Bacteriolytic effect of teicoplanin

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The glycopeptide antibiotic teicoplanin belongs to the same group as vancomycin and ristocetin and is a valuable tool for studying the autolytic system of sensitive Gram-positive bacteria. Teicoplanin, at a concentration of 1 µg ml⁻¹, caused rapid lysis of exponential phase cells of *Streptococcus faecalis*. *Bacillus* spp. were most sensitive to the antibiotic; effective lysis occurred at 0.1 µg teicoplanin ml⁻¹. The bacteriolytic effect depended on the antibiotic concentration, the growth phase and growth rate of the target organism. Antibiotic added to overnight cultures did not cause lysis. Mg²⁺ (50 mM) was unable to prevent lysis. Mutants with decreased autolytic activity were more resistant to teicoplanin and lysed more slowly than the wild-type. Growth of bacteria in slightly acidic medium protected the cells against the lytic effect of teicoplanin typically observed at pH 7 or 8. This pH-dependent antibiotic tolerance was demonstrated with both bacilli and streptococci. Bacterial lysis was prevented by the presence of Ac-L-Lys(Ac)-D-Ala-D-Ala and normal growth was observed when this peptide was added simultaneously with teicoplanin. Bacteria pretreated with teicoplanin, washed and transferred to fresh medium or buffers behaved as if the antibiotic was still present; in neutral or slightly alkaline conditions strong lysis occurred, whereas in acidic buffer only bacteriostasis was observed. In contrast to vancomycin, teicoplanin induced some lysis of bacteria in hypertonic media, presumably by affecting the integrity of the cell membrane.

Introduction

Teicoplanin is an antibiotic chemically related to the group of glycopeptides which also includes vancomycin and ristocetin. It was isolated from the fermentation broth of *Actinoplanes teichomyceticus* sp. nov. (Parenti *et al.*, 1978; Bardonne *et al.*, 1978) and has outstanding bactericidal activity against Gram-positive pathogenic bacteria (Pallanza *et al.*, 1983; Neu & Labthavikul, 1983; Fietta *et al.*, 1983). The antibiotic is a mixture of five components of very similar polarity and a sixth, more polar component, all with a similar *M_r* of about 1900 (Borghi *et al.*, 1984). The name teicoplanin describes the mixture of six components.

The structural unit common to all teicoplanin components consists of a linear heptapeptide aglycone in which two chlorinated β-hydroxytyrosine units and five substituted phenylglycine residues are interconnected in a tetracyclic structure, carrying D-mannose and N-acetyl-D-glucosamine moieties. An additional acyl glucosamine residue is present in some components and all contain one of five fatty acid residues. The main feature distinguishing teicoplanin from the other glycopeptide antibiotics is the presence of glucosamine and of aliphatic acid residues (Barna *et al.*, 1984).

The mode of action of teicoplanin was investigated by Somma *et al.* (1984), who presented data showing that: (a) teicoplanin inhibits peptidoglycan synthesis in intact cells and in 'cell-free' systems; (b) inhibition by teicoplanin is reversed by addition of cell wall material; (c) the antibiotic binds to walls and whole cells, rendering them resistant to the action of lysozyme; and (d) a complex is formed between teicoplanin and the peptide Ac-L-Lys(Ac)-D-Ala-D-Ala, a synthetic analogue of the terminal peptide moiety of the pentapeptide peptidoglycan precursor. Greenwood *et al.* (1987) reported that teicoplanin induced lysis of growing *Staphylococcus aureus* and *Streptococcus faecalis* and caused morphological changes in these bacteria.

Teicoplanin should be a valuable biochemical tool in the study of peptidoglycan metabolism and its correlation with the autolytic process. The aim of the present study was to investigate some factors that influence teicoplanin-induced lysis in sensitive bacteria such as *Bacillus* and *Streptococcus*.

Methods

Bacterial strains. *Bacillus subtilis* 168 and the autolysis-defective mutant *B. subtilis* FJ6 were from the Bacillus Genetic Stock Center

Fig. 1. Bacteriolytic effect of teicoplanin on exponential phase bacterial cells. (a) *Strep. faecalis* CCM 1875; (b) *B. pumilus* CCM 1697. To a culture (10 ml) of early exponential phase cells (approx. 2×10^8 ml⁻¹) growing in MBD LYC medium, teicoplanin (100 µl of an aqueous solution of appropriate concentration) was added at time zero. ●, Untreated cells; ○, cells treated with teicoplanin at the indicated concentration (µg ml⁻¹).

