimarial agents. Sulphadoxine is a sulphonamide used in combination with pyrimethamine, a compound that inhibits a second key reaction in the synthesis of folic acid. This combination (called Fansidar) was for some time second only to chloroquine as the most common antimarial in use (Kremser & Krishna, 2004).

The importance of sulphonamides in the treatment of bacterial and malarial infections during the 70 years since their implementation validates the mechanisms involved in the utilization of essential nutrients as targets for antimicrobial agents. The uptake mechanisms and the enzymes involved in the metabolism of nutrients identified as essential for the growth of pathogenic microorganisms provide us with novel targets for future antimicrobial drug development.

Pantothenic acid and the biosynthesis of coenzyme A (CoA)

While investigating the nutritional requirements of yeast, Williams et al. (1933) discovered an acidic substance that stimulated the growth of a strain of Saccharomyces cerevisiae. Williams et al. named the growth factor pantothenic acid, from the Greek word pantothen, meaning from everywhere, because of its widespread occurrence in living matter. Subsequently, pantothenic acid was found to stimulate the growth of a wide range of organisms, from lactic acid bacteria to plants and animals (Williams & Rohrman, 1935; Snell et al., 1938, 1939; Lythgoe et al., 1940).

Pantothenic acid (also known as vitamin B₅, or, when ionized, as pantothenate; 1) has since been shown to be a precursor to CoA (2), an essential enzyme cofactor in many, if not all, living organisms. For the catalysis of certain reactions, enzymes often require the participation of cofactors. CoA functions as an acyl group carrier and carbonyl-activating group in numerous reactions central to cellular metabolism, and provides the 4'-phosphopantetheine prosthetic group incorporated by carrier proteins that play key roles in fatty acid, polyketal and nonribosomal peptide biosynthesis. A survey of the BRENDA database (www.brenda-enzymes.info/) of all known enzyme activities shows that 9% of the approximately 3500 identified activities use CoA or a CoA thioester as a cosubstrate (E. Strauss, personal communication).

The different mechanisms by which organisms obtain the pantothenic acid required for the biosynthesis of CoA and the pathway by which organisms convert pantothenic acid to CoA are summarized in Fig. 1. Many organisms, including animals and several prokaryotic and eukaryotic pathogens, rely on uptake of exogenous pantothenic acid. Some bacteria, fungi and plants, however, are capable of synthesizing pantothenic acid de novo from β-alanine (3) and α-ketoisovalerate (4). Briefly, α-ketoisovalerate, an intermediate of branched chain amino acid biosynthesis, is converted to ketopantoate (5) by the enzyme ketopantoate hydroxymethyltransferase (KPHMT). Reduction of the ketopantoate by ketopantoate reductase (KPR) subsequently yields pantopate (6), which is condensed with β-alanine by pantothenate synthetase (PS) to form pantothenate. A detailed review of the enzymology of the pantothenate biosynthesis pathway is provided in Webb et al. (2004).

As shown in Fig. 1, CoA is synthesized in vivo from pantothenic acid by a universal series of five enzymatic steps (Genschel, 2004). The first step of the pathway, and the step that determines the rate of CoA biosynthesis in most organisms, is the pantothenate kinase (PanK)-catalysed phosphorylation of pantothenate to 4'-phosphopantetheinate (7) (Jackowski & Rock, 1981; Robishaw et al., 1982). 4'-Phosphopantotheinate is subsequently condensed with a cysteine molecule by phosphopantetheinylcysteine synthetase (PPCS) to yield 4'-phosphopantetheinylcysteine (8). In the third step of the pathway, the cysteine moiety of 4'-phosphopantetheinylcysteine is decarboxylated by phosphopantetheinylcysteine decarboxylase (PPCDC) to produce 4'-phosphopantetheine (9). Phosphopantetheine adenyltransferase (PPAT) transfers an adenyl group to 4'-phosphopantetheine, generating dephospho-CoA (10). Finally, dephospho-CoA is phosphorylated at the 3'-position of the ribose, by dephospho-CoA kinase (DPCK), to yield CoA. Leonardi et al. (2005a) have recently published a detailed review of the universal CoA biosynthesis pathway.

Bacteria: the requirement for pantothenic acid, uptake and biosynthesis

A few years after its discovery as a growth factor for yeast, pantothenic acid was observed to be essential for the growth of a range of lactic acid bacteria (Snell et al., 1937, 1938, 1939). Subsequently, pantothenic acid was shown to stimulate the growth of many disease-causing bacteria including, but certainly not limited to, Corynebacterium diptheriae (Mueller & Klotz, 1938), Streptococcus haemolyticus (McIlwain, 1939; Woolley & Hutchings, 1940) and Clostridium tetani (Mueller & Miller, 1941). Certain strains of Corynebacterium diptheriae were found to be capable of utilizing β-alanine, although somewhat less effectively, for growth in the absence of pantothenic acid (Mueller, 1937; Mueller & Cohen, 1937). Lactic acid bacteria, and Streptococcus haemolyticus, however, were unable to utilize β-alanine for this purpose (Snell et al., 1938; McIlwain, 1939; Cheldelin...
Fig. 1. Schematic depicting pantothenate uptake, biosynthesis and transformation into CoA via the CoA biosynthesis pathway. Many organisms rely on the uptake of exogenous pantothenate (1), and virtually all organisms have the capacity for pantothenate uptake. Some bacteria and fungi are also capable of de novo pantothenate synthesis from β-alanine (3) and α-ketoisovalerate (4). α-Ketoisovalerate is first converted to ketopantoate (5) by the enzyme KPHMT. The resultant ketopantoate is reduced by KPR, yielding pantoate (6). Pantoate is subsequently condensed with β-alanine by PS, to form pantothenate. The β-alanine required for the synthesis of pantothenate is obtained both by the uptake of exogenous β-alanine and by de novo β-alanine synthesis. Bacteria synthesise β-alanine from L-aspartate (11). The decarboxylation of L-aspartate to β-alanine is catalysed by ADC. Fungi utilise a different route to β-alanine. Saccharomyces cerevisiae synthesises β-alanine from spermine (12), and Schizosaccharomyces pombe and Saccharomyces kluveri synthesise β-alanine from uracil (13). Pantothenate that is taken up from the extracellular environment or synthesized de novo enters the universal CoA biosynthesis pathway. In the first step of this pathway, pantothenate is phosphorylated by PanK. The 4′-phosphopantothenate (7) generated is condensed with a cysteine molecule by PPCS, yielding 4′-phosphopantetheinoylcysteine (8). The cysteine moiety of 4′-phosphopantetheinoylcysteine is decarboxylated by PPCDC to form 4′-phosphopantetheine (9) to which an adenylyl group is transferred by the enzyme PPAT. The dephospho-CoA (10) generated by this reaction is phosphorylated at the 3′-position of the ribose by DPCK to yield CoA (2). Bacteria are also capable of taking up pantetheine (14) or pantethine (15; the oxidized form of pantetheine). PanK phosphorylates pantetheine/pantethine, generating 4′-phosphopantetheine, which can be adenylylated and phosphorylated by PPAT and DPCK, respectively, to yield CoA. The 4′-phosphopantetheine moiety of CoA is required for the activation of various carrier proteins, including ACP. Acps catalyses the transfer of the 4′-phosphopantetheine moiety from CoA to a conserved serine residue of apo-ACP, thereby converting the enzyme to holo-ACP, activated for fatty acid synthesis. The names of the Escherichia coli genes encoding each enzyme are shown in brackets.
et al., 1945). It was proposed that certain bacteria, but not others, could convert β-alanine, known to be a component of the pantothenic acid molecule, to pantothenic acid in vivo (Mueller & Klotz, 1938).

As the early nutrition studies had implied, certain species of bacteria are capable of de novo pantothenic acid biosynthesis from β-alanine. Escherichia coli is in this group. In Escherichia coli, the β-alanine utilized for the synthesis of pantothenic acid is produced from the decarboxylation of L-aspartate (11). This reaction is catalysed by the enzyme aspartate α-decarboxylase (ADC). In the early 1980s, analysis of the phenotypes of mutant strains of Escherichia coli auxotrophic for pantothenate led to the identification of genes encoding three of the enzymes of the pantothenate biosynthesis pathway in Escherichia coli: KPHMT (panB), PS (panC) and ADC (panD) (Cronan, 1980; Cronan et al., 1982). The gene coding for KPR (panE) was identified several years later (Elischewski et al., 1999). Homologues of these genes are present in the genomes of a wide distribution of bacteria, consistent with the genes being present in a wide range of bacteria (Gerdes et al., 2002; Genschel, 2004). Genes encoding KPHMT, KPR, PS and ADC appear, however, to be absent from some bacterial genomes, for example those of Streptococcus pneumoniae and Haemophilus influenzae, consistent with previous nutrition studies that had indicated that certain species of bacteria required an exogenous supply of pantothenate for growth. The pantothenate biosynthesis pathway is similarly absent from humans (Gerdes et al., 2002; Genschel, 2004).

The genes encoding the four enzymes of the pantothenate biosynthesis pathway in Escherichia coli have now all been overexpressed, and the encoded proteins have been purified and characterized functionally (reviewed in Begley et al., 2001; Webb et al., 2004). The crystal structures of the four enzymes have also been determined (Albert et al., 1998; Matak-Vinkovic et al., 2001; von Delft et al., 2001; Lobley et al., 2005; Ciulli et al., 2007). The Mycobacterium tuberculosis KPHMT, PS and ADC enzymes have been characterized functionally and the crystal structures of KPHMT and PS, and more recently, ADC have been solved (Chopra et al., 2002; Chaudhuri et al., 2003; Sugantino et al., 2003; Wang & Eisenberg, 2003, 2006; Gopalan et al., 2006). The crystal structure of an ADC from Helicobacter pylori has also been determined (Kwon et al., 2002; Lee & Suh, 2004), and a PS from Staphylococcus aureus was crystallized recently (Seetharamappa et al., 2007).

Gerdes et al. (2002) demonstrated that when any of the four genes encoding pantothenate biosynthesis enzymes in Escherichia coli were disrupted, the cells remained viable. The pantothenate biosynthesis pathway is nonessential in Escherichia coli because exogenous pantothenate can be transported into the cell. Pantothenate uptake mechanisms are present in virtually all bacteria, and are essential for bacteria without the capacity for de novo pantothenic acid synthesis, for example Streptococcus pneumoniae and Haemophilus influenzae (Gerdes et al., 2002; Genschel, 2004).

Uptake of pantothenate by Escherichia coli is mediated by a high-affinity (∞m 0.4 μM) Na+-stimulated pantothenate transporter, which transports pantothenate at a maximal velocity of 1.6 pmol min⁻¹ 10⁸ cells⁻¹ (Vallari & Rock, 1985a). The panF gene encodes this ‘pantothenate permease’, and when panF is disrupted, the resultant mutants cannot utilize exogenous pantothenate (Vallari & Rock, 1985b). Escherichia coli cells in which both the panF gene and the gene encoding PS (panC) have been disrupted are not viable, highlighting the importance of having either a functional pantothenate biosynthesis pathway or a functional pantothenate transporter. The Escherichia coli panF gene has been cloned, sequenced and overexpressed (Jackowski & Alix, 1990). Homologues of the Escherichia coli panF gene are present in a wide range of bacteria (Gerdes et al., 2002). The bacterial pantothenate transporters share sequence similarity with the Na⁺-dependent multivitamin transporters found in mammalian cells (Prasad et al., 1998). In addition to the pantothenate transporter, Escherichia coli also has the capacity to take up β-alanine for the biosynthesis of pantothenate. The amino acid carrier CycA was recently shown to mediate the transport of β-alanine into Escherichia coli with low affinity (∞m 2.4 mM; Schneider et al., 2004).

**Bacteria: the synthesis of 4′-phosphopantothenate from pantothenate**

In the first step of the pathway from pantothenate to CoA, pantothenate is phosphorylated by the enzyme PanK. An Escherichia coli panK gene (designated coaA) was first identified in 1987 (Vallari & Rock), some years after the coaA gene of Salmonella typhimurium had been identified (Dunn & Snell, 1979). Both genes were identified through analysis of temperature-sensitive bacterial strains with mutations that caused a temperature-dependent inactivation of PanK. The Escherichia coli coaA gene has subsequently been shown to encode a PanK that binds pantothenate with a ∞m of 36 μM, and ATP with a ∞m of 136 μM (Song & Jackowski, 1994). Even before the gene encoding Escherichia coli PanK had been identified, PanK had been shown to be the primary rate-controlling enzyme of the CoA biosynthesis pathway in Escherichia coli (Jackowski & Rock, 1981). The activity of this protein is tightly regulated by CoA feedback inhibition, both in vitro and in vivo (Vallari et al., 1987; Song & Jackowski, 1992). Escherichia coli PanK is also inhibited by CoA thioesters, but less potently than by free CoA (Vallari et al., 1987). CoA binds to PanK, inhibiting competitively the binding of ATP, which is required for the
phosphorylation of pantothenate (Vallari et al., 1987; Song & Jackowski, 1994).

In 2000, structures were determined for the Escherichia coli PanK enzyme in complex with a nonhydrolysable ATP analogue, and in complex with CoA (Yun et al., 2000). Subsequently, the structure of the Escherichia coli PanK with pantothenate and ADP bound to the active site was solved (Ivey et al., 2004). The Escherichia coli PanK crystal structure indicated that it belonged to the P-loop kinase superfamily (Leipe et al., 2003). The structures of the PanK complexes shed light on the structural basis for the binding of pantothenate, ATP and CoA, and provided an explanation for the competition between ATP and CoA.

Homologues of the Escherichia coli coaA gene are present in the genomes of many bacteria, including Salmonella typhimurium (mentioned above), Haemophilus influenzae, Mycobacterium tuberculosis, Streptococcus pneumoniae and Vibrio cholerae (Gerdes et al., 2002; Genschel, 2004). The primary structure of these bacterial ParKs is distinct from the primary structure of eukaryotic PanKs (Calder et al., 1999; Rock et al., 2000; Gerdes et al., 2002; Genschel, 2004). The PanK enzyme from Mycobacterium tuberculosis has now been overexpressed, purified, characterized and the crystal structure of the enzyme in complex with a CoA derivative has been solved (Das et al., 2005, 2006; Kumar et al., 2007). Although several differences between the Escherichia coli and Mycobacterium tuberculosis structures were observed, the CoA-binding region of the two enzymes was almost completely conserved.

Although the Escherichia coli PanK is considered to be the prototypical bacterial PanK, homologues of the Escherichia coli coaA gene are missing from the genomes of many bacteria (Gerdes et al., 2002; Genschel, 2004). A coaA gene, unrelated to the Escherichia coli coaA gene, but moderately related to eukaryotic PanK genes, such as the mammalian panK genes and the putative Drosophila melanogaster panK gene (fumble), was identified in the genome of Staphylococcus aureus (Gerdes et al., 2002; Choudhry et al., 2003). The Staphylococcus aureus coaA gene was overexpressed, and the encoded protein was purified and characterized (Choudhry et al., 2003; Leonardi et al., 2005b). The enzyme catalyses the same reaction as the Escherichia coli PanK, and binds the reaction substrates, pantothenate and ATP, with an affinity comparable to the affinity with which Escherichia coli PanK binds. The $K_m$ values for pantothenate and ATP are 27 and 93 $\mu$M, respectively, as reported by Choudhry et al. (2003), or 23 and 34 $\mu$M, respectively, as reported by Leonardi et al. (2005b). A homologue of the Staphylococcus aureus coaA gene has been identified in the genome of Bacillus anthracis, the bacterium responsible for the disease anthrax; however, the gene product is reported to lack PanK activity (Nicely et al., 2007). The main distinction between the PanKs of Escherichia coli and Staphylococcus aureus is in the regulation of PanK activity. Staphylococcus aureus PanK is refractory to feedback regulation by CoA and its thioesters, and as flux through the pathway is not regulated at downstream steps, CoA accumulates (Leonardi et al., 2005b). This property is thought to relate to the function of CoA in maintaining the reducing environment in Staphylococcus aureus, which lacks glutathione (Newton et al., 1996; deCardayre et al., 1998; Leonardi et al., 2005b). The lack of CoA feedback inhibition distinguishes the Staphylococcus aureus PanK from the mammalian PanKs with which it shares limited sequence homology. Similar to the Escherichia coli PanK, mammalian PanK activity is regulated by CoA and CoA thioesters (Halvorsen & Skrede, 1982; Fisher et al., 1985; Rock et al., 2000, 2002; Zhang et al., 2005, 2006; Hong et al., 2007).

The structure of Staphylococcus aureus PanK in complex with the nonhydrolysable ATP analogue, AMP-PNP, was determined recently (Hong et al., 2006). The structure determined for the Staphylococcus aureus PanK places this protein in the acetate and sugar kinase/heat shock protein 70/actin (ASKHA) superfamily (Bork et al., 1992; Hurley, 1996). The structure of the PanK in complex with AMP–PNP, combined with the results of kinetic analysis of Staphylococcus aureus PanK mutants, revealed the identity of potential key residues contributing to substrate binding and catalysis.

A panK gene that shares no sequence homology with the panK genes described above was recently identified in the genome of Bacillus subtilis (Yocum & Patterson, 2004). A coaA gene homologous to the coaA gene characterized in Escherichia coli previously, is also present in the genome (Genschel, 2004; Yocum & Patterson, 2004). Although disruption of the novel panK gene (designated coaX) had no effect on the growth of Bacillus subtilis, the coaX gene could not be disrupted in a strain of Bacillus subtilis with a pre-existing deletion in the coaA gene, consistent with the coaX gene encoding a PanK that becomes essential in the absence of another wild-type panK gene. CoaX homologues have been identified in the genomes of a number of other bacteria, for example Mycobacterium tuberculosis, which, like Bacillus subtilis, also possess a gene with homology to the Escherichia coli coaA gene. Homologues of the Bacillus subtilis coaX gene have also been identified in the genomes of bacterial pathogens such as Helicobacter pylori, Pseudomonas aeruginosa and Bordetella pertussis, in which no panK genes have been identified previously, and in the genome of Bacillus anthracis where, as mentioned above, a homologue of the Staphylococcus aureus coaA gene has been identified (Yocum & Patterson, 2004; Nicely et al., 2007). The coaX homologue in the genome of Bordetella pertussis, the causative agent of whooping cough, has been demonstrated to be essential for the viability of Bordetella pertussis, consistent with the gene encoding the only functional PanK of this organism (DeShazer et al., 1995; Wood & Friedman, 2000;
Staphylococcus aureus and Escherichia coli pantothenate with comparable affinity to the dependent phosphorylation of pantothenate, and bound to Helicobacter pylori encoded PanKs also differ from the Bacillus subtilis involved in pertussis toxin production (DeShazer et al., 2005). Previous studies, however, have characterized this gene as coding for a Bvg accessory factor involved in pertussis toxin production (DeShazer et al., 1995; Wood & Friedman, 2000).

Brand & Strauss (2005) cloned and overexpressed the coaX gene from Bacillus subtilis, and its homologue from Helicobacter pylori. Both expressed proteins catalysed the ATP-dependent phosphorylation of pantothenate, and bound to pantothenate with comparable affinity to the Escherichia coli and Staphylococcus aureus PanKs characterized previously. $K_m$ values for pantothenate of 168 and 101 $\mu$M were measured for the Bacillus subtilis and the Helicobacter pylori proteins, respectively. In contrast to the Escherichia coli and Staphylococcus aureus PanKs, however, the PanKs encoded by the coaX genes of Bacillus subtilis and Helicobacter pylori display a surprisingly low affinity for ATP ($K_m$ values of 3 and 10 $\mu$M, respectively). The Bacillus subtilis and Helicobacter pylori coaX-encoded PanKs also differ from the Escherichia coli and Staphylococcus aureus PanKs in that the phosphorly transfer reaction they catalyse is dependent on the presence of a monovalent cation (Hong et al., 2006). Furthermore, the activity of the coaX-encoded PanKs was not regulated by CoA or by acetyl-CoA. The PanKs expressed from the coaX genes of Bacillus subtilis and Helicobacter pylori therefore exhibit characteristics distinct from both types of bacterial PanKs (exemplified by the Escherichia coli and Staphylococcus aureus PanKs) characterized previously.

The crystal structures of three homologues of the Bacillus subtilis coaX-encoded PanK, from Thermotoga maritima, Pseudomonas aeruginosa and Bacillus anthracis, were solved recently (Hong et al., 2006; Yang et al., 2006; Nicely et al., 2007). All three proteins were also demonstrated to possess PanK activity. The structure of these PanKs places them in the ASKHA superfamily that the Staphylococcus aureus PanK is also a member of. The structure of the Pseudomonas aeruginosa PanK crystallized with pantethenate in the active site revealed a number of key interactions that contribute to pantethenate binding. A combination of modelling pantethenate and ATP into the active sites of the structures, and mutagenesis studies with the Pseudomonas aeruginosa and Helicobacter pylori PanKs, led to the identification of potential key catalytic residues in this type of PanK. Although the Staphylococcus aureus PanK and the Thermotoga maritima, Pseudomonas aeruginosa and Bacillus anthracis PanKs belong to the same structural superfamily, when the structures of the Staphylococcus aureus and Pseudomonas aeruginosa PanKs were compared, the pantethenate and ATP-binding pockets were observed to differ dramatically, shedding light on the structural basis for the diverse biochemical properties of these two types of PanKs (Hong et al., 2006). The noted absence of a hydrophobic pocket, similar to the pocket that accommodates the thiol tail of CoA in the Escherichia coli PanK structure, from the Thermotoga maritima PanK crystal structure, provides a possible explanation for the lack of CoA feedback inhibition of coaX-encoded PanKs (Yang et al., 2006).

Bacteria: the synthesis of 4′-phosphopantetheine from 4′-phosphopantothenate

Following the PanK-catalysed step of the CoA biosynthesis pathway, 4′-phosphopantothenate is condensed with a cysteine molecule by PPCS and the cysteine moiety is decarboxylated by PPCDC. A bifunctional enzyme, coded for by the coaBC gene (formerly the $d$pf gene), catalyses both these reactions in Escherichia coli (Kupke et al., 2000; Strauss et al., 2001). The $d$pf gene was originally identified for its ability to complement the temperature-sensitive phenotype of an Escherichia coli mutant strain with a defect in DNA synthesis (Spitzer & Weiss, 1985). The gene encoded a flavin mononucleotide-containing protein believed to play a role in DNA and/or pantethenate metabolism (Spitzer & Weiss, 1985; Spitzer et al., 1988). In 2000, Kupke et al. showed that the amino terminal domain of the protein coded for by the $d$pf gene catalysed the decarboxylation of 4′-phosphopantothenoyl cysteine. The carboxy terminal domain of the protein was later shown to catalyse the formation of 4′-phosphopantothenoylcysteine from 4′-phosphopantothenate and l-cysteine (Strauss et al., 2001).

