THE "GLUCOSE EFFECT" ON GROWTH INHIBITION OF ESCHERICHIA COLE BY STREPTOMYCIN, TRIMETHOPRIM AND SULFAMETHOXAZOLE, RESPECTIVELY

AXEL DALHOF

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1. Introduction

The presence of different carbon sources in growth media does not only influence growth rate and biomass production but it often has a severe effect on catabolic enzyme expression. The presence of glucose strongly represses the expression of inducible enzyme synthesis — a phenomenon originally referred to as "glucose effect" and later on as "transient" and "permanent catabolite repression", respectively [1-3].

Early steps in catabolism are binding of substrates to and transport into bacteria. Not only carbon and energy sources but also other often structurally related substances are transported or cotransported into cells; streptomycin is taken up by Escherichia coli via an inducible polyamine transport system [4, 5] and it is very likely that sulfonamides and purine analogues enter cells through the aromatic amino acid permease system(s) which has been shown to be under adenosine 3',5'-monophosphate (cAMP) control [6].

The present study was designed to investigate the effect of glucose and cAMP respectively on the inhibitory effect of streptomycin (SM), sulfamethoxazole (SMZ) and trimethoprim (TMP) as these substances seemingly are transported into bacteria by inducible systems.

2. Material and Methods

E. coli ATCC 10536 was used throughout this study with the exception of one experiment where E. coli 83-1 (aromatic amino acids and vitamins auxotrophic mutant of E. coli K-12; this strain was kindly provided by Prof. Davis) and its parent strain were used. Cultures were routinely grown at 37°C with shaking in 300 ml erlenmeyer flasks containing 100 ml medium. Growth was followed by determining viable cell counts per ml medium.

A defined medium [7] containing 10 mM glucose as carbon source was used unless otherwise stated. SM was used in concentrations of 4 µg/ml, SMZ 160 µg/ml, TMP 125 µg/ml; these concentrations represent twice the respective MIC.

3. Results

3.1. Effect of streptomycin, trimethoprim and sulfamethoxazole on acetate-grown E. coli

As acetate does not decrease intracellular cAMP concentrations or inhibit inducible enzymes in E. coli [8] the effect of SM, SMZ and TMP, respectively on the growth of E. coli ATCC 10536 was monitored in media containing sodium-acetate (120 mM and 12 mM, respectively). TMP and SMZ showed typical bacteriostatic inhibition patterns whereas SM reduced the viable cell counts (Fig. 1); identical data were obtained with cultures containing both acetate concentrations.

3.2. Effect of glucose and cAMP on the effect of streptomycin

Cells used in these experiments were cultured with 10 mM glucose as carbon source; cAMP (1 · 10^{-3} M; 0.5 · 10^{-2} M; 1 · 10^{-3} M) was added to the cultures
Fig. 1. Effect of streptomycin, trimethoprim and sulfamethoxazole on *Escherichia coli* ATCC 10536 growing on 12 mM acetate. ○, control; Δ, streptomycin 4 μg/ml; ●, trimethoprim 125 μg/ml; •, sulfamethoxazole 160 μg/ml; CFU, colony forming units.

immediately. After an incubation period of 1.75 h cells were washed and cAMP was removed by centrifugation. The cell pellet was resuspended in fresh prewarmed medium containing 4 μg streptomycin/ml only. The culture which had not been preincubated with cAMP was bacteriostatically inhibited by SM (Fig. 2). In contrast, preincubation with cAMP caused lysis of the cells. The onset, but not the rate of lysis, was obviously dependent on the cAMP concentrations used during the preincubation period.

Similar results were obtained by simultaneous addition of cAMP and SM to the cultures containing glucose as carbon source (Fig. 3). Again SM alone exhibited only an inhibitory effect but the addition of cAMP caused a reduction of viable cell counts after a lag period of 1 to 2 h. Under these conditions neither the onset nor the rate of lysis seemed to be concentration-dependent.

The addition of cAMP \(1 \cdot 10^{-2} \text{ M}\) to the cultures 3 h after the addition of SM also caused a reduction of viable cell counts after a lag period of 1.5 h (Fig. 4).

3.3. Effect of glucose on the inhibition of cell growth by sulfamethoxazole or trimethoprim

Using glucose at high concentrations (50 mM) as carbon source neither SMZ nor TMP inhibited growth of *E. coli* (Fig. 5); on lowering the glucose concentrations 10-fold both substances exhibited the typical bacteriostatic effect which could also be observed by using the non-repressing carbon source acetate. The
same effect as that caused by lowering the glucose concentrations could be obtained by adding $5 \cdot 10^{-2}$ M cAMP to cultures containing 50 mM glucose (results not shown).

3.4. Influence of amino acids on the inhibitory effect of trimethoprim or sulfamethoxazole

Chemical similarities between two components should interfere with specific reactions common to both substances. It has already been shown by Hölter [5] that the polyamines putrescine, spermidine and spermine which share a common transport system with SM compete with the uptake of streptomycin. Similarly aromatic amino acids should compete with the inhibition of cells by SMZ or TMP. But on the other hand biosynthesis of aromatic amino acids and $p$-aminobenzoic acid share common enzymes of the shikimic acid pathway which are regulated via repression and/or feedback inhibition by the aromatic amino acids. The key enzyme of these first common biosynthetic steps is 3-deoxy-D-arabino-heptulosonic acid-7-phosphate (DAHP)-synthetase. Therefore the possibility has to be excluded that addition of aromatic amino acids inhibits the biosynthesis of $p$-aminobenzoic acid by feedback inhibition or repression of DAHP-synthetase thus preventing SMZ and TMP from competing with tetrahydrofolate biosynthesis. Though it is unlikely that aromatic amino acids completely repress biosynthesis of aromatic vitamins [9] the DAHP-synthetase-deficient mutant *E. coli* 83-1 was also included in this experiment. In order to enable normal growth of this mutant the medium was supplemented with phenylalanine, tyrosine and tryptophan (10 mM each) and shikimic acid ($10^{-6}$ M).

