Intrinsic antibiotic resistance of *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa*, an opportunistic pathogen, is one of the major causes of life-threatening bacterial infections in western society. It is particularly troublesome in those patients who are already debilitated by severe burns, cancer, cystic fibrosis, leukaemia, diabetes mellitus, or major surgery including transplantation. Owing to the high intrinsic resistance of *P. aeruginosa* to almost all commonly used antibiotics, infections caused by this pathogen, once established, are often fatal (Flick & Cluff, 1976). In contrast to many other bacteria, plasmid-mediated antibiotic resistance is relatively uncommon (Bryan, 1979). This led a number of researchers (Nordstrom and Sykes, 1974; Brown, 1975; Bryan, 1979) to propose that the cell wall of *P. aeruginosa* might be a barrier to antibiotic diffusion although, until recently, direct evidence to support this conclusion was lacking.

It is now well established that the predominant reason for this high intrinsic resistance to hydrophilic antibiotics, such as β-lactams, is the low rate of permeation of these antibiotics across the outer membrane (Angus et al., 1982; Yoshimura & Nikaido, 1982; Nicas & Hancock, 1983). The rate of permeation of β-lactam compounds across the outer membrane of *P. aeruginosa* is 12-100 fold lower than the rate of permeation of the same compounds across the outer membranes of *Escherichia coli*. The relationship between low outer membrane permeability and antibiotic resistance was confirmed by examination of a mutant strain of *P. aeruginosa* (Z61) which is highly susceptible to all 27 antibiotics studied (Zimmerman, 1979; Angus et al., 1982). The mutant has a six fold increase in outer membrane permeability to the chromogenic β-lactam nitrocefin, compared with its parent strain K799 which has normal antibiotic resistance (Angus et al., 1982).

In other Gram-negative bacteria, it has been demonstrated that 'porin' proteins, which form water-filled channels across the outer membrane, are responsible for the uptake, into the cell periplasm, of hydrophilic antibiotics, such as β-lactams (Nikaido & Vaara, 1985). A mutant strain of *P. aeruginosa* lacking a major outer membrane protein, designated protein F, was shown to have a seven fold decrease in outer membrane permeability compared to its parent strain (Nicas & Hancock, 1983). This suggests that protein F is the major protein responsible for the (albeit poor) outer membrane permeation pathway of *P. aeruginosa*. In addition, purified protein F has been demonstrated to form water-filled channels in model membranes by three different methods (Hancock, Decad & Nikaido, 1979; Benz & Hancock, 1981; Yoshimura et al., 1983).

There has been some controversy recently over the actual size of the water-filled channels across the outer membrane of *P. aeruginosa*. Our original data (Hancock & Nikaido, 1978; Hancock et al., 1979), based on the liposome exclusion assay, suggested that protein F and protein F-containing outer membrane fragments formed very large water-filled channels with an exclusion limit for saccharides of approximately 3000 daltons compared to the 600 dalton exclusion limit of *Esch. coli* pore-forming (porin) proteins. This paradox was resolved when data were presented (Benz & Hancock, 1981; Yoshimura et al., 1983) indicating that only a small percentage, perhaps as few as 0.4% of the approximately 200 000 protein F molecules per cell (Nicas & Hancock, 1983), formed these large channels. Therefore, while the *P. aeruginosa* outer membrane has a large exclusion limit due to these large channels, the small total area of channels available for diffusion of compounds as large as antibiotics results in low outer membrane permeability. Interestingly, we have recently obtained evidence suggesting that the other 99-6% of so of protein F channels are small and should be almost impenetrable by β-lactams (Woodruff et al., 1986), in contrast to our previous proposal that these other channels were closed (Nicas & Hancock, 1983).