Most bacteria have a gene with homology to the Escherichia coli coaBC gene, and are predicted to express the bifunctional PPCS/PPCDC enzyme. In Streptococcus pneumoniae and other streptococci and enterococci, however, separate genes are predicted to code for PPCS and PPCDC (Gerdes et al., 2002; Genschel, 2004). Interestingly, Bacillus anthracis and Bacillus cereus have two genes: one potentially coding for the bifunctional PPCS/PPCDC enzyme and the other potentially coding for a monofunctional PPCS enzyme. In contrast to the situation in most bacteria, in humans, two distinct enzymes catalyse the conversion of 4′-phosphopantothenate to 4′-phosphopantothenoylcysteine, and the subsequent decarboxylation (Daugherty et al., 2002). Although the PPCDC domain of the Escherichia coli bifunctional PPCS/PPCDC enzyme shares primary structure similarity with the human PPCDC monofunctional enzyme, there is little similarity between the primary structures of the Escherichia coli PPCS domain and the human PPCS (Daugherty et al., 2002; Gerdes et al., 2002; Genschel, 2004). The PPCS domain of the Escherichia coli bifunctional enzyme differs from the human PPCS enzyme also because it utilizes CTP rather than ATP as a cofactor for the synthesis of 4′-phosphopantothenoylcysteine (Strauss et al., 2001; Daugherty et al., 2002).
and characterized (Kupke, 2001, 2002). Using site-directed mutagenesis, the residues involved in the catalysis of both the formation of 4’-phosphopantothenoylcysteine from 4’-phosphopantothenate, and the decarboxylation of 4’-phosphopantothenoylcysteine have been identified, and the mechanistic details of the reactions have been elucidated (Kupke et al., 2000; Kupke, 2001; Strauss & Begley, 2001; Kupke, 2002, 2004). Recently, the PPCS and PPCDC domains of a PPCS/PPCDC bifunctional enzyme expressed by the archaebacterium Methanocaldococcus jannaschii were also overexpressed and characterized (Kupke & Schwarz, 2006). The crystal structure of the carboxy terminal PPCS domain of the Escherichia coli PPCS/PPCDC enzyme was solved in 2004 (Stanitzek et al., 2004). The structure of a PPCDC domain from a bacterial PPCS/PPCDC enzyme remains to be solved.

**Bacteria: the synthesis of dephospho-CoA from 4’-phosphopantetheine**

In 1999, PPAT, the enzyme that catalyses the reversible transfer of an adenylyl group from ATP to 4’-phosphopantetheine, was purified from *Escherichia coli*. The purified enzyme was subjected to N-terminal sequencing, and analysis of the sequencing results revealed that a gene named *kdhB*, predicted previously to encode a protein involved in lipopolysaccharide biosynthesis (Clementz & Raetz, 1991; Roncero & Casadaban, 1992), coded for the enzyme (Geerlof et al., 1999). The gene, renamed *coaD*, was cloned and the protein was overexpressed, purified and characterized (Geerlof et al., 1999). Kinetic parameters were determined for the reverse reaction; *Kₘ* values of 7 µM and 0.22 mM were measured for dephospho-CoA and pyrophosphate, respectively. In humans, PPAT forms part of a bifunctional enzyme (called CoA synthase) that also catalyses the final step in the CoA biosynthesis pathway, the phosphorylation of dephospho-CoA (Aghajanian & Worley, 2002; Daugherty et al., 2002; Zbyvoloup et al., 2002). The PPAT characterized by Geerlof et al., however, lacked DPCK activity, distinguishing it from the human PPAT/DPCK. Homologues of the *Escherichia coli* PPAT have been identified in a wide range of bacteria, as well as in the genomes of fungi and animals (Mishra et al., 2001; Gerdes et al., 2002; Genschel, 2004). The PPAT domain of the human bifunctional PPAT/DPCK enzyme shows no significant sequence similarity to the *Escherichia coli* PPAT, highlighting the potential for selective inhibition of bacterial PPATs (Daugherty et al., 2002; Gerdes et al., 2002; Genschel, 2004).

The *Escherichia coli* PPAT was the first enzyme from the CoA biosynthesis pathway of any organism to have its structure solved. Crystal structures have been obtained for *Escherichia coli* PPAT in complex with the reaction product dephospho-CoA and with the substrates 4’-phosphopantetheine and ATP (Izard & Geerlof, 1999; Izard et al., 1999; Izard, 2002). The crystal structures of the PPATs expressed by *Mycobacterium tuberculosis* and *Thermus thermophilus* have also been determined (Brown et al., 2004; Morris & Izard, 2004; Takahashi et al., 2004). The former was crystallized in the apo form, and the latter in complex with 4’-phosphopantetheine.

As described above, phosphorylation of pantothenate by PanK is the primary rate-limiting step in the CoA biosynthesis pathway in *Escherichia coli*. However, 4’-phosphopantetheine accumulates in *Escherichia coli* cells, consistent with the PPAT-catalysed reaction being a secondary target for the regulation of flux through the pathway (Jackowski & Rock, 1984). Geerlof et al. (1999) found that CoA was tightly bound to the *Escherichia coli* PPAT when it was purified from *Escherichia coli*, and it was postulated that like PanK, PPAT is subject to feedback inhibition by CoA.

The structure of PPAT in complex with CoA was determined, and revealed the structural basis behind the binding of CoA (Izard, 2003).

**Bacteria: the synthesis of CoA from dephospho-CoA**

DPCK catalyses the final step in the CoA biosynthesis pathway: the phosphorylation of the 3’-hydroxyl group of the ribose sugar moiety of dephospho-CoA. The *Escherichia coli* gene coding for DPCK was initially identified by searching for homologues of the *Corynebacterium ammoniagenes* DPCK for which an amino terminal sequence had been obtained by subjecting the protein, purified from *Corynebacterium ammoniagenes* cells, to N-terminal sequencing (Martin & Drueckhammer, 1993; Mishra et al., 2001). The *Escherichia coli* gene identified, previously designated *yacE* but renamed *coaE*, was cloned and overexpressed. The purified protein was shown to catalyse the formation of CoA from dephospho-CoA and ATP, and *Kₘ* values of 0.74 and 0.14 mM were measured for dephospho-CoA and ATP, respectively (Mishra et al., 2001). Homologues of the *Escherichia coli* DPCK gene have since been identified in the genomes of a wide range of bacteria, as well as in the genomes of fungi and animals (Mishra et al., 2001; Gerdes et al., 2002; Genschel, 2004). The *Escherichia coli* DPCK also shares similarity with the DPCK domain of the human bifunctional CoA synthase (Daugherty et al., 2002; Gerdes et al., 2002; Genschel, 2004).

The structures of DPCKs from *Escherichia coli*, *Haemophilus influenzae* and *Thermus thermophilus* have been solved (Obmolova et al., 2001; O’Toole et al., 2003; Seto et al., 2005). The *Escherichia coli* DPCK was crystallized in the apo form, the *Haemophilus influenzae* DPCK with ATP bound to the active site and the DPCK from *Thermus thermophilus* crystallized both in the apo form and in complex with ATP.
Bacteria: the pathway from pantothenate to CoA is essential

By various methods, the four genes coding for the enzymes that catalyse the conversion of pantothenate to CoA in *Escherichia coli* have been shown to be essential for the viability of *Escherichia coli* (Vallari & Rock, 1987; Freiberg et al., 2001; Hare et al., 2001; Gerdes et al., 2002). The CoA biosynthesis pathway has also been demonstrated to be essential for *Mycobacterium tuberculosis* (Sassetti et al., 2003). Most bacteria cannot utilize CoA and the phosphorylated intermediates of the pathway when supplied exogenously, and hence the pathway is likely to be essential for the viability of many more bacteria.

Pantetheine (14) and the corresponding disulphide, pantetheine (15), have been shown to stimulate the growth of lactic acid bacteria, with varying efficiency in the absence of pantetheine (Snell et al., 1950; Craig & Snell, 1951). Jackowski & Rock (1984) demonstrated that *Escherichia coli* was able to utilize extracellular pantetheine for the synthesis of CoA. The *Escherichia coli* PanK has recently been shown to catalyse the phosphorylation of pantetheine (Worthington & Burkart, 2006). A $K_m$ of 91 $\mu$M was measured for pantetheine; the enzyme therefore binds pantetheine with approximately threefold lower affinity than it does pantothenate. 4'-Phosphopantetheine generated from the phosphorylation of pantetheine re-enters the CoA biosynthesis pathway at the PPAT-catalysed step. Although the utilization of pantetheine renders PPDC and PPCDC redundant, PanK, PPAT and DPCK remain indispensable.

Bacteria: acyl carrier protein (ACP) is activated by 4'-phosphopantetheine

As mentioned earlier, the 4'-phosphopantetheine moiety of CoA is required for the activation of a number of carrier proteins. ACP is the acyl group carrier in bacterial fatty acid synthesis. ACP exists in an inactive apo form until a 4'-phosphopantetheine prosthetic group from CoA is covalently bound to the terminal sulphydryl group of the 4'-phosphopantetheine prosthetic group of holo-ACP. The transfer of the 4'-phosphopantetheine moiety of CoA to ACP is catalysed by a phosphopantetheinyl-transferase called ACP synthase (AcpS; Elovson & Vagelos, 1968). The *Escherichia coli* gene coding for AcpS (acpS) has been cloned, overexpressed and characterized (Lambalot & Walsh, 1995; Flugel et al., 2000). Homologues of the *Escherichia coli* acpS gene have been identified in a range of bacterial genomes (Lambalot et al., 1996; McAllister et al., 2006). The *Escherichia coli* acpS gene has been shown to be essential for the viability of *Escherichia coli* (Lam et al., 1992; Takiff et al., 1992; Gerdes et al., 2002).

Fungi: the requirement for pantothenic acid, uptake and biosynthesis

Pantothenic acid was initially observed to stimulate the growth of several strains of *Saccharomyces cerevisiae* in a very striking way (Williams et al., 1933, 1940; Williams & Saunders, 1934; Leonian & Lilly, 1942). Subsequently, Lochhead & Landkerkin (1942) reported that pantothenic acid also stimulated the growth of several species of osmophilic yeast. For some yeast, β-alanine, the precursor of pantothenic acid, could replace pantothenic acid as a growth factor (Williams & Rohrman, 1936; Weinstock et al., 1939; Williams et al., 1940; Lochhead & Landkerkin, 1942; Saret & Cheldelin, 1945a), consistent with at least some yeast being capable of pantothenic acid biosynthesis from β-alanine.

In 1999, a gene encoding a KPHMT from *Aspergillus nidulans* was identified by complementation of the pantothenate auxotrophy of an *Aspergillus nidulans* panB mutant. The gene was overexpressed in *Escherichia coli* and the purified protein was demonstrated to possess KPHMT activity (Kurtov et al., 1999). Genschel et al. (1999) identified a PS gene from *Saccharomyces cerevisiae* (Yil145c) through complementation of an *Escherichia coli* panC mutant auxotrophic for pantothenate. Homologues of the *Escherichia coli* genes coding for KPHMT, KPR and PS have been identified in the genomes of *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and Neurospora crassa (Genschel, 2004). The genomes of *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and Neurospora crassa, however, lack a homologue of the *Escherichia coli* gene coding for the ADC enzyme that catalyses the synthesis of β-alanine from 1-aspartate (Genschel, 2004). Furthermore, biochemical evidence was consistent with *Saccharomyces cerevisiae* requiring an exogenous supply of pantothenate or β-alanine for growth (Stolz & Sauer, 1999). Hence, it was believed that fungi lacked the ability to synthesize β-alanine *de novo*. However, White et al. (2001) showed *Saccharomyces cerevisiae* to be capable of β-alanine synthesis, and found that the mechanism for β-alanine synthesis in *Saccharomyces cerevisiae* is distinct from that in *Escherichia coli*. It was demonstrated that β-alanine was produced by the degradation of the polyamine spermine (12; Fig. 1), and that this reaction was dependent on the amine oxidase FMS1. Certain yeast, for example *Schizosaccharomyces pombe* and *Saccharomyces kluyveri*, have been shown to utilize uracil (U; Fig. 1) for the synthesis of β-alanine (Gojkovic et al., 2000, 2001; Stolz et al., 2004).

Despite the presence of a pantothenate synthesis pathway, yeast take up pantothenate via pantothenate transporters localized to the plasma membrane. In *Saccharomyces cerevisiae*, pantothenate is transported into the cell by the protein...
product of the FEN2 gene, Fen2p (Stolz & Sauer, 1999). Mutation in the FEN2 gene is associated with resistance to the antifungal agent fluropropiomorph (Marcireau et al., 1996). Fen2p is a high-affinity H⁺: pantothenate symporter (Kₘ for pantothenate 3.5 μM) that allows efficient uptake of pantothenate at physiological concentrations (Stolz & Sauer, 1999). The protein is unrelated to the pantothenate transporter from Escherichia coli (Jackowski & Alix, 1990; Reizer et al., 1990) and the Na⁺-dependent multivitamin transporter from mammals (SMVT; Prasad et al., 1998). Yeast are also capable of uptake of exogenous β-alanine. In Saccharomyces cerevisiae, the uptake of β-alanine is mediated primarily by the yeast general amino acid permease, Gap1p (Stolz & Sauer, 1999).

The liz1 gene of Schizosaccharomyces pombe encodes a protein that is localized to the plasma membrane of Schizosaccharomyces pombe and is closely related to the Fen2p protein of Saccharomyces cerevisiae (Moyninhan & Enoch, 1999; Stolz et al., 2004). When this gene is disrupted, cell division defects, similar to those observed in Schizosaccharomyces pombe mutants with defects in fatty acid biosynthesis, are observed (Moyninhan & Enoch, 1999; Stolz et al., 2004). When expressed in Saccharomyces cerevisiae FEN2 mutants, Liz1 transported pantothenate into the cells with high affinity (Kₘ for pantothenate 2.75 μM) via a mechanism consistent with proton symport (Stolz et al., 2004). The phenotype of the liz1 mutants highlights the importance of the pantothenate uptake mechanism for Schizosaccharomyces pombe.

Fungi: the biosynthesis of CoA from pantothenate

A panK gene from the fungus Aspergillus nidulans was the first eukaryotic PanK to be expressed and characterized (Calder et al., 1999). The gene was identified by screening a cDNA library from Aspergillus nidulans for the ability to complement functionally the temperature-sensitive phenotype of an Escherichia coli PanK mutant strain (called ts9). The corresponding protein phosphorylated pantothenate with a Kₘ of 60 μM (at saturating concentrations of ATP). The distinct primary structure of the Aspergillus nidulans PanK distinguished it from the Escherichia coli PanK that had been expressed and characterized previously (Song & Jackowski, 1992), as did its unique regulatory properties. While the activity of Escherichia coli PanK is inhibited potently by nonesterified CoA, and to a lesser extent by CoA thioesters, acetyl-CoA is a much more potent inhibitor of Aspergillus nidulans PanK than CoA. The Aspergillus nidulans PanK shares sequence similarity with the mammalian PanKs (Rock et al., 2000; Ni et al., 2002). The sensitivity to inhibition by acetyl-CoA relative to free CoA is reminiscent of the regulatory properties determined for several mammalian PanKs (Halvorsen & Skrede, 1982; Fisher et al., 1985; Rock et al., 2000, 2002; Zhang et al., 2005).

Although a PanK from Saccharomyces cerevisiae is yet to be characterized, a homologue of the Aspergillus nidulans panK gene is present in the genome (designated Ydr531w; Calder et al., 1999). This gene is essential for viability of the yeast, as demonstrated by a large-scale deletion study of Saccharomyces cerevisiae (Giaever et al., 2002; Saccharomyces genome database: www.yeastgenome.org). A second gene in the genome of Saccharomyces cerevisiae (Ygr205w), not essential for viability, encodes a protein with a three-dimensional structure closely resembling that of the Aspergillus coli PanK (de La Sierra-Gallay et al., 2004). It has not yet been determined whether this protein catalyses the phosphorylation of pantothenate. If the protein codons for Ygr205w is a PanK, it is unclear whether the protein can catalyse the reaction of pantothenate to CoA, as E. coli panK mutants are able to compensate functionally for the Ydr531w gene product when this gene is disrupted.

Homologues of the human PPCS and PPCDC genes, and the individual domains of the human bifunctional PPAT/DPCK gene, have been identified in the genome of Saccharomyces cerevisiae (Genschel, 2004). The Saccharomyces cerevisiae genes putatively encoding PPCS (Yil083c), PPCDC (Ykl088w), PPAT (Ygr277c) and DPCK (Ydr196c) are all essential for the viability of Saccharomyces cerevisiae (Giaever et al., 2002; Saccharomyces genome database: www.yeastgenome.org). However, these four genes are yet to be expressed and characterized. Saccharomyces cerevisiae has also been reported to express a multienzyme CoA-synthesizing complex that catalyses the conversion of pantothenate to CoA via a pathway in which the order of reactions is distinct from that reported in other organisms (Bucovaz et al., 1997). However, a gene coding for such a complex has not yet been identified, and the biochemical data supporting the existence of this complex have been queried (Leonardi et al., 2005a).

Avian malaria parasites: pantothenic acid and the biosynthesis of CoA

While investigating the requirements of the avian malaria parasite, Plasmodium falciparum, maintained for short periods of time intraerythrocytically in vitro, Trager (1943) showed that the survival of the parasite was enhanced by the addition of calcium pantothenate to an appropriate culture medium. Following this finding, it was shown that the development of erythrocytic stage Plasmodium gallinaceum, in chickens, was inhibited by a pantothenic acid deficiency in the host that lowered the concentration of pantothenate in the blood (Brackett et al., 1946). These discoveries implicated pantothenic acid as a growth factor required by the erythrocytic stage malaria parasite.
Although it was shown that *Plasmodium* species developing within their host erythrocyte require pantothenic acid as a growth factor, in experiments in which blood-stage *Plasmodium lophurae* parasites were grown extracellularly in vitro, growth was enhanced by CoA but not by pantothenic acid (Trager, 1952, 1954). It was therefore concluded that *Plasmodium lophurae*, and possibly other malaria parasites, require exogenous CoA rather than its precursor pantothenic acid, and that the pantothenic acid requirement of the intraerythrocytic parasite reflects the pantothenic acid needed by the host erythrocyte for synthesis of CoA within the host cell compartment. It is the host-derived CoA that is taken up by the parasite. Consistent with this, a pantothenic acid analogue that inhibited the growth of *Plasmodium lophurae* developing intracellularly had no effect on the growth of parasites maintained extracellularly (Trager, 1966). The analogue presumably exerted its antiplasmodial effect by inhibiting the host erythrocyte's CoA biosynthesis, thereby reducing the CoA available to the parasite. Biochemical analysis later revealed that while the activity of all five enzymes of the CoA biosynthesis pathway could be detected in uninfected and *Plasmodium lophurae*-infected duck erythrocytes, none of the enzymes could be detected in *Plasmodium lophurae* parasites isolated from their host erythrocyte, further support for the parasite's dependence on the host erythrocyte for CoA (Bennett & Trager, 1967; Brohn & Trager, 1975; Trager & Brohn, 1975). The mechanism by which erythrocytic stage *Plasmodium lophurae* parasites, developing within duck erythrocytes, are believed to obtain the CoA required for growth is summarized in Fig. 2a.

**The human malaria parasite: pantothenic acid and the biosynthesis of CoA**

After a method for the continuous cultivation of intraerythrocytic stage *Plasmodium falciparum* parasites (within human erythrocytes) was established in 1976 (Trager & Jensen, 1976), it was demonstrated conclusively that *Plasmodium falciparum*, like *Plasmodium lophurae*, requires pantothenic acid to support its intracellular growth in vitro. Pantothenic acid was one of only a few low-molecular-weight compounds, and the only water-soluble vitamin, for which the intracellular parasite was shown to have an absolute requirement (Divo et al., 1985; Saliba et al., 2005).

Pantothenate is taken up rapidly by *Plasmodium falciparum*-infected erythrocytes but not by normal uninfected erythrocytes (Saliba et al., 1998). As shown in Fig. 3, the uptake occurs via the 'new permeability pathways' (NPP) induced in the erythrocyte membrane by the maturing parasite (Saliba et al., 1998). These NPP have a broad specificity and confer upon the host cell membrane an increased permeability to a range of low-molecular-weight solutes including various nutrients, metabolic wastes and inorganic ions (Ginsburg et al., 1983, 1985; Cabantchik, 1990; Ginsburg, 1994; Kirk et al., 1994). By contrast, normal uninfected erythrocytes lack the NPP and do not appear to express a functional pantothenate transporter.

Once inside the infected erythrocyte, pantothenate is phosphorylated by PanK. Phosphorylation was shown to occur primarily in the parasite and not the erythrocyte cytosol (Saliba et al., 1998). From the erythrocyte cytosol...
pantothenate is presumed to enter the parasitophorous vacuole (a vacuole that forms when the parasite invades the erythrocyte via an endocytosis-like mechanism and in which the parasite remains enclosed) by diffusion through low-selectivity channels in the parasitophorous vacuole membrane (Desai et al., 1993). From there, pantothenate is transported across the parasite plasma membrane into the parasite where it is phosphorylated. The transport into the parasite is mediated by a $\text{H}^+:\text{pantothenate}$ symport mechanism with a 1:1 stoichiometry that couples the transport of a proton, down its electrochemical gradient, to the transport of pantothenate (Saliba & Kirk, 2001).

These findings contrast markedly with the situation in \textit{Plasmodium lophurae}-infected erythrocytes, in which, because activity of the CoA biosynthesis enzymes could not be detected in \textit{Plasmodium lophurae} parasites isolated from their host cell, the parasite is predicted to rely on the host erythrocyte for its supply of CoA (discussed previously). Consistent with the differences observed between \textit{Plasmodium lophurae} and \textit{Plasmodium falciparum}, in contrast with \textit{Plasmodium lophurae}, erythrocytic stage \textit{Plasmodium falciparum} maintained extracellularly do not require exogenous CoA (Trager, 2002). The biochemical characteristics of the pantothenate transporter and PanK expressed by \textit{Plasmodium falciparum} differ significantly from those of their mammalian counterparts.