To study the effect of amino acids on the action of TMP or SMZ the 21 amino acids (100 µg/ml each) of the triose-, α-ketoglutarate-, pyruvate- and pentose
family, respectively, were added separately to the cultures but simultaneously with TMP or SMZ. Viable cell counts were determined after incubating the cultures for 18 h. The initial cell count was about $10^5$ cells/ml; in all control cultures containing amino acids, but no TMP or SMZ, the cell counts increased to approx. $10^6$ cells/ml. Cell counts of approx. $10^5$ cells/ml were determined in the cultures containing the amino acids of the triose-α-ketoglutarate and pyruvate family e.g. the drugs exhibited a bacteriostatic effect without being affected by those amino acids. Only the aromatic amino acids of the pentose family (phenylalanine, tryptophan, tyrosine and histidine) antagonized completely the inhibition of cell growth caused by TMP or SMZ in *E. coli* K-12 as well as *E. coli* 83-1. This indicates that regulation of the DAHP synthetase activity is not involved in the antagonistic effect of aromatic amino acids on the efficacy of TMP or SMZ.

4. Discussion

The data of this study indicate that the bactericidal or bacteriostatic effects of SM, TMP and SMZ, respectively are dependent on the carbon source and its concentration. Though aminoglycosides are bactericidal acting agents SM only inhibits cell growth bacteriostatically if glucose is used as carbon source. Similarly relatively high glucose concentrations completely antagonize the effect of SMZ or TMP. This result is in good agreement with earlier findings [10]. Thus not only vitamins, ions, salts and other well known factors influence the efficacy of different chemotherapeutics but also the carbon source strongly alters the effect of the drugs studied under these experimental conditions. Obviously in contrast to acetate glucose acts as a repressor substance. This assumption is strongly supported by the findings of Alper and Ames [6] which indicate that mutants in the cAMP control system in *Salmonella typhimurium* lacking either adenyl cyclase or cAMP receptor protein were partially or fully resistant to 22 antibiotics including SM when grown on glucose-containing medium. Substantial evidence has now accumulated in the literature to indicate that the phenomenon of “catabolite repression” can be explained by a glucose-mediated decrease of intracellular cAMP levels (reviewed in [11] and [12]). The function of cAMP in these regulatory processes is also well established; it was found to abolish catabolite repression. Cyclic AMP also overcomes catabolite repression of the antibacterial activity of SM, TMP or SMZ.

It seems likely that glucose acts as a catabolite-repressor of inducible enzymes involved in transport processes of SM, TMP and SMZ, respectively as Bryan et al. [4] and Höltje [5] have demonstrated that SM is transported via inducible enzymes into *E. coli*. Additional evidence has been obtained in this study that TMP and SMZ are also transported into *E. coli* since aromatic amino acids compete with their anti-
bacterial effect in the parent strain as well as in the DAHP-synthetase negative mutant so that regulatory processes can very likely be excluded. Therefore it might be assumed that these substances share common transport systems which are, according to the results obtained by Alper and Ames [6], under cAMP control. The antagonistic effect of histidine can perhaps be explained by the phenomenon of metabolic interlock [13,14].

Additional evidence that glucose interferes with the activity of drugs by repressing inducible transport enzymes has been obtained in studies of the effect of fosfomycin. Addition of glucose to the test medium strongly reduced the zones of inhibition around fosfomycin discs [15]. It has been shown by Kahane et al. [16] that glucose inhibits the 1-α-glycerophosphate-permease system by which fosfomycin is transported into bacteria.

As there are a lot of inducible enzymes directly or indirectly involved in the action of SM, TMP or SMZ they too might be subjected to catabolite repression. Therefore, at the present stage of investigations it is almost impossible to explain on a molecular basis the described phenomena and considerable caution must be employed in rationalizing such data in terms of a theory.

This study only indicates that growth inhibition of *E. coli* ATCC 10536 by SM, TMP and SMZ is catabolite-repressible under these experimental conditions. Therefore even under conditions of routinely used sensitivity testing, regulatory processes have also to be taken into account and one should be aware of the regulatory properties of repressors or inducers present in the medium affecting the antibacterial effect of different drugs. Already in 1971 Harwood and Smith [17] reported that cAMP regulates the levels of chloramphenicol acetyl transferase and streptomycin adenyl transferase produced by *E. coli* harbouring a drug resistance factor. Data obtained by de Crombrugge and coworkers [18] indicate that chloramphenicol acetyl transferase is controlled by both cAMP and guanosine 5’-diphosphate-3’-diphosphate (ppGpp). On the other hand antibiotics may influence control mechanisms, e.g. the mode of action of SM is mediated through the formation and activity of ppGpp [19,20].

It will be of interest to find out how antibiotics interfere with control systems and vice versa.

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References