Further evidence for the proposed large exclusion limit was provided by Miller & Becker (1978), who demonstrated that *P. aeruginosa* grew as well on pentamethionine as it did on the free amino acid methionine, whereas an *Esch. coli* methionine auxotroph grew well on trimeth-

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**Leading articles**

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Further evidence for the proposed large exclusion limit was provided by Miller & Becker (1978), who demonstrated that *P. aeruginosa* grew as well on pentamethionine as it did on the free amino acid methionine, whereas an *Esch. coli* methionine auxotroph grew well on trimeth-
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of these saccharides over a long period. The leakage of saccharides is expressed relative to that of sucrose. The conclusion that the P. aeruginosa exclusion limit is small is based on the assumption that larger substrates which can permeate across the outer membrane should permeate at the same rate as sucrose, an assump-

tion that is clearly incorrect given the theoretical considerations of Renkin (1954), who demon-

strated that friction of a trisaccharide passing through a pore of limited dimensions must be very much greater than friction on a disaccharide passing through the same channel. In addition, outer membrane permeability measurements cannot be made on cells that have been washed after loading with radiolabelled compounds, since the time required for half equilibration of solutes across the outer membrane is in the order of a few seconds. To estimate the exclusion limit, the paper of Yoneyama et al. (1986) utilizes plasmolysis experiments, based on the ability of large concentrations of saccharides to pass across the outer but not the inner membrane, causing water to leave the cytoplasm and consequent collapse of the inner membrane. However both plasmolysis and outer membrane collapse reflect the relative rate of trans-outer membrane permeability to the sugar in question compared with the rate of water movement across the cytoplasmic membrane. Thus, for P. aeruginosa these experiments can be interpreted by propos-

ing that the rate of penetration, even of sucrose, across the outer membrane is too slow to pre-

vent the collapse of the outer membrane due to water leakage across the cytoplasmic and, sub-

sequently, the outer membrane. It should be noted that the above papers differ in their esti-

mates of the size of sugar that can permeate the P. aeruginosa outer membrane: disaccharides in the case of Caulcott et al. (1984) and mono-
saccharides in the case of Yoneyama et al. 1986.

One important consideration is whether low outer membrane permeability alone is sufficient to account for the level of resistance to all antibi-

iotics observed in P. aeruginosa. Despite the low rate of diffusion across the P. aeruginosa outer membrane one can calculate that the ß-lactam nitrocefin, at an external concentration of 5 mg/l (given the measured outer membrane permeability coefficient C of $1.3 \times 10^{-3}$ min$^{-1}$ mg cells$^{-1}$ ml$^{-1}$) would equilibrate across the outer membrane in 21 sec. Thus low outer mem-

brane permeability will slow down the rate of uptake into the periplasm (to 116 molecules of ß-
lactam taken up/cell/sec in the above example) but will not prevent uptake. A secondary defence mechanism such as the presence of ß-lactamase is required to prevent the build-up of ß-lactams in the periplasm to the same concentration as that in the external medium. This is emphasized by the demonstration of Vu & Nikaido (1985) that strains of ß-lactamase-derepressed Enterobacter cloacae are resistant to third generation cephalosporins as a result of low outer mem-

brane permeability combined with very slow hydrolysis in the periplasm. These observa-

tions have been recently confirmed for ß-lactamase derepressed isolates of P. aeruginosa (Livermore, 1985; Bayer, A. S., Parr, T. R. Jr., Chan, L. and Hancock, R. E. W. unpublished, observations). Therefore it appears there are at least two readily demonstrable factors which interact to give rise to resistance to ß-lactams in P. aeruginosa low outer membrane permeability and hydrolysis of incoming ß-lactams by peri-

plasmic ß-lactamase.