Coenzyme A biosynthesis inhibitors as antimicrobial agents

Fig. 3. Pantothenate uptake and utilization by the \textit{Plasmodium falciparum}-infected human erythrocyte. Pantothenate enters the host erythrocyte via the NPP (a). From the erythrocyte cytosol, pantothenate is presumed to diffuse across the parasitophorous vacuole membrane into the parasitophorous vacuole, where it is taken up by the parasite via a $\text{H}^+:\text{pantothenate}$ symport mechanism (b). It is then converted into CoA in the parasite via a series of five enzyme-mediated steps (c–f, h). The enzymes catalysing the conversion of pantothenate to dephospho-CoA are predicted to be localized to the parasite cytosol while the enzyme catalysing the conversion of dephospho-CoA to CoA is predicted to localise to the parasite’s apicoplast. The dephospho-CoA presumably enters the apicoplast, via an as yet unknown mechanism (g), before being converted to CoA. A 4′-phosphopantetheine prosthetic group derived from CoA is transferred to apo-ACP (the inactive form of ACP), thereby converting it to holo-ACP (i, the functional form of ACP). The parasite’s apicoplast may be the site of ACP activation.
counterparts. In mammalian cells, uptake of pantothenate is mediated by Na⁺:pantothenate symporters; i.e. transport of pantothenate is Na⁺-dependent rather than H⁺-dependent. The mammalian pantothenate symporters are characterized by high substrate affinities; for example, the Na⁺-coupled multivitamin transporter transports pantothenate with a K_m of 2–5 μM (Prasad et al., 1998, 1999; Wang et al., 1999), while the H⁺:pantothenate symporter of Plasmodium falciparum transports pantothenate with low affinity, K_m of 23 mM (Saliba & Kirk, 2001). The molecular identity of this transporter remains to be determined. The PanK expressed by Plasmodium falciparum has a very high affinity for pantothenate, K_m of 0.3 μM (Saliba & Kirk, 2001). The mammalian PanKs that have been characterized bind pantothenate with a > 10-fold lower affinity (Halvorsen & Skrede, 1982; Fisher et al., 1985; Zhang et al., 2005, 2006). However, similar to the mammalian PanKs, the activity of Plasmodium falciparum PanK is inhibited by CoA and its thioesters (Lehane et al., 2007).

The genes predicted to code for the five enzymes of the Plasmodium falciparum CoA biosynthesis pathway share sequence similarity with the genes coding for the human CoA biosynthetic enzymes (Genschel, 2004). The enzyme with the lowest similarity to its human counterpart is PPAT. By contrast to the situation in humans, separate genes are predicted to code for PPAT and DPCK. Although PanK activity has been detected in the parasite, biochemical evidence for the presence of the remaining four CoA biosynthetic enzymes in the parasite is yet to be reported. There are no candidate genes coding for KPR, KPHMT and PS, the enzymes involved in pantothenate biosynthesis, in the parasite’s genome (Genschel, 2004; Muller & Kappes, 2007), consistent with previous studies that have implicated the parasite as a pantothenate auxotroph (Divo et al., 1985; Saliba et al., 2005).

The final enzyme in the Plasmodium falciparum parasite’s CoA biosynthesis pathway, DPCK, is predicted to possess an apicoplast Leader sequence, consistent with an apicoplast localization for the protein (Ralph et al., 2004). The localization of DPCK to the apicoplast, a vestigial nonphotosynthetic plastid that is the site of fatty acid biosynthesis, would allow CoA to be generated in the same organelle in which CoA- and 4′-phosphopantetheine-utilizing enzymes, such as ACP, are localized. The localization of a DPCK to an intracellular compartment has been reported previously; the human bifunctional PPAT/DPCK enzyme (CoA synthase) is localized to the mitochondrial outer membrane (Zhivoloup et al., 2003).

The localization of DPCK to the apicoplast, hints at the existence of an uptake mechanism that allows dephospho-CoA, synthesized in the cytosol by the first four enzymes of the pathway (none of which have an obvious apicoplast Leader sequence), to be transported into the apicoplast. It has been postulated that obligate intracellular bacterial pathogens such as Mycoplasma, Rickettsia and Chlamydia, which appear to lack all the enzymes of the CoA biosynthesis pathway with the exception of DPCK, also possess such a mechanism for dephospho-CoA uptake across the plasma membrane (Daugherty et al., 2002; Gerdes et al., 2002). The ability of Plasmodium lophurae to utilize exogenous CoA implies the existence of a CoA transporter on this parasite’s plasma membrane.

**CoA biosynthesis as a target for antimicrobial drug discovery**

CoA is an enzyme cofactor in numerous metabolic reactions and a source of 4′-phosphopantetheine in multiple biosynthetic pathways. The importance of CoA is reflected in the conservation of the CoA biosynthesis pathway across animals, plants and microorganisms (Genschel, 2004). Microorganisms generally lack mechanisms for exogenous CoA salvage and therefore must rely on the endogenous CoA biosynthesis pathway from pantothenic acid, for the production of CoA. Attempts to disrupt genes encoding the enzymes in this pathway, in a variety of microorganisms, have invariably failed or resulted in lethal phenotypes, consistent with the CoA biosynthesis pathway being essential for viability. Hence, the CoA biosynthesis pathway presents multiple targets for the development of novel antimicrobial agents. Despite the universal biochemistry of the CoA biosynthesis pathway, there is little sequence similarity between prokaryotic and eukaryotic enzyme counterparts (Genschel, 2004). The low sequence homology between prokaryotic and eukaryotic enzymes, and differences in the regulation of the pathway, highlight the possibility that selective inhibition of CoA biosynthesis in pathogenic microorganisms might be achieved with small molecule inhibitors.

Pantothenate uptake, for those microorganisms that rely on pantothenate salvage, and pantothenate biosynthesis, for those microorganisms that synthesize pantothenate de novo, provide additional targets for the inhibition of CoA biosynthesis. The H⁺-dependent pantothenate transport mechanisms of yeast and Plasmodium have little in common with the Na⁺-dependent multivitamin transporters present in mammalian cells, highlighting the possibility that selective inhibition of pantothenate uptake could be achieved. The absence of a pantothenate biosynthesis pathway in humans highlights the pantothenate biosynthesis pathway present in several bacteria and fungi as an attractive target for the development of agents to combat these microorganisms. Although pantothenic acid uptake mechanisms, present in virtually all microorganisms, render the pantothenate biosynthesis pathway nonessential, the viability of targeting this pathway as a chemotherapeutic strategy is...
highlighted by the impaired survival and pathogenesis of a panC and panD knock-out strain of Mycobacterium tuberculosis in immunocompetent and immunocompromised mice (Sambandamurthy et al., 2002, 2006; Webb et al., 2004).

The knowledge that we have gained over the last few years on the molecular, biochemical and structural properties of the enzymes of the CoA biosynthesis pathway from both microbial pathogens and humans should allow us to develop effective inhibitors of CoA biosynthesis that act as selective and potent inhibitors of the growth of pathogens.

**PanK as a target for antimicrobial drug discovery**

As the key rate-determining enzyme of the CoA biosynthesis pathway in many pathogenic microorganisms, PanK is a prime target for the inhibition of CoA biosynthesis and, thereby, microbial growth. PanK genes have now been identified in the genomes of almost all pathogenic microorganisms, and several have been overexpressed and characterized. In recent years, the genes coding for the multiple PanKs expressed by humans and other mammals have been identified and several of the encoded proteins have been characterized (Rock et al., 2000, 2002; Zhou et al., 2001; Ni et al., 2002; Li et al., 2005; Zhang et al., 2005, 2006; Hong et al., 2007).

The homology between prokaryotic and eukaryotic PanK sequences is very weak and this is reflected in their distinct kinetic and regulatory properties. There are also significant differences in the kinetic and/or regulatory properties of the PanKs expressed by eukaryotic microorganisms, such as Plasmodium falciparum, and the PanKs expressed by humans, even though these PanKs are more closely related in sequence. It should therefore be possible to develop selective small molecule inhibitors of the PanKs expressed by the pathogenic microorganisms of interest. Consistent with this hypothesis, PanKs from different organisms are already known to differ markedly in their sensitivity to the natural inhibitors of PanK activity: CoA and the various CoA thioesters.

The structure of PanKs from six different pathogenic microorganisms, representing the three different classes of PanK enzymes that have been identified to date, have now been solved, as have the structures of two human PanK isoforms (Hong et al., 2007). The available structures should facilitate greatly the design of inhibitors of microbial PanKs.

**Pantothenic acid analogues as antibacterial and antifungal agents**

Since the structure of pantothenic acid was determined to be D-3-(2,4-dihydroxy-3,3-dimethylbutyramido)propionic acid (Fig. 4a; Williams & Major, 1940), many analogues of the vitamin have been prepared. While a few of these have been reported to possess growth-promoting activity for organisms that require an exogenous supply of pantothenic acid, and some have lacked activity, many have been inhibitory, antagonizing the growth-promoting activity of pantothenic acid. The preparation and testing of pantothenic acid analogues in the 10 years following the elucidation of the structure of pantothenic acid was the subject of a detailed review by Williams et al. (1950). A review by Copping (1966) reported on a selection of the analogues that had been tested during this period and also in the following 15 years. Here, a comprehensive review of the inhibitory activity of pantothenic acid analogues reported between 1940 and today is presented.

**Pantoyltaurine and related compounds**

The sulphonic acid analogue of pantothenic acid (16; Fig. 4b) was the first compound reported to exert an inhibitory effect on the growth of microorganisms that was antagonized specifically by pantothenic acid. This compound, named pantoyltaurine under the nomenclature of Barnett & Robinson (1942a), who introduced the name pantoyl for the 2,4-dihydroxy-3,3-dimethylbutyryl moiety of pantothenic acid (Fig. 4a), was first prepared by Snell (1941a). Pantoyltaurine inhibited the in vitro growth of various lactic acid bacteria, including Lactobacillus arabinosus, Streptococcus lactis and Leuconostoc mesenteroides, bacteria that all require an exogenous supply of pantothenic acid for growth (Snell, 1941a, b). By contrast, it was found that pantoyltaurine had no effect on the growth of organisms such as Escherichia coli and Staphylococcus aureus, which are capable of synthesizing their own pantothenic acid. The demonstration that the growth-inhibitory effect of pantoyltaurine was overcome by increasing the concentration of pantothenate was consistent with pantoyltaurine inhibiting the growth of these organisms by interfering with the utilization of pantothenic acid. Interestingly, Snell (1941b) found that the inhibitory activity of pantoyltaurine appeared to be a property chiefly (if not solely) of the dextrotoratory isomer. The activity of pantothenic acid similarly resides only in the dextrotoratory isomer (Stiller et al., 1940); hence, the stereochemistry required for inhibition appeared to be the same as that required for growth promotion. Subsequent reports confirmed the antibacterial activity of pantoyltaurine against additional bacteria, including Streptococcus haemolyticus, and strains of Corynebacterium diphtheriae (McIlwain, 1942a, b; Barnett & Robinson, 1942b). The potency of pantoyltaurine against the different bacteria varied. The growth of Lactobacillus arabinosus was inhibited completely at a d-pantoyltaurine concentration 1000 times the concentration of pantothenate in the medium, and complete inhibition of the growth of Streptococcus haemolyticus was observed at a concentration of d,l-pantoyltaurine 500 times the pantothenate concentration. By comparison,
Fig. 4. Chemical structures of pantothenic acid and analogues of pantothenic acid, pantetheine and pantethine. The structure of pantothenic acid is shown with the pantoyl moiety of the structure highlighted in blue and the β-alanine moiety highlighted in red (a). The structures of pantothenic acid, pantetheine and pantethine analogues that have been synthesized and tested for antimicrobial activity are shown in (b). The analogues reported to inhibit the growth of bacteria are shown in the blue rectangle, analogues reported to inhibit the growth of fungi are shown in the green rectangle and analogues reported to inhibit the growth of malaria parasites are shown in the red square (b). Analogues with activity against more than one type of organism are shown in the overlapping regions, and analogues that have been shown to lack antimicrobial activity are shown in the outer region. Stereochemistry is omitted from the pantothenic acid, pantetheine and pantethine analogue structures.
Coenzyme A biosynthesis inhibitors as antimicrobial agents

*Leucomostoc mesenteroides* was relatively resistant to the growth-inhibitory effects of pantoyltaurine; complete inhibition of growth was only observed at a concentration of $\delta$-pantoyltaurine 162,000-fold that of pantothenate. The reason for the differing sensitivities to pantoyltaurine was unclear. The sensitivity of different strains of *Corynebacterium diphtheriae* to inhibition by pantoyltaurine appeared to correlate with the pantothenic acid requirement of the strain. However, this relationship did not hold for all the bacteria tested.

Growth of the yeast *Saccharomyces cerevisiae* was also inhibited by pantoyltaurine (Snell, 1941b). As for the lactic acid bacteria, the inhibitory effect was antagonized by pantothenic acid. Complete inhibition of growth was observed when $\delta$-pantoyltaurine was present at a concentration 10,000-fold the concentration of pantothenate. Snell (1941b) also investigated the effect of pantoyltaurine on yeast growth when grown in the presence of $\beta$-alanine, which can promote the growth of *Saccharomyces cerevisiae* in the absence of pantothenate. He observed that the yeast was far less sensitive to pantoyltaurine when the growth-promoting compound in the medium was $\beta$-alanine and not pantothenate.

Pantoyltaurine was also shown to inhibit growth of bacteria *in vivo*. When administered in large and frequent subcutaneous doses (17 doses of 2.2 g kg$^{-1}$ body weight in a 96-h period), $d,l$-pantoyltaurine protected rats from infection with a virulent strain of *Streptococcus haemolyticus* (McIlwain & Hawking, 1943). Furthermore, by administering pantothenate simultaneously to the rats, the therapeutic effect of pantoyltaurine was reversed, demonstrating that the antibacterial effect *in vivo* resulted from inhibition of pantothenic acid utilization, as had been shown to be the case *in vitro*. $d,l$-Pantoyltaurine was inactive against *Streptococcus haemolyticus* in mice when administered intraperitoneally in doses of 2.5 g kg$^{-1}$ body weight three times daily. However, the concentration of pantothenate in the blood of mice is several-fold higher than the concentration in the blood of rats; hence, it is likely that the concentration of pantoyltaurine reached in the blood of the mice was insufficient to antagonize the high plasma level of pantothenate (McIlwain & Hawking, 1943).

Although the dose of pantoyltaurine required for therapy in rats infected with *Streptococcus* was fairly high, no adverse effects of this compound were observed. Rats receiving the compound continued to gain weight normally throughout the treatment (McIlwain & Hawking, 1943). Mice also tolerated high doses of the compound. Single doses of pantoyltaurine, whether administered intraperitoneally or orally, were reported to be without effect on mice and rats in doses of 2–5 g kg$^{-1}$ body weight (Barnett & Robinson, 1942b; Snell *et al.*, 1943). Snell *et al.* (1943) reported that long-term daily oral administration of pantoyltaurine (at doses of 200 mg kg$^{-1}$ body weight) caused symptoms of pantothenic acid deficiency in mice; after 3–4 weeks of treatment the mice ceased growing, their fur became coarse and porphyrin deposits appeared on their whiskers. However, these results were not in agreement with the findings of Woolley & White (1943), who reported no such symptoms in mice or hamsters administered daily with concentrations of $\delta$-pantoyltaurine up to 2000-fold that of pantothenate. Furthermore, Snell *et al.* (1943) did not show that the symptoms of pantothenic acid deficiency they observed could be reversed by administration of additional pantothenic acid or removal of pantoyltaurine, and hence must be considered with caution.

The activity of pantoyltaurine both *in vitro* and *in vivo*, and its relatively easy preparation, led to the synthesis of more pantothenic acid analogues, structurally very similar to pantoyltaurine. These analogues included pantoyltauramidine (17), the sulphonamide analogue of pantothenic acid, and compounds in which the sulphonic acid group of pantoyltaurine was replaced with other sulphur-containing functional groups, such as a thiol, a disulphide and sulphone (McIlwain *et al.*, 1942; Barnett & Robinson, 1942b; McIlwain, 1942b; Barnett, 1944; Madinaveitia *et al.*, 1945). Many of these compounds were designed with the objective of reducing the water solubility of pantoyltaurine, while maintaining a structural resemblance as close as possible to pantothenic acid. It was hoped that reducing the solubility of pantoyltaurine would result in a decrease in the rate of elimination of the compounds from the blood stream of animals, thereby allowing the blood concentration to be maintained at a level sufficiently high to antagonize the activity of pantothenate present, thereby improving antibacterial activity *in vivo*. As was the case with pantoyltaurine, several of the analogues inhibited growth of bacteria with a requirement for exogenous pantothenic acid, including *Streptococcus haemolyticus*, *Lactobacillus arabinosus*, *Lactobacillus casei* and certain strains of *Corynebacterium diphtheriae*, *in vitro*, with the inhibitory effect reversed by pantothenic acid. The structures of a few of the more active compounds (17–20) are shown in Fig. 4b. While some of the analogues (18, 19 and 20a) had activity comparable to pantoyltaurine *in vitro*, and 20b and 20c were slightly more active against *Lactobacillus casei* and *Streptococcus pyogenes in vitro*, of the compounds tested in rats infected with streptococci (18, 19, 20a and 20b), only 20b inhibited the growth of streptococci *in vivo*. Compound 20b increased the survival time of rats infected with *Streptococcus pyogenes* when administered in eight doses of 750 mg kg$^{-1}$ body weight over a 39-h period initiated after infection, however, much less effectively than pantoyltaurine. Although most compounds were well tolerated, rats administered with compound 18 displayed signs of toxicity (Barnett, 1944). Compound 18 was subsequently shown to inhibit the...
growth of rats by antagonizing the growth-promoting activity of pantothenic acid (Boxer et al., 1955). Aryl-substituted analogues of pantoyltaurine (21 and 22) were prepared with the same aim of reducing the solubility relative to pantoyltaurine (Barnett et al., 1944). However, compound 21 was essentially inactive against Lactobacillus arabinosus in vitro and inactive against Streptococcus haemolyticus both in vitro and in Streptococcus haemolyticus-infected rats. Compound 22 was similarly inactive in vitro and was not tested in vivo.

The activity of pantoyltauramide against malaria parasites (discussed in a later section) and, to a lesser extent, against bacteria, encouraged the synthesis of several N-substituted pantoyltauramides, such as 23a–23d (Winterbottom et al., 1947). The majority of the compounds were tested for both antibacterial activity (discussed in a later section) and antimalarial activity (discussed in a later section). With few exceptions, the N-substituted pantoyltauramides inhibited the growth of Streptococcus haemolyticus in vitro, with a potency greater than pantoyltaurine. Of the compounds screened, 12, including 23a–c, inhibited the growth of Streptococcus haemolyticus completely when present in 12–50-fold molar excess relative to pantotenate (White et al., 1946; Winterbottom et al., 1947). Streptococcus viridans and Streptococcus agalactiae were also susceptible. In addition, many of these compounds succeeded where pantoyltaurine had failed, protecting mice from infection with Streptococcus haemolyticus when administered as a single oral dose at the time of infection (White et al., 1946). Furthermore, no toxic effects were observed in mice and rats (White et al., 1946). The most active compounds, with median survival doses (the median dose resulting in 50% survival) of 200 and 300 mg kg⁻¹ body weight, were 23a and 23b, respectively. A correlation between the antibacterial activity of certain analogues in vivo, and the blood concentration of the compounds reached after oral administration, was observed (White et al., 1946). Where tested, the antibacterial activity of the pantoyltauramides, both in vitro and in vivo, was antagonized by pantotenate. All compounds were prepared and screened as dextrorotatory iso- mers rather than racemic mixtures. Two analogues were also prepared as levorotatory isomers; however, as was observed for pantoyltaurine, only the dextrorotary isomers of these analogues inhibited pantothenic acid utilization (Winterbottom et al., 1947).

**N-Pantooyl-substituted amines**

Pfaltz (1943) prepared the alcohol analogue of pantothenic acid, pantothenol (24), and showed that this compound was as effective as pantothenic acid in preventing achromotrichia (an absence or loss of pigment in hair) of black rats. This activity was demonstrated to be a result of the oxida-
Compound 25f was the only analogue that proved to be more effective than pantothanol against the growth of Leuconostoc mesenteroides. Shive & Snell (1945a) reported the maximum inhibition of Leuconostoc mesenteroides growth with 1,3,5-pantothanol at a molar ratio of analogue to pantothanol of 700, and with 25f (also as a mixture of isomers) at a molar ratio of 600. Lactobacillus arabinosus, an organism inhibited to a greater extent by pantoyltaurine than by pantothanol, was inhibited more potently by many of the N-pantoylarylalkylamines than by pantothanol. No definitive relationship between activity and length of the N-substituent was observed with this organism. The most effective N-pantoylarylamine against Lactobacillus arabinosus was 25h, which inhibited the growth of Lactobacillus arabinosus maximally at a concentration 4500-fold that of pantothanol in the medium, when present as a mixture of isomers. However, this compound, and other N-pantoyl-substituted amines that showed improved activity relative to pantothanol, were in general no more potent than pantoyltaurine.

The organism-specific sensitivity to these compounds, presumably a result of differences in compound permeability, quantitative differences in pantothenic acid utilization or structural differences between the target in different organisms (or a combination of any of the above), highlighted the fact that the potency of a series of analogues against a particular organism cannot be predicted reliably from the potency against another, and must be determined experimentally. It also raises the possibility that pathogenic bacteria may be targeted selectively without inhibiting other bacteria such as the desirable bacteria in the intestinal flora. With few exceptions, the N-pantoyl-substituted amines described here were not tested against yeast.