Many non-ß-lactam antibiotics probably also utilize the porin pathway of P. aeruginosa. However, polycationic antibiotics, such as aminoglycosides and polymyxins, traverse the outer membrane by a different route (Hancock, 1984). These antibiotics interact with the outer membrane at sites where divalent cations form cross-bridges between adjacent lipopolysac-

charide molecules (Peterson, Hancock & McGrosart, 1985; Moore, Bates & Hancock, 1986). The consequent displacement of these divalent cations by the bulkier polycationic anti-

biotics (Moore et al., 1986) results in an increase in outer membrane permeability (Hancock, Raffle & Nicas, 1981; Loh, Grant & Hancock, 1984) which has been proposed to result in increased uptake of the polycationic antibiotic itself (Hancock, 1984). Since these polycations are promoting their own uptake across the outer membrane we have called this phenomenon 'self-promoted' uptake. The presence of this alternative pathway for aminoglycosides may explain the relative efficacy of such compounds
against P. aeruginosa. Furthermore, since it has been demonstrated, that aminoglycosides will disrupt the outer membrane thereby increasing its permeability towards the β-lactam nitrocefin (Hancock et al., 1981), this presents a plausible explanation of the known synergy between β-lactams and aminoglycosides against P. aeruginosa (Sykes & Morris, 1975).

Since low outer membrane permeability presents a considerable problem in the therapy of P. aeruginosa infections and since activation of the ‘self promoted’ uptake pathway results in an increase in outer membrane permeability (Hancock et al., 1981; Loh et al., 1984), a new approach to P. aeruginosa therapy can be proposed. Presentation of an activator of the self-promoted pathway together with an antibiotic may result in increased efficacy of this antibiotic against P. aeruginosa infections. With this in mind, we (Hancock & Wong, 1984) instituted a screening systems for such activator compounds which we have termed ‘permeabilizers’. These compounds fall into four basic classes: polycations, divalent cation chelators, monovalent organic cations and, possibly, reducing agents. A number of these compounds including polymyxin B (Sykes & Morris, 1975), polymyxin B nonapeptide (Vaara & Vaara, 1983), aminoglycosides (Sykes & Morris, 1975), ascorbate (Rawal, McKay & Blackhall, 1974) and EDTA (Wilson, 1970) have demonstrated effective synergy with antibiotics.

While these studies have become quite sophisticated, I feel that they have only touched upon the problems involved in treating P. aeruginosa infections. The considerable difficulties that remain include the development of unstable resistance to antibiotics (adaptation to resistance that reverts upon antibiotic removal) (e.g. Gerber & Craig, 1982), the common isolation of low level antibiotic-resistant mutants with subtle surface and permeability alterations (Bryan, O’Hora & Wong, 1984; Godfrey, Hatledid & Bryan, 1984; Godfrey, 1984) and the poor relationship between laboratory-derived MICs and therapeutic efficacy (Davis, 1974; Flick & Cluff, 1976).

R. E. W. HANCOCK
Department of Microbiology
University of British Columbia,
Vancouver, British Columbia,
Canada, V6T 1W5

References


The prevention of wound infection after coronary artery bypass surgery

Sternal wound infection, one of the many possible complications of open heart surgery, has a sinister reputation and may progress to mediastinitis, osteomyelitis and bacteremia; it has a high mortality (7–45%) and the survivors often require extensive wound debridement (Sarr, Gott & Townsend, 1984). Risk factors for sternal sepsis have recently been reviewed by Sarr et al. (1984).

Although both coronary artery bypass graft (CABG) and valve replacement operations are conventionally “clean” and require cardio-pulmonary bypass (CPB), in CABG no intra-vascular prosthesis is involved but instead saphenous veins are harvested from the upper thigh. Two recent reports emphasized the differences between the two operations and related them to post-operative infection (Wells, Newsom & Rowlands, 1983; Farrington et al., 1985a). Both found sternal wound sepsis to be common after CABG (about 8%) and to be caused by Staphylococcus aureus or endogenous, antibiotic-sensitive coliforms; coliforms were also often isolated from leg incision infections. Sternal infection after valve replacement was less common (about 2%) and predominantly staphyloccocal. Available evidence suggests peri-operative implantation of pathogens; Kluge et al. (1974) found extensive contamination of chest wounds in theatre with skin commensals, but coliforms and S. aureus were occasionally also isolated. During CPB the patient may be exposed to wound contaminants via the blood stream since blood (with theatre air and other contents of the wound) is aspirated from around the heart and returned to the circulation. Wells et al. (1983) suggested that bowel flora was