Fissekis et al. (1960a) synthesized a series of N-pantoylarylalkylamines by condensing pantolactone with various arylalkylamines. These analogues also proved to be inhibitory to lactic acid bacteria (Leuconostoc mesenteroides, Lactobacillus arabinosus and Streptococcus lactis) and the inhibition was reversed competitively by pantothenate. A number of the N-pantoylarylalkylamines, including 25i and 25j, were found to be more inhibitory than many of the N-pantoylalkylamines and related compounds discussed in the above paragraph. To allow a direct comparison between the biological activity of the N-pantoylarylalkylamines and the N-pantoylalkylamines synthesized previously, Fissekis et al. (1960a) included 25d, the N-pantoylarylamine reported to be the most effective inhibitor of Leuconostoc mesenteroides and second only to 25h as the most effective inhibitor of Lactobacillus arabinosus, in their assays. Compound 25i was the most potent N-pantoylarylalkylamine against both Leuconostoc mesenteroides and Lactobacillus arabinosus, and was twice as effective as N-pantoylarylalkylamine 25d at inhibiting the growth of Leuconostoc mesenteroides, and five times as active as 25d at inhibiting the growth of Lactobacillus arabinosus. Fissekis et al. (1960a) reported that a 2.5- and 400-fold molar excess of 25i relative to pantothanol resulted in maximal inhibition of Leuconostoc mesenteroides and Lactobacillus arabinosus, respectively. However, they also reported that growth of the two organisms was completely inhibited in the presence of a five and 2000-fold molar excess of the reference N-pantoylarylalkylamine (25d) relative to pantothenate. By contrast, Shive & Snell (1945b) had reported that a 750- and 7500-fold molar excess of 25d was required for maximum inhibition of Leuconostoc mesenteroides and Lactobacillus arabinosus, respectively. The inhibitory concentrations reported by Fissekis et al. (1960a) and Shive & Snell (1945b) both represented the concentration of a D,L mixture of each analogue that was required for inhibition in the presence of D-pantothenate. Compound 25j was the most potent N-pantoylarylalkylamine against Streptococcus lactis; maximum inhibition was observed when the analogue was present at a concentration 50-fold that of pantothenate. Parker et al. (1963) went on to synthesize N-pantoyl(substituted-phenyl)arylalkylamines in which various functional groups, including chloro and nitro groups, were introduced into the phenyl nucleus of N-pantoylarylalkylamines. It was hypothesized that certain functional groups would interact irreversibly with the target enzyme, producing more potent inhibitors, the activity of which would no longer be antagonized by pantothenate. All compounds inhibited growth of lactic acid bacteria, and some analogues had increased potency relative to the corresponding unsubstituted N-pantoylarylalkylamines. However, the activity of these compounds was still antagonized competitively by pantothenic acid; hence, introduction of the selected functional groups had not converted these analogues to noncompetitive antagonists. A para-chloro substituent increased the inhibitory activity of the corresponding N-pantoylarylalkylamines against Leuconostoc mesenteroides approximately fivefold, while a para-nitro substituent enhanced only the activity of N-pantoylarylalkylamine 25j. para-Chloro and para-nitro substituents enhanced only the inhibitory activity against Lactobacillus arabinosus of N-pantoylarylalkylamines with very short arylalkyl chains (Parker et al., 1963).

It was predicted that pantothanol would not be an effective antibacterial agent in vivo because it would undergo oxidation to pantothenic acid in the host animal (Snell & Shive, 1945). The majority of the N-pantoyl-substituted amines that have since been reported to inhibit bacterial growth in vitro should, however, not be converted to pantothenic acid in vivo, and hence may be effective antibacterials in the in vivo situation. To the best of our knowledge, N-pantoyl-substituted amines have not been tested in vivo as antibacterial agents. In light of their activity in vitro, in vivo testing of these compounds may prove worthwhile.

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved
Pantohydrazide and related compounds

With the aim of preparing pantothenic acid analogues more active than pantoyltaurine in vivo, Madinaveitia et al. (1945) synthesized a number of compounds, among which was pantohydrazide (26). This analogue demonstrated inhibitory activity somewhat greater than pantoyltaurine against Lactobacillus casei in vitro, and the activity was antagonized specifically by pantothenate. In the presence of 10 μM D,L-pantothenate, pantohydrazide was only slightly more active than pantoyltaurine, but at an extracellular pantothenate concentration of 0.1 μM the analogue was more than 50 times more active. Pantohydrazide also inhibited growth of Streptococcus pyogenes in vitro, but failed to show antibacterial activity in rats infected with Streptococcus pyogenes when administered subcutaneously in four doses of 1 g kg\(^{-1}\) body weight over a 12-h period after infection. The simple pantothenic acid analogue pantamide (27), and compounds 28 and 29, were also shown by Madinaveitia et al. (1945) to possess antibacterial activity against Lactobacillus casei in vitro. The activity of the two former compounds was antagonized by pantoyltaurine, while the antibacterial activity of the latter appeared to be independent of the concentration of pantothenate present in the medium. Compounds 28 and 29 were shown to also inhibit the growth of Streptococcus pyogenes in vitro. The in vivo antibacterial activity of these compounds, which was generally less potent than pantoylhydrazide in vitro, was not investigated.

Pantothenedrates and related compounds

After it was shown that replacement of the carboxyl group of nicotinic acid and para-aminobenzoic acid with various ketone groups resulted in compounds that inhibited specifically the growth-promoting activity of these two compounds, Woolley & Collyer (1945) prepared and tested two ketone analogues of pantothenic acid. The first of these, a methyl ketone analogue called D-methylpantothenone (30), inhibited the growth of the bacterium Lactobacillus casei and the yeast Saccharomyces cerevisiae. Growth of these two organisms was reduced to half maximum at concentrations of D-methylpantothenone between 460 and 2300 μM. The activity, however, was not reversed by pantothenic acid, and it is possible that the inhibition resulted from a nonpantothenic acid-related toxic effect, perhaps caused by contaminants present in the crude preparation of the compound (Woolley & Collyer, 1945). The possibility that this analogue acts as a noncompetitive inhibitor of pantothenic acid utilization was not discussed, and this should perhaps be investigated further. The second analogue, a phenyl ketone analogue, termed D-phenylpantothenone (31), inhibited the growth of several bacteria with a requirement for exogenous pantothenic acid (Lactobacillus casei, Lactobacillus arabinosus, and a strain of haemolytic streptococcus) as well as several microorganisms capable of pantothenic acid biosynthesis (Escherichia coli, Staphylococcus aureus, Endomyces vernalis and Saccharomyces cerevisiae). In the presence of 0.2 μM pantothenic acid, the concentrations of D-phenylpantothenone resulting in 50% inhibition of the growth of Lactobacillus casei, Lactobacillus arabinosus, the strain of haemolytic streptococcus, Escherichia coli, Staphylococcus aureus, Endomyces vernalis and Saccharomyces cerevisiae were 190, 650, 220, 7200, 500, 140 and 120 μM, respectively. The toxicity of this compound against the organisms that required an exogenous supply of pantothenic acid for growth was antagonized by pantothentic acid. By contrast, the toxicity against Escherichia coli, Endomyces vernalis and Saccharomyces cerevisiae was not reversed with increasing concentrations of pantothenic acid. Interestingly, the growth-inhibitory effect against Staphylococcus aureus, growth of which is not dependent on an exogenous supply of pantothenic acid, but is somewhat stimulated in its presence, was relieved by pantothentic acid.

The activity of D-phenylpantothenone against avian malaria parasites (discussed in a later section) prompted the synthesis of a series of phenylpantothenic acid analogues, which were also tested for in vitro antibacterial activity against Lactobacillus casei (Lutz et al., 1947). While a variety of analogues were prepared (analogues in which the pantoyl moiety of phenylpantothenone was modified, analogues in which the propiophenone moiety was varied and analogues in which substituents had been introduced into the phenyl nucleus), only those analogues that retained the intact pantoyl moiety with D-configuration (the same configuration as the growth-promoting isomer of pantothenic acid) demonstrated any appreciable antibacterial activity against Lactobacillus casei. Of these, D-para-tolylpantothenone (32a), in which the phenyl nucleus of phenylpantothenone is substituted with a methyl group, was the most active antibacterial agent, and demonstrated a sevenfold improved activity relative to D-phenylpantothenone. D-para-Chlorophenylpantothenone (32b) was 2.7-fold more active than D-phenylpantothenone. Lutz et al. (1947) also synthesized a phenylpantothenone analogue named D-para-tolylpantothenol (33) that possessed a structure closely related to both D-para-tolylpantothenone and pantothanol. This compound was, however, not as effective as phenylpantothenone against Lactobacillus casei in vitro.

Analogues of pantothenic acid with a modified pantoyl moiety

In contrast to the variety of structural modifications to the β-alanine moiety of pantothenic acid that have produced inhibitory pantothenic acid analogues, only a limited
number of modifications to the pantoyl group of pantothenic acid (Fig. 4a) have yielded effective antagonists of pantothenic acid. A number of pantoyl-modified pantothenic acid analogues have been reported to possess slight growth-promoting activity (reviewed in Williams et al., 1950). Other pantothenic acid analogues, for example 34a and 34b, which lack the α-hydroxy group of pantothenic acid, have been shown to display slight inhibitory activity against certain bacteria; however, the growth-inhibitory effect cannot be reversed by pantothenic acid and hence growth inhibition is either a result of noncompetitive inhibition of pantothenic acid utilization or inhibition of an unrelated cellular function (McIlwain et al., 1942; Barnett & Robinson, 1942b; McIlwain, 1942b).

One pantoyl-modified pantothenic acid analogue that has been shown to be an effective antagonist of pantothenic acid is α-methylpantothenic acid (35), in which a methyl substituent is present on the third carbon unit from the amide carbonyl group. This compound, first prepared by Drell & Dunn (1946), has been shown to inhibit the in vitro growth of a range of lactic acid bacteria, including Lactobacillus arabinosus, Lactobacillus casei, Lactobacillus fermenti and Leuconostoc mesenteroides, in a manner competitive with pantothenic acid (Drell & Dunn, 1946, 1948). In the presence of pantothenate concentrations between 0.3 and 3.0 μM, molar ratios of D,L-α-methylpantothenic acid: D-pantothenate of 3000–5900, 260–1200, 1500–2400 and 3000–5900 were reported to inhibit completely the growth of Lactobacillus arabinosus, Lactobacillus casei, Lactobacillus fermenti and Leuconostoc mesenteroides, respectively (Drell & Dunn, 1946, 1948). Under the same conditions, pantoyltaurine was considerably less active against most organisms tested; D,L-pantoyltaurine was required in 4000–6200, 13000–15000, 130000–150000 and 250000–300000-fold molar excess to exert the same level of inhibition of the growth of Lactobacillus arabinosus, Lactobacillus casei, Lactobacillus fermenti and Leuconostoc mesenteroides, respectively (Drell & Dunn, 1948). By contrast with pantoyltaurine and many of the analogues reported previously, which varied in their effectiveness against any given bacteria, α-methylpantothenic acid demonstrated effective inhibition of all 23 lactic acid bacteria tested.

α-Methylpantothenic acid was also shown to protect mice from infection with β-haemolytic streptococcus when administered in the diet, at a concentration 200-fold that of pantothenic acid, for 4 days before infection (Drell & Dunn, 1948). When administered orally to chicks and mice, in a single dose of up to 8.4 and 10 g kg⁻¹ body weight, respectively, or intraperitoneally to chicks and mice in a single dose of up to 1 g kg⁻¹ body weight, α-methylpantothenic acid exhibited no (or only low) acute toxicity (Schinazi et al., 1950). Daily administration (over several weeks) of α-methylpantothenic acid in the diet of mice, however, revealed that the analogue also antagonized the action of pantothenic acid in animals; there was a decrease in food consumption, in weight gain and in the survival time of mice, with the extent of the decrease increasing proportionally with the ratio of the α-methylpantothenic acid concentration to the pantothenic acid concentration in the diet (Drell & Dunn, 1951). When the ratio of D,L-α-methylpantothenic acid to D-pantothenate was >100, symptoms of pantothenic acid deficiency ensued. The administration of pantothenate could relieve the effects of α-methylpantothenic acid, consistent with the compound inhibiting pantothenic acid utilization by the animals. By contrast, Bird et al. (1955) reported no symptoms of pantothenic acid deficiency in rats fed daily with α-methylpantothenic acid, even when a concentration of α-methylpantothenic acid 200 times the concentration of pantothenate was added to the diet. A pantothenic acid deficiency was reported to result in humans given a diet lacking in pantothenic acid and dosed daily with 0.5 g α-methylpantothenic acid (Bean & Hodges, 1954). However, as discussed by Bird et al. (1955), because the appropriate experimental controls were not used, the observed deficiency was not shown conclusively to result from administration of the pantothenic acid analogue.

Following the success of α-methylpantothenic acid in inhibiting the growth of a range of bacteria in vitro, and in inhibiting the growth of β-haemolytic streptococci in vivo, several more α-substituted analogues of pantothenic acid with alkylaryl substituents in place of the terminal methyl group of α-methylpantothenic acid were synthesized (Drell & Dunn, 1954a). Compounds in this series also incorporated other changes into the pantoyl moiety, namely the replacement of one or both the β-methyl substituents with hydrogen. Although none of the compounds were consistently more active than α-methylpantothenic acid (the compound with the closest structural resemblance to pantothenic acid), all analogues in this series inhibited growth of lactic acid bacteria. The structures of a number of these compounds (36a–c) are shown in Fig. 4b. With all but one compound (36b), the activity was competitively reversed with pantothenate. The activity of this group of compounds demonstrated that various α-substituents were permitted without significant loss of inhibitory activity, and furthermore, that the β-methyl substituents were not required for an analogue to antagonize the growth-promoting activity of pantothenic acid for lactic acid bacteria. Interestingly, substitution of one of the β-methyl groups for a hydroxymethyl group produced an analogue (called hydroxypantothenic acid) that possesses considerable growth-promoting activity, rather than inhibitory activity, for various species of lactic acid bacteria and for Saccharomyces cerevisiae (Mitchell et al., 1940). Replacement of the methyl groups with a cyclopentane ring, but not with a cyclohexane...
ring, however, also resulted in a compound (37) inhibitory to bacterial growth (Fissekis et al., 1960b). This compound inhibited the growth of *Streptococcus lactis* and *Lactobacillus arabinosus*, and the inhibitory effect was reversed with pantothentic acid; the growth of *Streptococcus lactis* and *Lactobacillus arabinosus* was reduced by 50% when the analogue (prepared as a racemic mixture) was present in 2500- and 3300-fold excess, respectively, relative to D-pantothentic acid. These findings therefore highlight the importance of the nature of the β-substituents in determining the activity of a pantothenic acid analogue.

The incorporation of pantoyl modifications into inhibitory pantothenic acid analogues with pre-existing variations in the β-alanine moiety of pantothenic acid has had mixed results. Homopantoyltaurine (38), in which an extra carbon unit is introduced into the pantoyl group of pantoyltaurine, is an inhibitor of pantothenic acid utilization and growth of *Streptococcus haemolyticus* (McIlwain et al., 1942; Barnett & Robinson, 1942b; McIlwain, 1942b). It is, however, much less active than pantoyltaurine. As an inhibitor of *Diplococcus pneumoniae* and certain strains of *Corynebacterium diptheriae*, homopantoyltaurine is also less effective than pantoyltaurine (McIlwain, 1942b). The introduction of a methyl substituent into the pantoyl moiety of pantoyltaurine, as in α-methylpantothentic acid, improves the antibacterial activity of pantoyltaurine against certain lactic acid bacteria, such as *Leuconostoc citrovorum* and *Streptococcus faecalis*, in vitro. Maximum inhibition of *Leuconostoc citrovorum* and *Streptococcus faecalis* was observed when this compound (α-methylpantoyltaurine; 39a) was present (as a mixture of isomers) at a concentration 2400 and 26 000 times the concentration of D-pantothenate, respectively (Drell & Dunn, 1948). Maximum inhibition of these two organisms with D,L-pantoyltaurine occurred only with concentrations 4200 and 35 000 times the concentration of D-pantothenate. However, 39a was significantly less effective than α-methylpantothentic acid against all bacteria tested, as were the α-substituted pantoyltaurine analogues 39b and 39c (Drell & Dunn, 1954a). α-Methylpantothenol (40) inhibited the growth of *Leuconostoc mesenteroides*, *Lactobacillus arabinosus* and *Lactobacillus casei* in vitro in a manner dependent on the pantothenic acid concentration of the assay medium (Drell & Dunn, 1954b). For *Lactobacillus casei*, the analogue was more active than pantothenol but less active than α-methylpantothentic acid; however, for *Leuconostoc mesenteroides* and *Lactobacillus arabinosus* the analogue was less effective than both pantothenol and α-methylpantothentic acid. α-Methylpantothenol was almost as effective as α-methylpantothenic acid in inhibiting the growth of mice, when incorporated into a daily pantothenic acid-deficient diet (Drell & Dunn, 1954b). The compound is predicted to be oxidized and yield α-methylpantothentic acid in vivo and may therefore prove to be effective as an antibacterial in animals.

### Pantothenamides and related compounds

The success with which pantothenic acid antagonists had been generated by simply modifying the β-alanine moiety of pantothenic acid led Clifton et al. (1970) to synthesize a series of N-pantoyl-substituted amines, in which the terminal carboxylic acid of pantothenic acid was replaced by various secondary amides. The analogues in this series, termed N-substituted pantothanemides, inhibited growth of the lactic acid bacteria *Lactobacillus arabinosus*, *Lactobacillus casei* and *Pediococcus cerevisiae* (formerly *Leuconostoc mesenteroides*), and the effect was antagonized competitively by pantothenic acid. The structures of a selection of the antibacterial pantothanemides (41a–f) reported by Clifton et al. (1970) are shown in Fig. 4b. Although the susceptibility to each compound varied between organisms, among the most active were N-nonylpantothanemide (41a) and N-phenylpantothanemide (41b). With these compounds, complete inhibition of growth of at least one of the above organisms was observed at a concentration of ~20 μM (~25-fold higher than the concentration of D,L-pantothene in the assay medium). A number of the pantothanemides, including N-pentylpantothanemide (41c) and N-heptylpantothanemide (41d), were, in contrast to many of the pantothenic acid analogues reported previously, also effective inhibitors of the growth of *Escherichia coli*. Furthermore, the analogues were unique among the pantothentic acid analogues inhibitory to *Escherichia coli*, as the growth-inhibitory effect exerted was, at lower concentrations of the analogues, reversed by pantothenic acid. At higher concentrations, however, the compounds were irreversibly toxic. The most potent pantothanemide for *Escherichia coli* was N-pentylpantothanemide, inhibiting growth completely at 2 μM, a concentration ~2.5-fold the concentration of D,L-pantothenate in the medium.

A series of pantothentic acid analogues similar to the pantothanemides, in which the N-substituted amide functionality introduced in place of the terminal carboxylic acid group of pantothenic acid was replaced with either an N-substituted carbamate or ureido group, was synthesized by Sargent et al. (1975). Although these analogues lacked the activity against *Escherichia coli* that the pantothanemides had been shown to possess, they were effective inhibitors of the growth of the lactic acid bacteria *Lactobacillus plantarum* and *Pediococcus cerevisiae*. A selection of the active carbamate and ureido analogues (42a–e and 43a–b, respectively) is shown in Fig. 4b. The inhibitory effect of all analogues was antagonized competitively by pantothentic acid. In general, the carbamate analogues
Coenzyme A biosynthesis inhibitors as antimicrobial agents

were more effective than the corresponding ureido analogues. In the presence of 0.3 μM extracellular pantothentic acid, the most active carbamate analogue (42a) inhibited the growth of Lactobacillus plantarum completely at a concentration of 2 μM, and several others, including compounds 42b–e, completely inhibited the growth of Lactobacillus plantarum and Pediococcus cerevisiae at concentrations below 25 μM.

There has been renewed interest in the pantothenamides recently, after a study performed by Strauss & Begley (2002) revealed that the pantothenamide N-pentylpantothenamide (41c) was converted in vitro to a CoA analogue, named ethyldethia-CoA (44; Fig. 5), by three of the enzymes of the Escherichia coli CoA biosynthesis pathway: PanK, PPAT and DPCK. Furthermore, the conversion occurred more rapidly than the conversion of pantothenate to CoA. Strauss & Begley (2002) postulated that N-pentylpantothenamide exerted its growth-inhibitory effect not by inhibiting CoA biosynthesis and thereby reducing the amount of CoA being synthesized in the cell, but rather through its biosynthetic conversion to a CoA analogue that they predicted would inhibit CoA-utilizing and acetyl-CoA-utilizing enzymes. The central role of CoA in cellular metabolism highlights the potential of CoA antagonists as antimicrobial agents. Structural analogues of large phosphorylated compounds like CoA analogues, however, are unlikely to be suitable for use as antibiotics because although they may be effective enzyme inhibitors, bacteria are unable to take them up. The ability of Escherichia coli to synthesize the CoA analogue from the pantothenamide de novo overcomes this obstacle of getting a CoA analogue across the cell membrane.

While Ivey et al. (2004) showed N-pentylpantothenamide and N-heptylpantothenamide (41d) to be effective competitive inhibitors of the Escherichia coli PanK-catalysed phosphorylation of pantothenate* (both with IC₅₀ values, the concentration resulting in 50% inhibition, of 60 μM), the inability of pantothenate to attenuate the antibacterial activity of the pantothenamides against Escherichia coli by more than twofold indicated that competitive inhibition of pantothenate phosphorylation was not the primary target for pantothenamide toxicity (Zhang et al., 2004). Further evidence that this was the case was provided by experiments showing that overexpression of the Escherichia coli coaA gene did not attenuate the activity of the pantothenamides against Escherichia coli (Zhang et al., 2004).

Zhang et al. (2004) confirmed what Strauss & Begley (2002) had predicted from their in vitro study, demonstrating that in vivo, N-pentylpantothenamide and N-heptylpantothenamide were converted to CoA analogues that were incorporated by, and inhibited the function of, ACP. As discussed in a previous section, apo-ACP is activated by the attachment of a 4’-phosphopantetheine moiety from CoA.

*Inhibition of pantothenate phosphorylation is observed when the enzyme PanK is inhibited (i.e. in a competitive or noncompetitive manner) by a compound, and when a compound competes for binding to the enzyme and is phosphorylated in place of the natural substrate, pantothenate. In the latter scenario the enzyme cannot be considered inhibited despite pantothenate phosphorylation being inhibited. In some cases it is known whether an inhibitor of pantothenate phosphorylation is an inhibitor or substrate of PanK, and this is described in the relevant places within the text. For many compounds discussed here, however, it has not been shown whether pantothenate phosphorylation is a result of a compound serving as an inhibitor or a substrate of PanK. Throughout this manuscript we have therefore described compounds to be ‘inhibitors of the PanK-catalysed phosphorylation of pantothenate’ rather than ‘inhibitors of PanK’.

Fig. 5. Miscellaneous chemical structures. The chemical structures of ethyldethia-CoA (44), the CoA analogue generated in vivo from N-pentylpantothenamide; three thioester analogues of pantothentic acid (47a–c) that are known to be substrates of Staphylococcus aureus PanK, a pantetheine analogue (56) known to inhibit Escherichia coli PanK, a 4’-phosphopantetheine analogue (58) known to be a potent inhibitor of the Escherichia coli PPAT, and the pantoyl adenylate reaction intermediate (75) are shown. Stereochemistry is omitted from the structures.
In the presence of the pantothenamides, however, a 4'-phosphopantothenamide moiety, which lacks the terminal sulphhydryl group of 4'-phosphopantetheine required for the attachment of acyl chains, is transferred to ACP from the corresponding CoA analogue (eg. ethyldethia-CoA (44) in the case of N-pentylpantothenamide) instead. As a result of ACP inactivation, fatty acid biosynthesis was inhibited in N-pentylpantothenamide-treated *Escherichia coli*. By reversing the inhibitory effect of N-pentylpantothenamide with exogenous fatty acid, Zhang *et al.* (2004) demonstrated that the inhibition of fatty acid synthesis was indeed the primary mechanism behind the growth-inhibitory activity of this compound, highlighting its primary role as a competitive substrate of panK in *vivo*.  

Pantothenamides, including N-pentylpantothenamide (41c) and N-heptylpantothenamide (41d), have also been shown to inhibit the growth of *Staphylococcus aureus*, and are inhibitors of the pantothenate phosphorylation reaction catalysed by the *Staphylococcus aureus* PanK (Choudhry *et al.*, 2003; Leonardi *et al.*, 2005b). Minimum inhibitory concentrations (MICs) of 25 and 0.16 μM were determined for *N*-pentylpantothenamide and N-heptylpantothenamide, respectively, against *Staphylococcus aureus* strain RN4200 (Leonardi *et al.*, 2005b). Despite distinct differences between the PanKs of *Staphylococcus aureus* and *Escherichia coli*, both at the primary sequence level and at the level of feedback regulation, N-pentylpantothenamide and N-heptylpantothenamide are substrates of both *Staphylococcus aureus* PanK and *Escherichia coli* PanK. As in *Escherichia coli*, the *Staphylococcus aureus* CoA biosynthesis pathway metabolizes N-heptylpantothenamide to yield a CoA analogue that is incorporated by ACP. The inhibition of fatty acid synthesis has been shown to be a primary mechanism for the toxicity of this compound to *Staphylococcus aureus* (Leonardi *et al.*, 2005b). Supplementation with pantothenate relieves the toxicity of pantothenamides against *Staphylococcus aureus* more effectively than in *Escherichia coli*, indicating that CoA synthesis from pantothenate also becomes limiting for the growth of *Staphylococcus aureus* in the presence of pantothenamides.

The recently characterized coaX gene-encoded PanKs, expressed by *Bacillus subtilis*, *Heliocobacter pylori* and *Pseudomonas aeruginosa*, are resistant to inhibition by N-pentylpantothenamide, and do not utilize this pantothenic acid analogue as a substrate (Brand & Strauss, 2005; Hong *et al.*, 2006). The growth of *Pseudomonas aeruginosa* has been shown to be unaffected by N-pentylpantothenamide (Hong *et al.*, 2006). Furthermore, expression of the *Pseudomonas aeruginosa* coaX gene in *Escherichia coli* strain DV70, in which the endogenous PanK is inactive at 42°C, renders the *Escherichia coli* strain resistant to growth inhibition by N-pentylpantothenamide at this temperature (Hong *et al.*, 2006). By contrast, expression of the wild-type *Escherichia coli* coaX gene renders the strain sensitive.

Virga *et al.* (2006) recently synthesized a series of 20 N-substituted pantothenamides. Although several inhibited the phosphorylation of pantothenate by the *Escherichia coli* and *Staphylococcus aureus* PanKs, and were shown to be substrates of the *Escherichia coli* PanK, none of the analogues tested inhibited the growth of the wild-type *Escherichia coli* strain UB1005 with MIC values below 200 μM. The most potent inhibitors of the growth of *Staphylococcus aureus* (strain RN4220) among this series, 41f and 41g (both with MIC values of 100 μM), were unfortunately not as active as N-pentylpantothenamide and N-heptylpantothenamide.  

A natural analogue of pantothenic acid analogues

Recently, a pantothenic acid analogue named CJ-15,801 (45), which differs from pantothenic acid only by the presence of a double bond in the β-alanine moiety of the structure, was isolated from the fungus *Sematosporium sp.* CL28611. While the analogue lacked appreciable activity against bacteria such as *Escherichia coli* and *Streptococcus pneumoniae*, it was found to inhibit the growth of multidrug-resistant strains of *Staphylococcus aureus* with MIC values between 30 and 230 μM (Sugie *et al.*, 2001). In light of the antibacterial activity of CJ-15,801, multiple synthetic routes to this compound have since been published (Han *et al.*, 2004; Nicolaou & Mathison, 2005; Lee *et al.*, 2006). The synthesis of a series of related enamides has also been reported (Villa *et al.*, 2007).

Structure-based design of pantothenic acid analogues

The structure determined for the *Escherichia coli* PanK with pantothenate and ADP bound to the active site (*Escherichia coli* PanK–ADP–pantothenate) revealed several key amino acid residues that interact with pantothenate and that are proposed to contribute to substrate specificity (Ivey *et al.*, 2004). Hydrogen-bonding interactions between the secondary hydroxyl group of pantothenate and the imidazole side chain of His-177, the amide carbonyl oxygen of pantothenate and the hydroxyl group of Tyr-175 (via a water molecule), the amide nitrogen with Asn-282 and Lys-145 and the carboxyl group with the side chains of Tyr-240 and Asn-282, are all predicted to contribute to the binding and orientation of pantothenate in the active site (Fig. 6a). The primary hydroxyl group of pantothenate also forms a hydrogen bond with Asp-127, which is predicted to promote phosphoryl transfer by increasing the nucleophilicity of this hydroxyl group for attack on the γ-phosphate of ATP. The absence of a side chain on Gly-146 provides room for two pantothenate methyl groups. The enzyme’s preference for R-pantothenate over its enantiomer S-pantothenate is explained by the H-bond interaction between the hydroxyl substituent on the chiral centre and His-177.
A comparison between the structure of the *Escherichia coli* PanK enzyme with the nonhydrolysable ATP analogue AMP–PNP bound to the active site (*Escherichia coli* PanK–AMP–PNP) and that of the *Escherichia coli* PanK-ADP-pantothenate complex revealed that upon binding of pantothenate, structural rearrangements occur to accommodate

---

**Fig. 6.** Binding interactions proposed to contribute to the substrate specificity of the *Escherichia coli* PanK (a), the *Staphylococcus aureus* PanK (b), the *Pseudomonas aeruginosa* PanK (c) and human PanK3 (d). Adapted from Ivey et al., 2004; Hong et al., 2006, 2007; Virga et al., 2006; Yang et al., 2006. A prime superscript indicates a residue from a second monomer.
analogue than CoA thioesters (Yun et al., 2000). The pantothenyl moiety of CoA binds in essentially the same position and orientation as pantothenate, while the β-mercaptoethylamine group extends up into the hydrophobic pocket, where it is tightly sealed from water molecules. The aromatic residues in the pocket interact with the thiol group of CoA; the tight interaction between the thiol group and the sidechains of the surrounding aromatic residues explains why unesterified CoA inhibits *Escherichia coli* PanK more effectively than CoA thioesters (Yun et al., 2000).

The structural basis for the ability of the pantothenic acid analogue N-pentylpantothenamide (41c) to bind and act as a substrate for *Escherichia coli* PanK was investigated by modeling N-pentylpantothenamide in the *Escherichia coli* PanK–ADP–pantothenate structure. From the *Escherichia coli* PanK–N-pentylpantothenamide model, it was predicted that the pantothenamide would bind in the same conformation as pantothenate, such that the terminal hydroxyl of N-pentylpantothenamide was oriented to form the hydrogen bond with Asp-127, and thereby oriented for phosphoryl transfer. The alkyl chain of N-pentylpantothenamide was predicted to extend into the hydrophobic pocket above the pantothenate-binding site, in a manner similar to the β-mercaptoethylamine moiety of CoA. These findings shed light on how the bulky N-pentylpantothenamide could bind the *Escherichia coli* PanK active site with comparable affinity to the natural substrate, pantothenate (K_m values of 17.1 and 16.7 μM, respectively, as determined by a two enzyme-coupled assay or 140 and 41 μM, respectively, as determined by a radiochemical assay; Strauss & Begley, 2002; Ivey et al., 2004).

A pantothenic acid analogue, called homopantothenic acid or HoPan (46), which is not an effective inhibitor or substrate of *Escherichia coli* PanK, and lacks antibacterial activity against *Escherichia coli*, was also modeled in the *Escherichia coli* PanK–ADP–pantothenate structure (Ivey et al., 2004). Owing to the extension of the β-alanine moiety by one carbon unit relative to pantothenate, hydrogen-bond interactions with Tyr-240 and Asn-282 are no longer possible, and the terminal hydroxyl group of HoPan is not oriented in a position for hydrogen bonding with Asp-127 or for phosphoryl transfer. The interaction between pantothenate and Tyr-240 is important for the structural rearrangements that occur on binding of pantothenate that allow the substrate to be accommodated in the active site. As such, the inability of HoPan to make this interaction presumably prevents the compound from forming a productive complex with the enzyme (Ivey et al., 2004). This structural study therefore provided a structure-based explanation for the differential activity of N-pentylpantothenamide and HoPan with regard to *Escherichia coli* PanK.

The knowledge gained from the *Escherichia coli* PanK crystal structure provides a basis for the rational design of future pantothenic acid analogues, whether they are intended as inhibitors of the enzyme or as substrates. Virga et al. (2006) used the structural information obtained on the *Escherichia coli* PanK-binding site (summarized in Fig. 6a) and the adjacent hydrophobic pocket to design a further series of pantothenamides that were subsequently tested as inhibitors of the *Escherichia coli* PanK-catalysed phosphorylation of pantothenate, as well as for inhibitory activity against the pantothenate phosphorylation reactions catalysed by *Staphylococcus aureus* PanK and by two eukaryotic PanKs: the *Aspergillus nidulans* PanK and the mouse PanK1ζ isoform. In this study Virga et al. (2006) also synthesized and tested HoPan (46), and a related analogue. A number of compounds as effective as N-pentylpantothenamide and N-heptylpantothenamide at inhibiting the *Escherichia coli* PanK-catalysed phosphorylation of pantothenate were identified. Pantothenamides with linear N-alkyl substituents that were not bulky or sterically hindered were generally more effective inhibitors of the *Escherichia coli* PanK-catalysed phosphorylation of pantothenate than pantothenamides with aromatic N-substituents (particularly when substituents were present on the aromatic group), and more effective than pantothenamides with N-substituents containing polar functional groups (Fig. 6a). The pantothenamides were found to exert their apparent inhibitory effect on pantothenate phosphorylation by acting as competitive substrates of the *Escherichia coli* PanK, as evidenced by their phosphorylation and metabolism by downstream enzymes in the CoA pathway.

Overall, many of the analogues synthesized by Virga et al. (2006) inhibited the phosphorylation of pantothenate, albeit with varying efficiency, whether it was catalysed by the *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus nidulans* or mouse PanK. The extent to which pantothenate phosphorylation by the four different PanKs was inhibited by the different types of analogues tested was indicative of differences in the pantothenate-binding site structure of the enzymes. The pantothenate phosphorylation reaction catalysed by the *Aspergillus nidulans* PanK for example, was the most resistant to the inhibitory effects of the pantothenamide series as a whole, while the most sensitive to inhibition by HoPan. Pantothenate phosphorylation by mouse PanK was more sensitive to inhibition by pantothenamides with bulky, sterically hindered N-substituents than were the reactions catalysed by the other three PanKs. Although the majority of analogues were effective inhibitors of the *Staphylococcus aureus* PanK-catalysed phosphorylation of pantothenate, the reaction was inhibited most potently by the...
pantothenamides with linear alkyl N-substituents. The Staphylococcus aureus PanK-catalysed phosphorylation of pantothenate was much more sensitive, than the reactions catalysed by Escherichia coli and the two eukaryotic PanKs, to inhibition by pantothenamides with polar functional groups in the N-substituent. HoPan, and pantothenamides with N-substituents containing nonsubstituted aromatic functional groups were also effective inhibitors of the reaction catalysed by this enzyme. Structure–activity relationships (SARs) developed for the Staphylococcus aureus PanK are summarized in Fig. 6b.

Despite the differences discussed above, the results of the Virga et al. (2006) study were also consistent with some overlap between the SARs of the PanKs from different organisms. For example, although not a very effective inhibitor of the pantothenate phosphorylation reaction catalysed by the Escherichia coli or mouse PanK, R-HoPan was a more effective inhibitor than S-HoPan of pantothenate phosphorylation, irrespective of which of the four PanKs catalysed the reaction; these PanKs therefore show similar stereoselectivity. The stereoselective binding of the R-isomer was consistent with the Escherichia coli PanK structure, in which the R-configuration allows the secondary hydroxyl group of pantothenate to form a hydrogen bond with His-177. The biological activity of pantothenate similarly resides in the R-isomer, and the antibacterial activity of several of the pantothenic acid analogues reviewed here has been shown to be a property only of the stereoisomer with configuration at the α-carbon corresponding to that of the growth-promoting isomer of pantothenic acid.

With the Escherichia coli PanK structure solved initially in 2000, and then again in complex with pantothenate and ADP in 2004, and the recent elucidation of the structures of PanKs from Mycobacterium tuberculosis, Staphylococcus aureus, Pseudomonas aeruginosa, Thermotoga maritima and Bacillus anthracis, we have available the structures of all three types of PanKs found in the bacterial kingdom. Although the structure of the Staphylococcus aureus PanK has been solved only in complex with the ATP analogue, AMP–PNP, the close structural homology between the Staphylococcus aureus and Pseudomonas aeruginosa PanKs allowed a model of the Staphylococcus aureus PanK–pantothenate complex to be generated (Hong et al., 2006). The model illustrated that the proposed pantothenate-binding site is not enclosed, and that the pantothenate carboxyl group is fully exposed. Mutagenesis data are consistent with Glu-70 fulfilling the role of the catalytic base, activating the primary hydroxyl group of pantothenate for phosphoryl transfer (Fig. 6b; Hong et al., 2006). Further characterization of the pantothenate-binding site should help with identification of the key residues that confer substrate specificity to the Staphylococcus aureus PanK, and other PanKs of this type.

The crystal structure of the Pseudomonas aeruginosa PanK with pantothenate bound in the active site (Hong et al., 2006) provides a basis for understanding specific interactions that confer substrate specificity to the Pseudomonas aeruginosa PanK as well as other PanKs of this class. In the Pseudomonas aeruginosa PanK structure, pantothenate sits in a buried pocket where it forms various specific interactions with the enzyme (Fig. 6c). The primary hydroxyl group of pantothenate is hydrogen bonded to Asp-101, and via a water molecule, to the side chain of His-156. The secondary hydroxyl group is also hydrogen bonded to Asp-101. The carbonyl oxygen of the amide group of pantothenate is suggested to form a key hydrogen bond, via a water molecule, with Asn-9. Mutation of this asparagine to a glycine results in almost complete loss of PanK activity (Hong et al., 2006). Via the same water molecule through which pantothenate is bonded to Asn-9, the pantothenate carbonyl oxygen is also hydrogen bonded to the side chain of Thr-157 and the carbonyl oxygen of the backbone of Val-55. The carboxyl group at the other end of the pantothenate structure interacts with the side chains of Tyr-92, Arg-102 and Thr-180 and with the amide nitrogen of the backbone of Gly-99. Finally, pantothenate is predicted to make van der Waals contacts with the side chains of Val-55, Ile-142 and Ile-160 (Hong et al., 2006).

Determination of the Thermotoga maritima PanK structure has also contributed to the knowledge of the substrate specificity of this most recently characterized class of PanK. Although the structure of the Thermotoga maritima PanK–pantothenate complex has not been determined, structural similarity between this PanK and other members of the acetate and sugar kinase/heat shock protein 70/actin (ASKA) superfamily allowed the substrate-binding site to be predicted. A model of the enzyme with pantothenate bound in the putative active site revealed Asp-105 as the potential residue responsible for activating the primary hydroxyl group of pantothenate for nucleophilic attack on ATP (Yang et al., 2006). Mutagenesis of the corresponding residue (Asp-87) in the closely related Helicobacter pylori PanK to an asparagine or glutamate resulted in a drastic reduction in PanK activity (Yang et al., 2006), consistent with the role of this residue in catalysis.

The proposed pantothenate-binding site of the very recently crystallized Bacillus anthracis homologue of the Pseudomonas aeruginosa and Thermotoga maritima PanKs has also been characterized (Nicely et al., 2007). The Bacillus anthracis PanK was crystallized in the apo form: however, a model of the Bacillus anthracis PanK–pantothenate complex was developed based on the pantothenate-bound Pseudomonas aeruginosa PanK structure. The side chain of Asp-109 (an aspartic acid residue conserved in the Pseudomonas aeruginosa and Thermotoga maritima PanK sequences) was positioned to hydrogen bond with both the primary and the secondary hydroxyl groups of the pantothenate molecule. A conserved glycine residue (Gly-107) was predicted to interact with the carboxyl group of the...
bound pantothenate, as were a conserved arginine (Arg-110) and Tyr-100. Gly-147 and Gly-148 provided a pocket for this carboxyl group.

PanKs of the type exemplified by the Pseudomonas aeruginosana PanK are resistant to inhibition by N-pentylpantothenamide (41c). The absence of a hydrophobic pocket capable of accommodating the extended tail of N-pentylpantothenamide, from the pantothenate-binding site of the Pseudomonas aeruginosana PanK, and the predicted binding sites of the Thermotoga maritima and Bacillus anthracis PanKs, provides a likely explanation for the resistance to this compound (Hong et al., 2006; Yang et al., 2006; Nicely et al., 2007). Similarly, the absence of a hydrophobic pocket capable of accommodating the thiol tail of CoA provides a structural basis for why this type of PanK is not feedback regulated by CoA or CoA thioesters. The enzyme structures should prove useful for designing pantothenic acid analogues that will bind and inhibit specifically this type of bacterial PanK.

The susceptibility of the murine PanK-catalysed phosphorylation of pantothenate to inhibition by several of the pantothenamides synthesized by Virga et al. (2006) highlighted the need for a crystal structure of one or more of the mammalian PanK isoforms. Crystal structures have now been determined for the catalytic core domains (residues 231–597 and 12–368, respectively) of the human PanK1 and PanK3 isoforms (Hong et al., 2007). The human enzymes were crystallized in complex with the feedback inhibitor acetyl-CoA, which allowed the acetyl-CoA-binding site to be characterized and key interactions between the feedback inhibitor and the human PanKs to be identified. The pantothenyl moiety of acetyl-CoA forms a number of hydrogen bonds with human PanK1 and PanK3: the amide group nearest the phosphate groups is hydrogen bonded to Ser-195 and Ala-269, and the amide nearest the thioester group hydrogen bonds with Arg-207 and Val-268. The carbonyl oxygen of the thioester group forms a hydrogen bond with Val-268; the inability of free CoA to fulfil this hydrogen bond provides an explanation as to why the mammalian PanK1 and PanK3 isoforms, by contrast with the Escherichia coli PanK, are inhibited more potently by acetyl-CoA than by free CoA. Acetyl-CoA is also involved in a number of van der Waals interactions with residues lining the acetyl-CoA-binding site, including Val-250, Ala-269, Ile-253, Tyr-254, Tyr-258 and Leu-263. Based on the assumption that pantothenate occupies a position in the human PanKs similar to the pantothenyl moiety of acetyl-CoA, interactions between the human PanKs and the pantothenate molecule could be predicted. A salt-bridge between the side chain of Arg-207 and the carboxyl group of pantothenate is proposed to anchor the carboxyl end of the pantothenate molecule in the binding site (Fig. 6d). Glu-138 is predicted to serve as the catalytic base that activates the primary hydroxyl group of pantothenate for nucleophilic attack; however, this residue was not located in a position to interact with the hydroxyl group. This was presumed to be because the acetyl-CoA-bound structure is in a catalytically inactive state.

Although the structures of human PanK1 and PanK3 in complex with acetyl-CoA provide an insight into the interactions that confer substrate specificity on the human PanK isoforms, the pantothenate-binding site should be verified by determining the structure of a human PanK in complex with pantothenate. Further characterization of the pantothenate-binding site should greatly facilitate the design of compounds that target selectively the PanK-binding sites of microbial pathogens. Whether mammalian cells are able to take up such compounds, and metabolize them, will also need to be investigated, as these may provide additional means of achieving selectivity.

**Structural considerations for inhibitor uptake**

The relationship between chemical structure and the ability of a compound to reach inhibitory concentrations within the target cell must be considered when designing antimicrobial compounds. Although many of the pantothenamides synthesized by Virga et al. (2006) were effective inhibitors of the Escherichia coli PanK-catalysed phosphorylation of pantothenate, and several were shown to be metabolized by PanK and downstream enzymes of the CoA biosynthesis pathway to form CoA analogues incorporated into ACP in vitro, the majority lacked antibacterial activity. The likely explanation for this is that Escherichia coli does not take them up efficiently and/or that they are effluxed from the cell. By contrast, Escherichia coli is obviously capable of taking up the closely related antibacterial pantothenamide N-pentylpantothenamide (41c) and maintaining an intracellular concentration of the analogue sufficient for inhibition. The mechanism by which Escherichia coli takes up N-pentylpantothenamide is unknown, but has been shown to be independent of PanF, the Escherichia coli pantothenate transporter (Zhang et al., 2004). The precise structural features that allow N-pentylpantothenamide to permeate the Escherichia coli cell efficiently are unclear, and will need to be determined in order to design analogues with potent antibacterial activity.

ToLC-dependent proteins present on the outer membrane of Gram-negative cells are involved in the efflux of small molecules and toxic compounds from the cell (reviewed in Andersen et al., 2000). Zhang et al. (2004) and Virga et al. (2006) showed that N-heptylpantothenamide (41d) is a substrate of a ToLC-dependent efflux pump(s), whereas N-pentylpantothenamide is not. The ToLC-dependent efflux of N-heptylpantothenamide renders the UB1005 wild-type Escherichia coli strain relatively resistant to the antibacterial...
effects of N-heptylpantothenamide (MIC > 200 µM). As a result, N-pentylpantothenamide (MIC 25–50 µM) is considerably more potent against the UB1005 strain of *Escherichia coli*. By contrast, N-heptylpantothenamide is much more effective than N-pentylpantothenamide as an inhibitor of the growth of *Staphylococcus aureus*, a Gram-positive bacterium. N-Heptylpantothenamide and N-pentylpantothenamide inhibit the growth of *Staphylococcus aureus* with MIC values of 0.16 and 25 µM, respectively (Leonardi *et al*., 2005b). The affinity of the comparatively more hydrophobic pantothenamide, N-heptylpantothenamide, for the ToLC-dependent efflux system of *Escherichia coli* may indicate that very hydrophobic pantothenamides will also be substrates for the ToLC-dependent efflux pumps, and hence will also fail to inhibit the growth of *Escherichia coli*. Virga *et al.* (2006) found at least four of the pantothenamides synthesized in their study to be substrates for ToLC-dependent efflux; however, no obvious correlation between hydrophobicity and susceptibility to ToLC-dependent efflux was observed.

It is important that new compounds designed as inhibitors or substrates of PanK be tested not only in enzyme assays but also for activity against whole cells, in order to identify compounds that can overcome the uptake and efflux barriers.

**Designing pantothenic acid analogues as precursors to CoA analogues**

For pantothenic acid analogues to be converted to CoA analogues, they must be accepted as substrates of PanK, and the phosphorylated derivatives must be further metabolized by PPAT and DPCK. Relative to PanK, less is known about the substrate specificity of PPAT and DPCK. However, the range of N-substituted pantothenamides that have been shown to yield CoA analogues after incubation with *Escherichia coli* PanK, PPAT and DPCK is consistent with PPAT and DPCK also being somewhat promiscuous, accepting analogues of 4′-phosphopantetheine and dephospho-CoA, respectively, in which the β-mercaptoethylamine moiety has been modified, as substrates for the reactions they catalyse (Strauss & Begley, 2002; Virga *et al*., 2006).

In an attempt to improve the method of CoA analogue synthesis *in vitro*, van Wyk & Strauss (2007) prepared three thioester analogues of pantothenic acid (47a–c; Fig. 5) that they predicted would, after incubation with purified PanK, PPAT and DPCK, yield the corresponding thioester analogues of CoA that could be utilized in aminolysis reactions with amines to produce an array of CoA analogues. In effect, this technique would allow several CoA analogues to be synthesized from just one pantothenic acid analogue *in vitro*. Interestingly, however, van Wyk & Strauss (2007) found that, although the *Escherichia coli* PanK had been shown previously to accept a number of pantothenamides as substrates for phosphorylation, the enzyme did not phosphorylate compounds 47a–c. To overcome this problem, they investigated whether the *Staphylococcus aureus* PanK would phosphorylate the analogues, and found that this was indeed the case. The phosphorylated analogues were further metabolized by purified *Escherichia coli* PPAT and *Escherichia coli* DPCK, yielding the corresponding thioester analogues of CoA. The thioester analogue of CoA generated from 47c was used effectively to prepare several more CoA analogues via aminolysis reactions with amines. Aside from the importance of this study for the synthesis of CoA analogues *in vitro*, it also revealed further differences between the SARs of the *Escherichia coli* PanK and the *Staphylococcus aureus* PanK (Fig. 6). The effect of pantothenic acid analogues 47a–c on bacterial growth was not investigated.

Although van Wyk & Strauss (2007) showed that the *Escherichia coli* PanK was unable to phosphorylate pantothenate thioesters, the enzyme has recently been reported to phosphorylate the antibacterial pantothenic acid analogue, pantothenol (24) (Kumar *et al*., 2007). The related *Mycobacterium tuberculosis* PanK was also shown to accept pantothentic acid as a substrate for phosphorylation. Whether the 4′-phosphopantothenol generated by these enzymes was further metabolized by PPAT and DPCK was not investigated.

**Conclusions and future directions**

Since pantoyltaurine was synthesized in 1941, numerous pantothenic acid analogues that inhibit the ability of various bacteria to utilize pantothenic acid, and thereby inhibit bacterial growth have been synthesized. Antibacterial analogues have been obtained predominantly through modification of the β-alanine moiety of pantothenic acid, for example, replacement of the carboxylic acid group with a sulphonic acid, N-substituted sulphonamide, thiol, disulphide, sulphone, alcohol, ketone, N-substituted amide, N-substituted carbamate or an N-substituted ureido group. Limited modification of the pantoyl moiety, for example replacement of one or both of the methyl substituents with larger alkyl groups, a cyclopentane ring or hydrogens, as well as the introduction of alkyl or substituents, has also yielded antibacterial analogues of pantothenic acid.

It is evident from the research reviewed here that a number of the pantothenic acid analogues reported since 1941 are effective inhibitors of the growth of various bacterial species and deserve further characterization. Concluding, from the many *in vitro* investigations, which of these compounds are the most promising is, however, somewhat difficult. Factors such as the nature of the test organism, length of incubation, concentration of bacterial
inoculum and the stereochemistry of compound preparations have been shown to affect the amount of any given compound that is required to exert an antibacterial effect (Snell, 1941b; Snell & Shive, 1945; Shive & Snell, 1945a, b). It is therefore not a straightforward task comparing the activity of pantothenic acid analogues on the basis of quantitative data reported in different studies. Limited comparisons between studies are possible, however, because many analogues have been tested for activity against multiple species of bacteria, because reference compounds were often included in antibacterial screens, and because the stereochemistry of compound preparations is indicated in most reports.

Several of the compounds that have been demonstrated to possess effective antibacterial activity against lactic acid bacteria in vitro are not reported to have been tested against pathogenic species of bacteria for which antibacterial agents are sought, or for antibacterial activity in vivo. Prompted by the in vitro activity of pantothene (24), a number of series of N-pantoyl-substituted amines, which included compounds that, by contrast to pantothene, should not be oxidized to pantothenic acid in vivo, have been synthesized. Among these are compounds, including 25d, 25f and 25h–i, that are reported to possess improved antibacterial activity relative to pantothene against certain species of lactic acid bacteria. For this reason, such compounds should be among those tested against pathogenic species of bacteria in vitro. The activity of compounds 32a–b, the most effective of the pantotenone type analogues, at least against Lactobacillus casei, also warrants further testing of these compounds.

The majority of the studies reviewed here report only on the activity of analogues against whole organisms. Future studies investigating the antibacterial activity of pantothenic acid analogues would benefit from simultaneous investigations into the mechanism of action of these compounds. Furthermore, pending the availability of structural information on the target protein(s), simultaneous structure-based investigations should enhance significantly the process of lead optimization.

Pantoyltaurine (16), a number of N-substituted pantoyltauramides, including 23a–d, and ω-methylpantothenic (35) acid have been reported to cure rats or mice infected with streptococci, providing evidence that analogues of pantothenic acid that inhibit the ability of bacteria to utilize the vitamin can cure animals of bacterial infection. The in vivo activity of certain N-substituted pantoyltauramides, ω-methylpantothenic acid and, to a lesser extent pantoyltaurine, warrants further testing of these compounds in animal models of human infections, with the aim of using these compounds for the treatment of human bacterial infections. The dosages of these analogues required to affect antibacterial activity in humans may well be lower than those reported to be necessary in mice and rats. In vitro, the antibacterial effect of pantoyltaurine, N-substituted pantoyltauramides and ω-methylpantothenic acid is dependent on the ratio of analogue concentration : pantothene concentration. Hence, in humans, where the concentration of pantothene present in blood plasma is up to 10-fold lower than the concentration in mouse plasma (McIlwain & Hawking, 1943), a lower concentration of analogue should be necessary to maintain the analogue : pantothene ratio above the value required to exert the same effect in mice. Snell (1941b) reported that the levorotatory isomer of pantoyltaurine lacked appreciable inhibitory activity, and that the activity of pantoyltaurine could be attributed to the dextrorotatory isomer. Subsequently, similar findings were reported for other pantothenic acid analogues. Nonetheless, pantoyltaurine and ω-methylpantothenic acid were both prepared and administered to rats and mice as a mixture of isomers. Hence, the dosage of the resolved isomer required to suppress bacterial growth in vivo would be expected to be at least twofold lower than that required of the mixture of isomers.

It became evident during the preparation of this review that there has been relatively little research dedicated to investigating the effects of pantothenic acid analogues on the growth of yeast and other fungi. Three analogues, pantoyltaurine (16), methylpantothenone (30) and phenylpantothenone (31), are reported to inhibit the growth of Saccharomyces cerevisiae. The effect of methylpantothenone and phenylpantothenone is not, however, antagonized by pantothenic acid, and, therefore, whether these two analogues inhibit growth by interfering with pantothenic acid utilization is unclear. Owing to the observed variability in the efficacy with which many pantothenic acid analogues inhibit the growth of different species of bacteria, and due to the fact that a crystal structure of a fungal PanK is still not available, it is difficult to predict which compounds will prove to be the most effective antifungal agents. Perhaps a good starting point for further investigations would be to determine whether the pantotenamides reported by Virga et al. (2006) to inhibit pantothene phosphorylation catalysed by the Aspergillus nidulans PanK also inhibit growth of Aspergillus nidulans.

Pantetheine/pantethine analogues as antibacterial agents

The discovery that microorganisms can utilize pantetheine (14) or pantethine (15) with varying efficiencies in place of pantothenic acid presented new opportunities for the design of compounds inhibiting the biosynthesis of CoA. Although the structural similarity between pantothenic acid and panteth(e)line meant that many of the analogues modelled on pantothenic acid could also be considered panteth(e)line analogues, particularly some of the N-substituted pantothenamides (41), and pantoyltaurine analogues 18 and 19, a
number of antibacterial compounds were designed specifically as analogues of pantetheine and pantethine.

Wieland et al. (1952) were the first to prepare compounds intended as analogues of pantetheine, synthesizing three compounds in which the terminal sulphhydryl group of pantetheine had been replaced with other sulphur-containing groups. Replacement of the sulphhydryl group with a thiophenyl group, as in compound 48, but not with a thiomethyl or a thioethyl group, yielded a competitive inhibitor of the growth of Lactobacillus helveticus. Stewart et al. (1955) prepared a series of pantetheine and pantethine analogues, a number of which displayed slight growth-promoting activity for certain lactic acid bacteria but only one of which showed antibacterial activity. Oxypantetheine (49), in which a hydroxyl group replaced the terminal sulphhydryl group of pantetheine, inhibited the growth of Lactobacillus helveticus in a manner antagonized by pantetheine. Stewart et al. (1955) reported that a concentration of D-oxypantetheine 100–200 times the concentration of Lactobacillus helveticus sulphydryl group of pantetheine, inhibited the growth of pantetheine reduced the growth of tothenic acid. In the presence of either pantothetic acid or pantetheine, 6-methylpantothenic acid and 6-methylpantetheine were reported to be synergistic when tested against Leuconostoc citrovorum, consistent with the pantothetic acid and pantetheine analogues acting via distinct, yet complementary mechanisms, and highlighting the potential for analogues of pantothentic acid to be used in synergistic combinations with pantetheine analogues. Bird et al. (1955) also investigated the activity of a few pantetheine analogues, including a pantothenamide reported previously by Clifton et al. (1970) to have antibacterial properties (41e), oxypantetheine (49; discussed above), and the corresponding 6-methyl substituted analogues (54 and 55). The analogues inhibited the growth of Lactobacillus helveticus and Lactobacillus arabinosus in the presence of both pantetheine and pantothenic acid; however, they were more effective in the presence of the former than the latter. The 6-methyl-substituted analogues were less active than the corresponding analogues lacking the 6-methyl substituents.

6-Methylpantetheine, when given orally at a concentration of 0.05% of the diet to rats with a pantothenic acid deficiency, did not hasten the further development of the pantothenic acid deficiency. Similarly, when 41e and 49 were administered intraperitoneally to rats at a dosage of 6 mg day\(^{-1}\) the pantothenic acid deficiency was not accelerated; on the contrary, compounds 41e and 49 had a growth-promoting effect equivalent to that produced by pantothenate. The growth-promoting activity of compounds 41e and 49 was postulated to arise because they were cleaved to form pantothenic acid in the rats. Whether any of these compounds inhibited bacterial growth in vivo was not investigated.
More recently, pantetheine analogues with fluorescent (or bioorthogonal) labels have been designed as precursors to CoA analogues, to enable reporter labelling of carrier proteins with labelled CoA analogues that are invariably impermeable to cells (Clarke et al., 2005; Meier et al., 2006; Worthington & Burkart, 2006). This method of reporter labelling exploits the promiscuity of the CoA biosynthesis pathway; pantetheine analogues are transformed step-wise into CoA analogues by the CoA biosynthetic enzyme PanK. Worthington & Burkart (2006) has shown to accept several pantetheine analogues as substrates for phosphorylation. Furthermore, pantetheine analogues transformed by PanK, PPAT and DPCK, generating the corresponding labelled CoA analogues. Escherichia coli PanK has been shown to be true for at least pantothenamide analogues (Clarke et al., 2005), as well as pantothenic acid analogues (Meier et al., 2006; Worthington & Burkart, 2006). For the purpose of reporter labelling, it is desirable that the pantetheine analogues be of low toxicity to the target cell, and hence the potential of these compounds as antimicrobial agents has not been investigated. One pantetheine analogue (56; Fig. 5) that binds Escherichia coli PanK with a high affinity \((K_d \approx 0.76 \mu M)\), but is a poor substrate, has been shown to be a noncompetitive inhibitor of the enzyme. Whether this compound is also inhibitory to cell growth has not been reported; however, work in this area is ongoing. Compound 57 (Fig. 4b), a pantetheine analogue synthesized by Clarke et al. (2005), was reported to be inhibitory to the growth of Escherichia coli, however, only at concentrations > 180 \(\mu M\).

**Conclusions and future directions**

A number of pantetheine and pantethine analogues have been reported to inhibit the growth of lactic acid bacteria by inhibiting the ability of the bacteria to utilize panteth(e)ine. Despite reports of effective analogues such as oxypantheonetine (49) and homopantethine (50), the range of bacteria these pantetheine analogues have been screened against is limited and notably lacks species of pathogenic bacteria. Furthermore, the antibacterial activity of such compounds is yet to be investigated in vivo.

Owing to the ability of bacteria to utilize both pantothenic acid and panteth(e)ine as precursors to CoA, inhibition of panteth(e)ine utilization in isolation may not be a feasible antibacterial strategy in vivo. Bacteria do, however, utilize pantothenic acid and panteth(e)ine with varying efficacy and as such, inhibition of panteth(e)ine utilization may for some species of bacteria be sufficient to limit growth even in the presence of pantothenic acid. Moreover, because of the significant structural overlap between the CoA precursors pantothentic acid, pantetheine and pantethine, it is likely that pantetheine and pantethine analogues also inhibit the ability of bacteria to utilize pantothentic acid. This was shown to be true for at least pantothenamide 41e, oxypantheonetine (49), \(\alpha\)-methylpantetheine (53) and \(\alpha\)-methylpantheonetine analogues 54 and 55. Bird et al. (1955) showed, however, that all the aforementioned analogues were less potent against the bacteria tested when pantothenic acid was present in place of pantetheine as the precursor to CoA in the assay. This highlights the importance of screening panteth(e)ine analogues (as well as pantothenic acid analogues) for activity in the presence of a combination of CoA precursors that is relevant to the environment in which these analogues are required to be active if used for chemotherapy, for example in the blood stream of humans.

Bird et al. (1955) reported that \(\alpha\)-methylpantothenic acid (35), a pantethine analogue, and \(\alpha\)-methylpantethine (53), a pantetheine analogue, act synergistically against Leuconostoc citrovorum in the presence of either pantetheine or pantothanic acid. This finding is consistent with compounds that inhibit the growth of bacteria by inhibiting the utilization of panteth(e)ine, when combined with inhibitors of pantothenic acid utilization, acting synergistically. Whether this relationship exists between other panteth(e)ine and pantothanic acid analogues has not been investigated, and presumably will vary for each pair of analogues. It will be important to understand fully the mechanism(s) by which panteth(e)ine and pantothanic acid analogues exert their antibacterial effect, as compounds with overlapping mechanisms of action may, by contrast, exhibit antagonistic behaviour when combined.

At present, little is known about the mechanism(s) by which the panteth(e)ine analogues discussed here exert their antibacterial effect. Stewart & Ball (1966) showed that the phosphorylated form of oxypantheonetine (49) was converted by enzymes present in beef liver extracts to the corresponding CoA analogue, and Clarke et al. (2005), Worthington & Burkart (2006) and Meier et al. (2006) have demonstrated that several of the pantetheine analogues that have been synthesized for the purpose of labelling carrier proteins (including analogues 56 and 57) are metabolized to CoA analogues by the CoA biosynthetic enzyme PanK, PPAT and DPCK. Hence, at least some of the panteth(e)ine analogues discussed here are proposed to inhibit bacterial growth by being converted to CoA analogues that inhibit CoA-utilizing enzymes. CoA analogues corresponding to panteth(e)ine analogues 49, 51 and 52 have been reported to inhibit competitively the acetylation of CoA in a phosphotransacetylase assay, lending support to this hypothesis.

Recent investigations with pantetheine analogues focus on their utility as precursors to labelled-CoA analogues in *in vitro* and *in vivo* protein labelling strategies. Analogues generated for this purpose should also be screened for antibacterial activity *in vitro*. This may greatly facilitate the identification of pantetheine analogues that possess potent antibacterial activity. One pantetheine analogue synthesized for the purpose of protein labelling (compound 57) has...
already been shown to possess antibacterial activity, and another compound (56) was identified as a noncompetitive inhibitor of *Escherichia coli* PanK, and hence may prove to possess potent antibacterial activity.

### 4′-Phosphopantetheine analogues as antibacterial agents

As PPAT catalyses a rate-limiting step in the biosynthesis of CoA, and the bacterial and mammalian PPAT enzymes share little sequence similarity, the enzyme is an attractive target for the development of antimicrobial agents. Zhao *et al.* (2003) used the available crystal structure of the *Escherichia coli* PPAT in complex with 4′-phosphopantetheine to design and synthesize a library of dipeptides that were structurally related to 4′-phosphopantetheine and would participate in key substrate–enzyme interactions. Co-crystallization of *Escherichia coli* PPAT with the most active PPAT inhibitor from the initial library, followed by further structure-based design, led to the identification of a potent inhibitor of *Escherichia coli* PPAT (compound 58; Fig. 5) with an IC$_{50}$ of 6 nM against the enzyme, which was inactive against porcine PPAT. Unfortunately, the active inhibitors of *Escherichia coli* PPAT lacked antibacterial activity, perhaps because they are unable to enter the cell.

While this study highlights the effectiveness of a structure-based inhibitor design, it also draws attention to the difficulties associated with designing antibacterial analogues of a metabolite that is membrane impermeant. It may be worthwhile to investigate whether further modifications of the structure of 58 will yield compounds that retain the potency and selectivity of 58, but that have improved antibacterial activity. It may also be interesting to test whether the potent PPAT inhibitors identified by Zhao *et al.* (2003) possess activity against bacterial pathogens such as *Mycoplasma*, *Rickettsia* and *Chlamydia*, which, because they appear to lack all but one of the enzymes in the CoA biosynthesis pathway, are predicted to possess different capabilities from most organisms with respect to the uptake of intermediates in the CoA biosynthesis pathway.

### Inhibitors of pantothenic acid biosynthesis as antibacterial and antifungal agents

Cheldelin & Schink (1947) were, to the best of the authors’ knowledge, the first to synthesize compounds designed to inhibit the biosynthesis of pantothenic acid. Three analogues of pantoic acid, a direct precursor of pantothenic acid, were prepared and shown to inhibit growth of the bacterium *Acetobacter suboxydans* and the yeast *Saccharomyces cerevisiae* (see compounds 59–61; Fig. 7). Against *Acetobacter suboxydans* and *Saccharomyces cerevisiae*, two organisms that synthesize pantothenate from pantoate and β-alanine, the compounds were generally more effective in the absence of pantothenic acid, when the growth-promoting factor was pantoic acid or β-alanine, respectively, and were predicted to inhibit the coupling of pantoate with β-alanine. The inhibition of *Acetobacter suboxydans*, a bacterium that utilizes exogenous pantoic acid (Sarett & Cheldelin, 1945b), was reversed competitively by pantoic acid. Compounds 59, 60 and 61, when present in 10-, 1000- and 12 000-fold molar excess relative to pantoic acid, respectively, inhibited the growth of *Acetobacter suboxydans* by 50% and, when present in 100 000-, 25 000- and 40 000-fold molar excess relative to β-alanine, respectively, inhibited growth of *Saccharomyces cerevisiae* by 50%. As could be expected, the analogues lacked inhibitory activity against *Lactobacillus arabinosus*, which relies on the uptake of extracellular pantothenic acid.

A few years before the study by Cheldelin & Schink (1947), it was reported that the antibacterial activity of salicylic acid (62), a compound that inhibits the growth of *Escherichia coli* and *Staphylococcus aureus*, was, at low concentrations, antagonized by pantothenic acid and to a lesser extent by pantoic acid or pantolactone (Ivanovics, 1942a,b). Accordingly, it was postulated that this compound inhibited the synthesis of pantothenic acid, either by inhibiting the synthesis of pantoic acid or its conversion to pantothenic acid.

Several analogues of β-alanine, including both α- and β-amino acids, have been observed to inhibit the growth of yeast in media containing low concentrations of β-alanine, but not in the presence of pantothenic acid (Nielsen *et al.*, 1944; Hartelius, 1946; Williams *et al.*, 1950). Examples include, but are certainly not limited to, l-asparagine (63), l-glutamic acid (64), l-aspartic acid (65), d,l-serine (66), β-aminobutyric acid (67) and isoserine (68). The β-alanine analogue taurine (69) was reported to inhibit the β-alanine-stimulated growth of one strain of *Saccharomyces cerevisiae*; in the presence of 0.3–0.7 μM β-alanine 80–95% inhibition of growth was observed with 1 mM taurine (Sarett & Cheldelin, 1945a). Taurine was, however, without effect on the sixteen other strains of yeast tested. d-Serine has also been shown to inhibit the growth of *Escherichia coli in vitro* in a manner that is antagonized competitively by β-alanine and noncompetitively by pantothenic acid (Davis & Maas, 1949; Maas & Davis, 1950; Cosloy & McFall, 1973). The growth-inhibitory effect against *Escherichia coli* and *Saccharomyces cerevisiae* of propionic acid (70), another compound similar in structure to β-alanine, is antagonized by β-alanine and pantothenic acid (Wright & Skeggs, 1946; King & Cheldelin, 1948). At low β-alanine concentrations, a molar ratio of sodium propionate to β-alanine of 100:1 caused 50% inhibition of the growth of *Saccharomyces cerevisiae*, and a molar ratio of 10 000:1 inhibited growth completely.
Pantothenic acid was, however, more effective at reversing the activity of sodium propionate. It was concluded that propionic acid inhibited the growth of these organisms by inhibiting the conversion of β-alanine to pantothenic acid (King & Cheldelin, 1948). Several β-alanine analogues including aspartic acid, taurine, serine, isoserine, γ-amino-β-hydroxybutyric acid (71), γ-aminobutyric acid (72) and gluconic acid (73) have since been shown to inhibit the condensation of pantoate and β-alanine catalysed by PS purified from *Escherichia coli* (Miyatake et al., 1979; reviewed in Webb et al., 2004). The *Mycobacterium tuberculosis* PS has subsequently been shown to utilize β-alanine analogues, including several of those mentioned above, as alternative substrates to β-alanine (Zheng & Blanchard, 2001).

α,β-Methyleneadenosine 5′-triphosphate (AMP-CPP; 74), a nonhydrolysable analogue of ATP, is reported to inhibit...
Mycobacterium tuberculosis PS (Zheng & Blanchard, 2001). AMP-CPP competes with ATP for binding to PS (Kᵢ of 290 μM).

Tuck et al. (2006) have recently described a series of compounds that inhibit the activity of Escherichia coli PS. The PS-catalysed condensation of pantoate and β-alanine is ATP-dependent and proceeds via a pantoyl adenylate reaction intermediate (75; Fig. 5). Initially, pantoate is adenylated with the concomitant loss of a pyrophosphate molecule. Subsequent nucleophilic attack on the pantoyl adenylate intermediate by β-alanine gives rise to pantothenate and AMP. The PS inhibitors synthesized by Tuck et al. (2006) were designed as analogues of the pantoyl adenylate reaction intermediate. Compounds in which the phosphodiester group of pantoyl adenylate was replaced with an ester group and the pantoyl moiety of the structure had been modified (e.g. compound 76) were shown to be competitive inhibitors of Escherichia coli PS, with respect to ATP, and to bind the enzyme with moderate affinity (Kᵢ values of 2–18 mM). Replacement of the phosphodiester group with a sulphamoyl group yielded inhibitors that bound the enzyme with higher affinity than the ester analogues. The sulphamoyl analogues bind PS in a manner competitive with both ATP and pantoate. The most potent inhibitor of PS was compound 77, the analogue most similar in structure to the pantoyl adenylate reaction intermediate; Kᵢ values of 300 and 800 nM were measured vs. ATP and pantoate, respectively. Whether the PS inhibitors also inhibited the growth of Escherichia coli was not reported.

Baillie et al. (1992) had previously prepared three phosphonate analogues of pantoate and/or pantothenate that were designed as inhibitors of PS (78–80). Adenylation of the analogues by PS was predicted to yield mimics of the pantoyl adenylate intermediate. Unfortunately, however, the compounds did not inhibit Escherichia coli PS. Whether the analogues failed because they were not adenylated and the unmodified analogues did not bind Escherichia coli PS, or because the adenylated analogues did not bind the Escherichia coli PS, was not investigated.

Nafronyl oxalate (81), a vasodilator utilized in the treatment of vascular and cerebral disorders, has recently been reported as an inhibitor of Mycobacterium tuberculosis PS (White et al., 2006). This compound was among 4080 compounds screened in a high-throughput assay. A Kᵢ value of 75 μM was determined for nafronyl oxalate, based on a competitive mode of PS inhibition. When screened for antimycobacterial activity in vitro at a concentration of 13 μM, however, the compound was without effect. The effect of higher concentrations of this drug, which is well tolerated in humans, is yet to be investigated.

The l-aspartic acid analogues β-hydroxyaspartic acid (82), meso-diaminosuccinic acid (83) and l-cysteic acid (84) all inhibit the growth of Escherichia coli in vitro (Ravel & Shive, 1946; Shive & Macow, 1946). The antibacterial activity of these compounds is reversed competitively by l-aspartic acid. The molar ratio of analogue: l-aspartic acid reported to produce maximum growth inhibition was between 10 and 30 for β-hydroxyaspartic acid, between 100 and 200 for meso-diaminosuccinic acid and between 30 and 100 for l-cysteic acid. β-Alanine and pantothentic acid were also shown to antagonize the activity of β-hydroxyaspartic acid and l-cysteic acid, except at high concentrations of the analogues. These findings were consistent with the compounds exerting their antimicrobial effect by inhibiting the synthesis of β-alanine from l-aspartic acid. Williamson & Brown (1979) showed that β-hydroxyaspartic acid and l-cysteic acid, along with d-serine, l-glutamic acid, succinic acid (85), oxaloacetic acid (86) and l-serine, are competitive inhibitors of the Escherichia coli ADC-catalysed synthesis of β-alanine from l-aspartic acid. Kᵢ values of 0.13, 0.08 and 0.16 mM were determined for β-hydroxyaspartic acid, l-cysteic acid and d-serine, respectively.

Using MS, Webb et al. (2003) screened compounds for the capacity to bind to the active site of the Escherichia coli ADC. Fifty-five compounds, all similar in structure to l-aspartic acid and possessing a primary amine group, were tested and a small selection was shown to bind to the enzyme. The compounds identified as binding to ADC included β-hydroxyaspartic acid and d-serine, validating the effectiveness of this approach. This technique proved valuable in elucidating structural requirements for enzyme binding: these will hopefully help guide the design and synthesis of inhibitors of ADC that bind with high affinity.

Conclusions and future directions

Pantothentic acid biosynthesis has generated interest as a potential antimicrobial target, largely as a result of the importance of pantothenic acid for the growth of microorganisms, and the absence of this pathway from humans and other animals. The finding that the survival and pathogenesis of a strain of Mycobacterium tuberculosis defective in de novo pantothentic acid biosynthesis, as a result of the deletion of the genes encoding ADC and PS, is limited in both immunocompetent and immunocompromised mice (Sambandamurthy et al., 2002), is consistent with pantothentic acid biosynthesis being a valid antimicrobial drug target, despite the existence of pantothenate salvage mechanisms in microorganisms. Under defined conditions in vitro, a number of analogues of the pantothentic acid precursors β-alanine, pantoic acid and l-aspartic acid are reported to inhibit the growth of bacteria, and analogues of β-alanine and pantothetic acid the growth of yeast. Such analogues have been shown to be most effective in the absence of the
products of reactions in the pathway downstream of those they inhibit. For example, the β-alanine analogue propionic acid (70), and pantoic acid analogue 59 were more active against *Escherichia coli* and *Acetobacter suboxydans*, respectively, in the absence of pantothenic acid, and l-aspartic acid analogues β-hydroxyaspartic acid (82) and l-cysteic acid (84) were more active in the absence of β-alanine and pantothenic acid. In order to evaluate the potential of any antimicrobial β-alanine, pantoic acid or l-aspartic acid analogues in the chemotherapy of bacterial and fungal diseases, antimicrobial activity should be quantified in the presence of a standardized, physiological concentration of pantothenic acid and pantothenic acid precursors to determine whether any compounds, when present at practical concentrations, inhibit pantothenic acid biosynthesis effectively enough that pantothenic acid salvage is insufficient to maintain growth. Even the growth of the *Mycobacterium tuberculosis* pantothenic acid auxotroph developed by Sambandamurthy et al. (2002) was unimpaired *in vitro* when sufficient exogenous pantothenic acid was supplied, highlighting the importance of using physiological concentrations of metabolites in such *in vitro* screens. It is also clearly advantageous to screen compounds against different pathogenic organisms for which therapy is sought, including *Mycobacterium tuberculosis*. These microorganisms presumably differ somewhat in their dependence on the biosynthesis and salvage of pantothenic acid.

Compounds including analogues of β-alanine and ATP (substrates for PS), and analogues of the intermediate in the PS-catalysed reaction, are reported to inhibit the activity of purified *Escherichia coli* or *Mycobacterium tuberculosis* PS. Despite the potent activity of sulphamoyl analogues of the pantoyl adenylate intermediate against the *Escherichia coli* PS enzyme, the antibacterial activity of these compounds has not been reported. These analogues should be screened for activity against both *Escherichia coli* and *Mycobacterium tuberculosis* *in vitro*. If they lack antimicrobial activity, it will be important to determine why (e.g., whether the analogues are not taken up by the bacteria) so that potent inhibitors of PS that also possess antibacterial activity can be designed.

Competitive inhibitors of the *Escherichia coli* ADC-catalysed decarboxylation of l-aspartate, and compounds that bind ADC and are likely to also inhibit competitively the ADC-catalysed decarboxylation reaction, have been identified. The effects of a number of these compounds on pantothenic acid biosynthesis, and on the growth of bacteria, still need to be determined. The possibility that inhibitors of the ADC-catalysed reaction, when used in combination with inhibitors of the PS-catalysed condensation of β-alanine and pantoate, enhance inhibition of pantothenic acid biosynthesis and antibacterial activity should perhaps be investigated.

**Pantothenic acid analogues as antimalarial agents**

Trager’s discovery that calcium pantothenate favoured the survival of *Plasmodium lophurae* *in vitro* (1943) implicated pantothenic acid as a requirement of the erythrocytic stage malaria parasite and led naturally to interest in examining the antimalarial activity of compounds that could inhibit the utilization of pantothenic acid. At this time, the pantothenic acid analogues pantoyltaurine and pantoyltauramide had already been prepared and shown to be active against various bacteria with a requirement for pantothenic acid (Snell, 1941a, b; McIlwain et al., 1942; Barnett & Robinson, 1942b; McIlwain, 1942a, b). Pantoyltaurine (16), pantoyltauramide (17) and several N-substituted pantoyltauramides were therefore the first pantothenic acid analogues screened against malaria parasites (McIlwain & Hawking, 1943; Mead et al., 1946; Mead & Koepfi, 1947). Because procedures for the *in vitro* cultivation of malaria parasites were not yet established, the antiplasmodial activity of analogues was initially investigated in ducks, chickens and canaries infected with avian species of malaria.

When tested for antiplasmodial activity in *Plasmodium relictum*-infected canaries, *Plasmodium lophurae*-infected ducks and *Plasmodium gallinaceum*-infected chickens, pantoyltaurine was found to be largely inactive (McIlwain & Hawking, 1943; Berliner et al., 1946). D-Pantoyltauramide, when incorporated into the diet of *Plasmodium gallinaceum*-infected chicks, was also inactive; however, when administered intravenously at doses of 2 g kg⁻¹ body weight day⁻¹, D-pantoyltauramide suppressed parasite growth in *Plasmodium gallinaceum*-infected chickens (Blanchard & Schmid, 1946). Cantrell (1949) reported suppression of the proliferation of *Plasmodium gallinaceum* in chickens administered intravenously with pantoyltauramide in doses of 400 mg kg⁻¹ body weight day⁻¹, and showed that the antiplasmodial effect was nullified by coadministering the compound with increasing concentrations of pantothenate. The latter finding was consistent with the antiplasmodial activity of pantoyltauramide resulting from the inhibition of pantothenate utilization. A few of the N-substituted pantoyltauramides also suppressed parasite growth, for example, 23e suppressed the growth of *Plasmodium gallinaceum* in chicks administered intravenously with 1 g kg⁻¹ body weight day⁻¹; however, none were considerably more active than pantoyltauramide (Berliner et al., 1946; Blanchard & Schmid, 1946).

Winterbottom et al. (1947) prepared a further series of N-substituted pantoyltauramides, which were predicted to have improved absorption/excretion characteristics relative to pantoyltaurine, and hence to be more active when administered orally. The new series did prove to be more active than pantoyltaurine, and pantoyltauramide, with all
compounds tested showing some degree of antiplasmodial activity in *Plasmodium gallinaceum*-infected chickens when incorporated into the diet. A few of the active *N*-substituted pantoyltaramides synthesized by Winterbottom et al. (1947) are shown in Fig. 4b (23a–d). Some of the compounds were even more active than the standard antimalarial, quinine, when tested against a standardized, blood-induced infection of *Plasmodium gallinaceum*, in which peak parasitaemia was reached 4 days postinfection (Brackett et al., 1946). The most active compound (23b) was four times more potent than quinine in this test, and sixteen times more potent against a blood-induced infection of *Plasmodium gallinaceum* in which peak parasitaemia was reached 7 days postinfection. The latter test is a more sensitive test for the antiplasmodial activity of slower acting compounds (Brackett et al., 1946). A daily intake of 23b as low as 6.5 mg kg$^{-1}$ body weight and 1.4 mg kg$^{-1}$ body weight was reported to reduce the parasitaemia of chickens to \( \leq 0.5\% \) of the parasitaemia of untreated chickens in the 4- and 7-day tests, respectively. Compound 23b was also active when administered as a single oral dose/day; however, a higher amount was necessary than was required when the compound was incorporated into the diet. Interestingly, as discussed in a previous section, 23b was also a potent antibacterial agent. The suppression of parasite growth induced by the analogues was antagonized by the addition of pantothenic acid (in large amounts) to the diet, providing confirmation that the compounds were exerting their effect by interfering with pantothenic acid metabolism (Brackett et al., 1946). Furthermore, it was observed that the dose of the analogues that was toxic to the host (detected as reduced food intake/weight gain and in some cases death) was far above the dose required for effective parasite suppression (Brackett et al., 1946). For example, chickens with a daily intake of 40 mg kg$^{-1}$ body weight of 23b gained weight normally, and the weight of chickens with a daily intake of 120 mg kg$^{-1}$ body weight was reduced only slightly. The findings highlighted the potential of these compounds as chemotherapeutic agents. When 23a and 23b were tested for toxicity in rhesus monkeys, both compounds were better tolerated than quinine (Berliner et al., 1946).

Phenylpantotheonone (31) was another compound screened against malaria parasites after being shown to exert an inhibitory effect (antagonized by pantothenic acid) against various bacteria *in vitro* (Woolley & Collyer, 1945). The analogue was inactive when incorporated into the diet of *Plasmodium lophurae*-infected ducks; however, when incorporated into the diet of chickens infected with *Plasmodium gallinaceum* or *Plasmodium lophurae*, \( \alpha \)-phenylpantotheonone suppressed parasite growth with an activity equivalent to or greater than quinine, respectively (Berliner et al., 1946; Blanchard & Schmid, 1946). As with the pantoyltaramides, the compound’s inhibitory effect was negated by coadministration of pantothenic acid. \( \alpha \)-Phenylpantotheonone was also tested in humans infected with *Plasmodium vivax*, and although it was tolerated well, with no signs of toxicity observed in humans administered with 2 g day$^{-1}$ for 4 days, it only partially suppressed parasite growth when administered orally (Berliner & Butler, 1946). \( \alpha \)-Phenylpantotheonone was also shown to be significantly less toxic than quinine to rats and monkeys (Berliner & Butler, 1946; Berliner et al., 1946).

Following on from the demonstration of the antiplasmodial activity of \( \alpha \)-phenylpantotheonone, several structural analogues of this compound were synthesized (Lutz et al., 1947; Senear et al., 1947). While \( \alpha \)-phenylpantotheonone, and analogues in which the pantoyl moiety had been modified, were inactive, the majority of the phenylpantotheonone analogues, which included analogues in which substituents had been introduced into the phenyl nucleus (32a–c), and a series of sulphur analogues (87a–d) also closely related to pantoyltaurine, were active against *Plasmodium gallinaceum* in *Plasmodium gallinaceum*-infected chickens when incorporated into the diet or administered orally. The activity of the \( \alpha \)-phenylpantotheonone analogues, like that of the lead compound, was generally equivalent to or greater than the activity of quinine (Berliner et al., 1946; Lutz et al., 1946; Senear et al., 1947). However, \( \alpha \)-para-chlorophenylpantotheonone (32b) was the only compound with significantly improved activity relative to \( \alpha \)-phenylpantotheonone, suppressing parasite growth with activity threefold that of quinine, when tested against a standardized, blood-induced infection of *Plasmodium gallinaceum*, in which peak parasitaemia was reached 5 days postinfection. The activity of a selection of the analogues was shown to be specific relative to pantothenic acid, as inhibition was suppressed when the diet was supplemented with pantothenic acid (Berliner et al., 1946; Senear et al., 1947). Interestingly, while compounds 32a and 32b proved to be both antibacterial and antiplasmodial, 32a was the most potent antibacterial of the two, and 32b was the most potent antiplasmodial. \( \alpha \)-para-Tolylpantotheonol (33), another phenylpantotheonone analogue synthesized by Lutz et al. (1947), also proved to be active against *Plasmodium gallinaceum* in *Plasmodium gallinaceum*-infected chicks. Although this compound had antiplasmodial activity equivalent to quinine, it was not tolerated as well by chicks. When the toxicity of compound 87a, a representative of the phenylpantotheonone analogues, was investigated in animals, the analogue was shown to display very low acute and chronic toxicity in several different animals (Singher et al., 1948). The maximum tolerated dose (the dose at which the final weight of the animal is equal to the starting weight) of another phenylpantotheonone analogue (87b) in chicks was found to be similar to that of quinine (Berliner et al., 1946).
activity and low-toxicity profiles of many of the D-phenylpantothenone analogues and N-substituted pantoyltauramides, they appear not to have been tested in humans (or other primates) infected with malaria.

The study of the antiplasmodial activity of the above compounds was limited because procedures for the in vitro cultivation of malaria parasites were not yet established. As in vivo cultivation of parasites was used, it was unclear whether differences in antimalarial activity were due to differences in the inherent antiplasmodial activity of the compounds or differences in their absorption/excretion characteristics in the host. Furthermore, the marked difference in the susceptibility of *Plasmodium lophurae* to phenylpantothenone when developing within chickens and when developing within ducks demonstrated that the nature of the host could dramatically affect a parasite’s susceptibility to a given compound. The development of methods for maintaining erythrocytic stage malaria parasites (various species, for at least one cycle of development in vitro (Trager, 1966), allowed the antiplasmodial activity of pantothenic acid analogues to be investigated in an in vitro culture system (Trager, 1966, 1971). Initially, 23b, one of the N-substituted pantoyltauramides that had proven to be a potent inhibitor of *Plasmodium gallinaceum* in vivo, was tested against erythrocytic stage *Plasmodium lophurae* parasites developing intracellularly in vitro (Trager, 1966). Consistent with the in vivo result against *Plasmodium gallinaceum*, the compound inhibited the proliferation of *Plasmodium lophurae* in vitro (when added to the culture at a concentration of 360 μM), and the effect was antagonized by the addition of 40 μM pantothenate to the culture medium. As mentioned in a previous section, the same pantothenic acid analogue had no effect on the erythrocytic stage parasite’s extracellular growth in vitro (Trager, 1966). Pantothenic acid analogues were therefore predicted to exert their antiplasmodial effect, at least against *Plasmodium lophurae*, by inhibiting CoA biosynthesis in the host erythrocyte, thereby reducing the CoA available to the parasite.

Compound 23b was also tested in *in vitro* cultures of the monkey malaria parasite *Plasmodium coatneyi*, and the clinically important, human malaria parasite *Plasmodium falciparum*, developing within monkey, and chimpanzee/human erythrocytes, respectively (Trager, 1966). The inhibitory effect of this compound was even more pronounced against these parasites. After a 2-day incubation with concentrations of 23b between 220 and 820 μM, a striking reduction in the parasitaemia of *Plasmodium coatneyi* and *Plasmodium falciparum* cultures was observed. The antiplasmodial activity, at least against *Plasmodium coatneyi*, was partially negated by the addition of pantothenate (40–65 μM) to the cultures. As it was not possible to maintain *Plasmodium coatneyi*, and *Plasmodium falciparum* extracellularly, it was unclear whether the action of this analogue was directly on the parasite, or whether, as seemed likely for *Plasmodium falciparum*, the primary site of action was the host erythrocyte’s CoA biosynthetic machinery, reducing the CoA available to the parasite. Additional pantothenic acid analogues, para-tolypantothenone (32a) and N-substituted pantoyltauramides 23d, both of which were reported previously to inhibit proliferation of *Plasmodium gallinaceum* in the chick (Berliner et al., 1946; Brackett et al., 1946; Lutz et al., 1947), were also tested and found to possess antiplasmodial activity against *Plasmodium coatneyi* and *Plasmodium falciparum in vitro* (Trager, 1966). Although perhaps less effective than 23b, para-tolypantothenone, when tested at a concentration of 410 μM, reduced significantly the parasitaemia of *Plasmodium coatneyi* cultures, and 23d, when tested at 830 μM, reduced the parasitaemia of *Plasmodium falciparum* cultures. The finding that compounds, such as 23b, 23d and 32a, which possess activity against *Plasmodium gallinaceum* and/or *Plasmodium gallinaceum* parasites developing intraerythrocytically, also inhibit the growth of *Plasmodium coatneyi* and/or *Plasmodium falciparum* hinted that more of the pantothenic acid analogues shown previously to inhibit the growth of avian species of malaria would also be active against human malaria parasites.

Trager (1966) also reported that dodecyl-β-alanine (88), a pantothenic acid analogue that retains the β-alanine moiety of pantothenic acid intact but in which the pantoyl moiety is replaced by a 12-carbon unit chain, inhibited the growth of *Plasmodium coatneyi* in vitro. In the presence of dodecyl-β-alanine, at a concentration of 470 μM, an approximately fourfold reduction in the parasitaemia of *Plasmodium coatneyi* cultures was observed after a 2-day incubation. This was the first report of an analogue that deviated from the structure of pantothenic acid in the pantoyl moiety, inhibiting the proliferation of malaria parasites. Several years before this study, Schinazi et al. (1950) had shown that the antibacterial pantothenic acid analogue, α-methylpantothenic acid (35), in which the pantoyl moiety contains an additional methyl substituent, was inactive against *Plasmodium lophurae* in chicks when administered orally (in doses of 5 g kg⁻¹ body weight day⁻¹ for 5 days) or intraperitoneally (in doses of 2 g kg⁻¹ body weight twice daily for 4 days). Whether dodecyl-β-alanine was inhibiting parasite growth via an effect on pantothenic acid utilization was not investigated.

Razdan et al. (1970) prepared a further series of N-substituted pantoyltauramides, which, like the majority of N-substituted pantoyltauramides prepared by Winterbottom et al. (1947), were N-arylsubstituted pantoyltauramides. The corresponding N-substituted pantoyltauramides with α-methylsubstituents on the pantoyl moiety were also synthesized, and all compounds were tested for antiplasmodial activity in *Plasmodium berghei*.
infected mice as well as *Plasmodium gallinaceum*-infected chicks. When administered as a single subcutaneous dose of 640 mg kg\(^{-1}\) body weight to mice (72 h after infection) or to chicks (immediately after infection), none of the analogues, including 23b, which had been shown previously to suppress parasite growth in *Plasmodium gallinaceum*-infected chickens, were active. The failure of these compounds in *in vivo* was predicted to be a result of the method of drug administration, which, differed from that used in previous *in vivo* screens in which the analogues were generally administered in the diet over several days. These results highlight the importance of investigating different drug administration procedures. Two years before this study, Eslager *et al.* (1968) had also prepared and screened a selection of \(N\)-arylsubstituted pantoyltauramides, which included 23b, but had been unable to prolong significantly the survival time of *Plasmodium berghei*-infected mice with a single subcutaneous dose of 640 mg kg\(^{-1}\) body weight of any of the compounds. Although subsequently a representative group of analogues were administered to *Plasmodium berghei*-infected mice in the diet (in doses up to 366 mg kg\(^{-1}\) body weight day\(^{-1}\) for 6 days), no significant suppression of parasite proliferation was observed. It is possible that the failure of these compounds to suppress the proliferation of *Plasmodium berghei* in mice even when a drug administration procedure similar to that used by researchers such as Brackett *et al.* (1946), who found several \(N\)-arylsubstituted pantoyltauramides to be active in *Plasmodium gallinaceum*-infected chicks, is due to the high concentration of pantothentic acid in the blood of mice. McIlwain & Hawking (1943) reported previously that the antibacterial pantothenic acid analogue pantoyltaurine failed to inhibit the proliferation of streptococci in mice while a similar dosage had been effective at protecting rats from streptococcal infection.

Trager (1971) extended the *in vitro* study of the antiplasmodial activity of pantothenic acid analogues against *Plasmodium coatneyi* and *Plasmodium falciparum* developing intraerythrocytically, assessing the activity of several more compounds. Among the analogues tested were a number of the \(N\)-arylsubstituted pantoyltauramides reported by Eslager *et al.* (1968) and Razdan *et al.* (1970) to lack antiplasmodial activity in *Plasmodium gallinaceum*-infected chicks and/or *Plasmodium berghei*-infected mice under the experimental conditions tested. Many of the \(N\)-arylsubstituted pantoyltauramides, which were screened at concentrations up to 450 \(\mu\)M (~10-fold the concentration of pantotenate present in the culture medium), inhibited the growth of both *Plasmodium coatneyi* and *Plasmodium falciparum* developing within monkey and human erythrocytes, respectively. The *in vitro* study highlighted a few general trends in the inherent antiplasmodial activity of the analogues. Among the \(N\)-arylsubstituted pantoyltauramides, the nature of the substituents on the aryl group greatly influenced the activity; for example, the compounds with chlorophenyl \(N\)-substituents (23b and 23f) were more active than the corresponding compounds with fluorophenyl \(N\)-substituents. The activity of the chlorophenyl compound (23b) was surpassed by 23g, a pantoyltauramide in which the sulphonamide nitrogen is substituted with a methoxyquinolyl group. This compound, which was the most active compound of this series against both *Plasmodium coatneyi* and *Plasmodium falciparum*, reduced significantly the number of parasites of ‘normal appearance’ in cultures at a concentration of 60 \(\mu\)M (only ~1.5 times the concentration of pantotenate in the cultures). In this study, the activity of 23g approached that of the standard antimalarial agent, primaquine, *in vitro*. Limited modification to the pantoyl moiety of a few of the \(N\)-substituted pantoyltauramides had little effect on activity; for example, the compound corresponding to 23b with a \(\alpha\)-methyl substituent and the compound corresponding to 23g with the secondary hydroxy group removed retained antiplasmodial activity. However, a loss of antiplasmodial activity was observed when the sulphonamide group of pantoyl-modified \(N\)-substituted pantoyltauramides was replaced with the corresponding sulphone. Whether the activity of compounds in this series of pantothentic acid analogues could be antagonized by pantothenic acid was not investigated.

Compound 23g was tested for antiplasmodial activity in *Plasmodium knowlesi*-infected monkeys, but was found to be ineffective when administered by mouth in doses of 10 mg kg\(^{-1}\) body weight for 7 days. However, when tested in *Plasmodium cynomolgi*-infected monkeys under the same dosage regime, 23g suppressed parasitaemia and, in one monkey, elicited a cure (Trager, 1971). Interestingly, 23g was one of the compounds reported by Razdan *et al.* (1970) that had failed to inhibit the proliferation of *Plasmodium berghei* in mice or *Plasmodium gallinaceum* in chicks when administered in a single subcutaneous dose, consistent with the screening method used by Razdan *et al.* being an ineffective way of identifying pantothentic acid analogues that may possess antiplasmodial activity against primate species of malaria *in vivo*.

In view of the urgent need for new antimalarial agents effective against drug-resistant strains of *Plasmodium falciparum*, and the recent developments in understanding *Plasmodium falciparum*’s pantothentic acid requirement and the mechanisms by which it transports and metabolizes the vitamin, the activity of pantothentic acid analogues against the human malaria parasite has recently been revisited. Saliba *et al.* (2005) showed that pantothénol (24), the provitamin of pantothentic acid, previously recognized for inhibiting the growth of pantothentic acid-requiring bacteria *in vitro* (Snell & Shive, 1945), also inhibits the growth of...
Plasmodium falciparum in vitro (IC₅₀ value of 60 μM measured against the FAP6 strain). The antiplasmodial activity of this analogue is antagonized by pantothenic acid consistent with the analogue inhibiting parasite growth via an effect on the parasite's utilization of pantothenic acid. Importantly, pantothenol is selective; the concentrations required to inhibit parasite proliferation were without effect on the proliferation of a human cell line (the Jurkat, leukaemic T-lymphocyte cell line). Further characterization of the action of pantothenol revealed that it had little, if any, effect on the transport of pantothenate into the parasite but, rather, inhibited the phosphorylation of pantothenate by the parasite's PanK, and thereby its accumulation within the parasite (Saliba et al., 2005). Pantothenol inhibited pantothenol phosphorylation by competing with pantothenate for PanK and was, in the process, phosphorylated by the enzyme (Lehane et al., 2007). Whether pantothenol is further metabolized by downstream enzymes in the parasite's CoA biosynthesis pathway is yet to be determined. The direct activity of pantothenol against the intracellular Plasmodium falciparum parasite is in stark contrast with the mechanism by which pantothenic acid analogues are reported to exert their effect on Plasmodium lophurae parasites developing within avian erythrocytes.

Despite the close structural resemblance between pantothenol and pantothenic acid, Lehane et al. (2007) have shown recently that the mechanism by which pantothenol is taken up by the malaria parasite is distinct from the mechanism by which the parasite acquires pantothenate (discussed in a previous section). This was the first study to investigate the mechanism by which an antimicrobial pantothenic acid analogue is taken up by the target organism. Moreover, Lehane et al. (2007) showed that pantothenate uptake across the parasite plasma membrane can be inhibited without affecting pantothenol uptake. This highlights the possibility that compounds that inhibit specifically the uptake of pantothenate might act synergistically with compounds (such as pantothenol) that inhibit the metabolism of pantothenate, in inhibiting parasite growth.

When administered orally to Plasmodium vinckei vinckei-infected mice (1.4 g kg⁻¹ body weight day⁻¹ for 4 days), pantothenol was tolerated well and significantly inhibited the growth of parasites, however, not enough to elicite a cure (Saliba et al., 2005). In mammalian cells, pantothenol is oxidized to pantothenic acid by the enzyme alcohol dehydrogenase (Abiko et al., 1969). The parasite has, in contrast, been shown to be incapable of this conversion (Saliba et al., 2005). The conversion of pantothenol to pantothenate would not only reduce the effective concentration of pantothenol in the blood, but would also convert it to the very compound that antagonizes its activity. This provides a possible explanation for pantothenol's selectivity and for the relatively weak inhibition observed in vivo. The high pantothenate plasma concentration of mice may also contribute to the latter (McIlwain & Hawking, 1943). The activity of pantothenol is, nonetheless, consistent with the view that inhibiting pantothenate utilization may be an effective antimalarial strategy.

A dozen more pantothenic acid analogues, which, like pantothenol differ from pantothenic acid by having simple modifications to the β-alanine moiety, have since been shown to inhibit Plasmodium falciparum in vitro, the majority with IC₅₀ values in the range 15–160 μM in the presence of 1 μM extracellular pantothenate (Saliba & Kirk, 2005; Spry et al., 2005). These compounds include: CJ-15 801 (45), the fungal metabolite reported to inhibit the growth of multidrug-resistant strains of Staphylococcus aureus; N-pantoyl-substituted amines 25a–e and 25k, some of which had been shown previously to inhibit the growth of lactic acid bacteria in vitro; and pantoylhydrazide (26), shown by Madinaveitia et al. (1945) to inhibit the growth of Lactobacillus casei and Streptococcus pyogenes in vitro. With the exception of 26, the activity of all compounds was antagonized by pantothenic acid. These analogues generally appear to act by a mechanism similar to pantothenol, and display similar or improved selectivity for the parasite in vitro. Limited structure–activity information was also gained from the series of N-pantoyl-substituted amines; for example, introduction of a polar substituent into the carbon two carbon units removed from the amide nitrogen in the alkyl chain, as in 25a, substantially reduced antiparasomal activity. Although a preliminary screen of in vivo antiparasomal activity in Plasmodium vinckei vinckei-infected mice with a selection of these analogues (25b–e and 25k) did not result in significant inhibition of parasite proliferation (Spry et al., 2005), it is possible that with other compounds in this series, or with a different dosage regime, an antiparasomal effect would be seen in vivo. Some of the mice administered with a single oral dose (of up to 3 g kg⁻¹ body weight) of the N-pantoyl-substituted amines 25b–d and 25k exhibited signs of acute toxicity, the pharmacological basis for which is yet to be determined. However, there were no signs of toxicity observed in mice administered with lower dosages of these compounds, or in mice administered orally with N-pantoyl-substituted amine 25e (2.8 g kg⁻¹ body weight day⁻¹ for 4 days). The in vivo antiparasomal activity of these compounds (most of which, in contrast to pantothenol, are unlikely to undergo conversion to pantothenate in vivo) and related analogues should be characterized in more detail in the future.

Conclusions and future directions

Pantothenic acid analogues, particularly N-substituted pantoylauramides and phenylpantothenones, have a history of antiparasomal efficacy against an assortment of
Plasmodium species. Nonetheless, only in the past few years, when it has become evident that pantothenic acid uptake and metabolism are promising targets for much-needed antimalarial chemotherapy (Kirk & Saliba, 2007), has interest in pantothenic acid analogues as antimalarial agents resurged. The early studies identified a number of promising antiplasmodial pantothenic acid analogues, and these warrant further characterization. Compounds 23b–d and 32b, for example, demonstrated activity superior to the standard antimalarial quinine when tested against Plasmodium gallinaceum in chickens, and compounds 23b and 23d have also been shown to inhibit the proliferation of Plasmodium falciparum in vitro. Another compound, 23g, is reported to inhibit proliferation of Plasmodium falciparum in vitro, at a concentration only ~1.5 times the concentration of pantothenate in the culture medium, and to suppress the proliferation of Plasmodium cynomolgi in monkeys when administered orally.

The activity of many pantothenic acid analogues discussed in this section has, because a suitable system for the continuous culture of malaria parasites in vitro was not developed until 1976, been investigated only in animal hosts, predominantly avian. It is therefore important to screen such analogues against Plasmodium falciparum in vitro. This is necessary for two reasons. Firstly, it has been shown that Plasmodium species, namely Plasmodium lophurae and Plasmodium falciparum, differ substantially with regard to the mechanisms by which they obtain the CoA required for growth, and hence it should be confirmed that compounds including N-substituted pantoyltauramide 23c, α-phenylpantothenone (31) and phenylpantothenone analogues 32b–c, 33 and 87a–d, which are reported to inhibit proliferation of Plasmodium gallinaceum and/or Plasmodium lophurae as effectively as (or more effectively than) quinine, also possess activity against species of Plasmodium that infect humans. Secondly, the early in vivo antimalarial screens did not allow compounds to be screened for inherent antimalarial activity independently of host pharmacokinetics and therefore it would be prudent to confirm that a compound like α-methylpantothenic acid (35), which is reported to cure mice of infection with streptococci and yet failed to suppress parasitaemia in Plasmodium lophurae-infected ducks, does in fact lack antiplasmodial activity. The activity of a few of the pantothenic acid analogues demonstrated to be active against Plasmodium gallinaceum has been shown to be active against Plasmodium falciparum growing in early culture systems, developed before 1976, that allowed the development of Plasmodium falciparum through at least one cycle in vitro. The activity of these compounds (23b, 23d, 32a), and the other pantothenic acid analogues reported by Trager (1971) to inhibit Plasmodium falciparum in vitro, particularly 23g, should be confirmed against Plasmodium falciparum parasites in continuous culture, where parasites continue to reproduce asexually approximately every 48 h as they do in an infected host. With the development of a method for the continuous culture of the erythrocytic stages of Plasmodium falciparum (Trager & Jensen, 1976) and subsequently, high-throughput techniques for assaying the sensitivity of this parasite to chemical compounds (Desjardins et al., 1979; Bennett et al., 2004; Smilkstein et al., 2004), the antiplasmodial activity of pantothenic acid analogues can now be readily determined against various strains (drug-resistant and sensitive) of the virulent species of the human malaria parasite in vitro. The effects of pantothenate supplementation on the activity of compounds should also be investigated, in order to determine whether (as suspected) such analogues are inhibiting growth by inhibiting pantothenate utilization. Compounds revealed/confirmed to be effective at inhibiting the in vitro growth of Plasmodium falciparum by inhibiting pantothenic acid utilization should, pending satisfactory selectivity, be tested in mammalian models of malaria with the ultimate aim of using them in chemotherapy of humans. As the blood plasma concentration of pantothenic acid in mice is significantly higher than that in humans, caution is required when screening pantothenic acid analogues for antiplasmodial activity in mice, to avoid rejecting compounds that may prove valuable in treating humans infected with malaria. Owing to the differences in the mechanisms by which avian and human malaria parasites utilize CoA from pantothenic acid, and because primates are not amenable for initial screens of in vivo antiplasmodial activity, Plasmodium berghei and Plasmodium vinckei-infected mice remain the best animal models for testing inhibitors of pantothanic acid utilization for antiplasmodial activity in vivo. When a lack of toxicity allows, high dosages of compounds that yield plasma concentrations sufficient to antagonize the high plasma levels of pantothenic acid in mice may need to be tested.

The studies published in the last few years have focused on investigating the antiplasmodial activity of antibacterial pantothenic acid analogues including pantothenol (24), CJ-15,801 (45) and other N-pantoyl-substituted amines not tested previously for antiplasmodial efficacy. The activity of these analogues in vitro, and in the case of pantothenol also in vivo, has reinforced the view that analogues of pantothenic acid may be effective antimalarial agents.

Concluding remarks: future directions

In the past seven decades, a large number of pantothenic acid analogues have been synthesized by researchers with interests in various organisms with a common aim of identifying compounds that inhibit the utilization of pantothenic acid. Among the analogues prepared, a remarkable number have demonstrated antimicrobial activity...
against bacteria, fungi or malaria parasites, and some have shown activity against multiple organisms. Unfortunately, many compounds with promising antimicrobial activity in vitro appear to have been abandoned before testing in vivo. Other compounds that have demonstrated antimicrobial activity in animal models of human infections, in some cases enough to provide a cure, appear to have been abandoned before detailed studies in humans or other primates. This review attempts to bring together the results of these investigations, not only to highlight the potential of pantothenic acid utilization as a target for antimicrobial chemotherapy but also in the hope that interested researchers will take advantage of the findings of earlier researchers when embarking on the further synthesis and screening of potential inhibitors of pantothenic acid utilization. Analogues of precursors to pantothenic acid that inhibit pantothenic acid biosynthesis are also reported to possess antibacterial and antifungal activity in vitro. The activity of these compounds in vitro highlights the potential of pantothenic acid biosynthesis as a target for antibacterial and antifungal chemotherapy. However, whether such compounds are effective in vivo needs to be established.

A selection of the vast number of compounds that have been shown previously to inhibit the in vitro growth of different microorganisms should be tested for antimicrobial activity against the respective microorganisms in vivo. Compounds such as N-substituted pantoyltauramides 23a and o-methylpantothenic acid (35), which have been shown to possess antibacterial activity in mice infected with streptococci, and N-substituted pantoyltauramides 23b and 23g, and o-para-chlorophenylpantothenone (32b), which have been shown to possess antimalarial activity in various animal models of malaria, should be subjected to more rigorous animal testing to determine whether they may be suitable for trials in humans. The importance of pantothenic acid as a precursor to CoA in virtually all organisms, and the inhibitory activity of pantothenic acid analogues against a diverse range of microorganisms, implicates inhibitors of pantothenic acid utilization as potential broad-spectrum antimicrobial agents. As such, it may prove valuable to screen analogues that have been reported to inhibit the growth of a particular microorganism against other microorganisms for which new chemotherapies are required. As illustrated in Fig. 4b, there are many known inhibitors of bacteria that are yet to be tested against malaria and fungi, and there are some effective inhibitors of the growth of malaria parasites that have not been tested against bacteria and fungi.

In vitro screening procedures should involve testing against more than one species of any particular pathogen including, if possible, the clinically relevant species. If the in vitro activity of an analogue warrants testing in animal models of human infections, compounds should be tested in more than one animal host. This should help to avoid the exclusion of compounds that may be potent inhibitors of only certain species of a particular pathogen, or compounds that may be active in humans but not in certain animal hosts, for example because of differences in the plasma pantothenic acid concentration or differences in absorption, distribution, excretion and/or metabolism of the compound. For the same reasons as outlined above, compounds that have been reported to be inactive against a particular organism, or have failed to display antimicrobial activity in a certain animal host should not be forsaken before further characterization.

The compounds shown previously to be antimicrobial can be adopted as lead compounds for the further development of inhibitors. If the reported synthetic route to the chosen lead compound can be easily modified to produce a number of related compounds it is likely that the process of lead optimization can be fast-tracked. A significant body of information on SARs, and the selectivity, toxicity and pharmacokinetics of certain compounds is available and should help to direct lead optimization.

Since pantothenic acid was first shown to support the growth of various microorganisms, considerable progress has been made towards understanding the mechanisms by which organisms obtain the pantothenic acid they require, and how they utilize pantothenic acid for the synthesis of CoA. Recently the genes coding for the enzymes of the CoA biosynthesis pathway have been identified, and the crystal structures of all the Escherichia coli enzymes, with the exception of PPCDC, have been determined, bringing renewed interest in the pathway as a target for antimicrobial chemotherapy. In many organisms, PanK catalyses the key rate-limiting step of the CoA biosynthesis pathway. Pantothenic acid analogues have been shown to be effective inhibitors of the pantothenate phosphorylation reactions catalysed by the PanKs from Escherichia coli, Staphylococcus aureus, Plasmodium falciparum and Aspergillus nidulans. These pantothenic acid analogues exert their antimicrobial effect on Escherichia coli, Staphylococcus aureus and Plasmodium falciparum, by inhibiting pantothenate phosphorylation and/or by being phosphorylated by PanK and subsequently converted to CoA analogues that inhibit CoA-utilizing enzymes. It is likely that the mechanism by which many more of the antimicrobial pantothenic acid analogues reviewed here exert their antimicrobial effect involves inhibition of PanK-catalysed pantothenate phosphorylation and/or conversion to the corresponding phosphorylated analogue by PanK.

The crystal structures of the Escherichia coli PanK with ADP and pantothenate, with an ATP analogue, or with CoA bound in the active site, have proved particularly useful for...
understanding how this enzyme is able to bind pantothenate, the feedback inhibitor CoA and pantothenic acid analogues. Knowledge of the Escherichia coli PanK active site structure provides a basis for the design of pantothenic acid analogues that will bind the enzyme. The unique ability of this enzyme to accommodate compounds much bulkier than the natural substrate suggests that the design of inhibitors will not be highly restricted. With the structures of PanKs from Mycobacterium tuberculosis, Staphylococcus aureus, Pseudomonas aeruginosa, Thermotoga maritima and Bacillus anthracis determined recently, there is now structural information on all three classes of PanK found among bacteria. This knowledge should help guide the design of inhibitors/substrates of bacterial PanKs, whether targeting a group of organisms expressing a particular type of PanK, in the case of selective antibacterial agents, or targeting bacteria expressing any of the three PanK types, in the case of broad-spectrum antibacterial agents. Although PanK activity has been detected in Plasmodium falciparum cell lysates, a Plasmodium PanK is yet to be purified or expressed heterologously. Until this enzyme is expressed and a crystal structure becomes available, the design of inhibitors of the Plasmodium PanK will rely on the results of SARs developed for this enzyme by screening pantothenic acid analogues against the enzyme in cell lysates. The crystal structures of the human PanK1a and PanK3 isoforms that were solved recently will hopefully aid the design of selective inhibitors/substrates of microbial PanKs.

Currently, the mechanisms by which pantothenic acid analogues gain entry to microbial cells are largely not understood. Knowledge of the uptake mechanisms of the inhibitors will also become important, and should aid in the design of new inhibitors that are efficiently taken up by the target microorganism, and perhaps not by the infected host.

To the best of the authors’ knowledge, the only compounds reported to inhibit the utilization of pantothenic acid by microorganisms are structural analogues of pantothenic acid. The majority of these are competitive inhibitors that are inhibitory only when the compound is present at a concentration several-fold higher than that of pantothenic acid. A few pantothenic acid analogues that inhibit the growth of microorganisms in a manner that cannot be antagonized by pantothenic acid have also been reported. These compounds, however, have been somewhat neglected, presumably because in earlier investigations antagonism with pantothenic acid was the primary mechanism for showing that an analogue inhibited growth by inhibiting pantothenic acid utilization. Hence, at the time, it was unclear whether such compounds were in fact irreversible inhibitors (or high-affinity competitive inhibitors) of pantothenic acid utilization or generally toxic to the microorganism. The activity of irreversible inhibitors of pantothenic acid utilization is independent of the pantothenic acid concentration and hence such compounds are attractive antimicrobial agents because high concentrations are not required to antagonize the pantothenic acid present in the host. It may be interesting to investigate the mechanism of action of compounds reported previously to be resistant to antagonism by pantothenate, to determine whether these compounds are in fact irreversible inhibitors. With biochemical assays available for measuring the activity of purified PanK, or PanK activity in cell lysates, it is possible to determine quite rapidly whether a compound is inhibiting PanK-catalysed pantothenate phosphorylation (by inhibiting PanK or acting as a substrate). To fast-track the identification of inhibitors of the PanK-catalysed pantothenate phosphorylation reaction, and pantothenic acid utilization in general, where possible, compound libraries can be screened in such enzyme assays. Screening compound libraries should not only facilitate the identification of competitive inhibitors of pantothenate phosphorylation of the type reviewed here but may also lead to the identification of irreversible inhibitors and/or inhibitors that are not substrate analogues.

Acknowledgements

This work was supported by a grant from the Australian National Health and Medical Research Council and a grant from the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

References


Burlet E (1944) The biological conversion of pantothenol (γ-hydroxypropylamidol of 2,4-dihydroxy-3,3-dimethylbutyric acid) to pantothenic acid. Z Vitaminforsch 14: 318–331.


© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved
Coenzyme A biosynthesis inhibitors as antimicrobial agents

99


Coenzyme A biosynthesis inhibitors as antimicrobial agents

99


Williamson JM & Brown GM (1979) Purification and properties of l-aspartate-α-decarboxylase, an enzyme that catalyzes the...