(Columbus, Ohio, USA). *B. pumilus* CCM 1697 and *Strep. faecalis* CCM 1875 were obtained from the Czechoslovak Collection of Micro-organisms (Bratislava, Czechoslovakia). *Micrococcus luteus* NCTC 2656 was from the National Institute for Medical Research (Mill Hill, London, UK). *Strep. faecalis* 8462 Vanco^R was supplied from the micro-organism collection of the Medical Academy of Gdańsk (Poland). *Strep. faecium* ATCC 9790 and the autolysis-defective mutant LYT-14 were kindly provided by G. D. Shockman (Temple University School of Medicine, Pennsylvania, USA).

Growth conditions. Bacteria were grown in Davis minimal broth (Difco) supplemented with 0.4% glucose, 0.2% Lab-Lemco (Oxoid), 0.2% yeast extract (Difco) and 0.2% Casamino acids (Difco). This medium was called MBD LYC. In 'poor' medium, for slow growth of bacteria, Lab-Lemco, yeast extract and Casamino acids were reduced to 0.02% and glucose to 0.2%. Hypertonic MBD LYC medium was supplemented with 500 mM-sucrose and 50 mM-Mg²⁺ (MgCl₂). For most experiments the pH of MBD LYC medium was between 7.0 and 7.1. For experiments of the pH-dependence of lysis, the pH of the MBD LYC medium was adjusted with 100 mM-buffers to the following values: 5.3 (Tris/maleate); 6.2, 6.6 and 7.0 (potassium phosphate); and 8.0 (Tris/HCl).

Overnight cultures of bacteria in MBD LYC medium were diluted to approximately 2×10^7 cells ml⁻¹ with fresh medium and incubated at 37 °C with vigorous agitation. Growth was followed by measuring the optical density of the culture at 660 nm with a Zeiss spectrophotometer. At the indicated times small volumes (100 µl) of an aqueous solution of teicoplanin, or other antibiotics, were added. Chloramphenicol was added as an ethanol solution (100 µl) of appropriate concentration.

Pretreatment with teicoplanin. Exponential phase bacteria (40 ml) were treated for 15 min with teicoplanin (2 µg ml⁻¹), centrifuged and washed. The cell suspension was then divided into four aliquots. Cells from each aliquot were harvested, washed and suspended in one of the following media: fresh MBD LYC medium containing *N*-Ac-L-Lys(Ac)-D-Ala-D-Ala in 250-fold molar excess relative to teicoplanin; fresh medium without peptide; 100 mM-BisTris/HCl at pH 6.0; and 100 mM BisTris/HCl at pH 7.5.

Chemicals. Teicoplanin was a gift from Gruppo Lepetit (Milano, Italy). Vancomycin, methicillin and chloramphenicol were from Sigma. The following peptides, related to the terminal moiety of peptidoglycan precursors, were synthesized in our laboratory: Ac-L-

Lys(Ac)-D-Ala-D-Ala; Ac-L-Lys(Ac)-D-Ala-L-Ala; Ac-L-Lys(Ac)-L-Ala-D-Ala; Ac-L-Lys(Ac)-D-Ala-D-Leu and Ac-L-Lys(Ac)-D-Ala-Gly.

Results and Discussion

Teicoplanin-induced lysis in exponential phase bacteria

It has often been observed that the addition of antibiotics inhibiting cell wall biosynthesis to exponential phase bacterial cultures results in a rapid and extensive lysis (Boman & Erikson, 1963; Kitano & Tomasz, 1979; Chmara & Borowski, 1986; van Heijenoort *et al.*, 1987).

The addition of teicoplanin to cultures of *Strep. faecalis* CCM 1875 in the early exponential growth phase caused complete lysis. Lysis was not instantaneous and did not begin until at least 15 min after antibiotic addition (Fig. 1a). Effective lysis was observed at antibiotic concentrations ranging from 1 to 10 µg ml⁻¹. Teicoplanin at a concentration of 0.1 µg ml⁻¹ caused lysis for 45 min followed by regrowth. Teicoplanin at 10 µg ml⁻¹ caused total lysis of *Strep. faecalis* with no regrowth, even after 24 h of incubation.

The clinical strain of *Strep. faecalis*, 8462 Vanco^R, did not lyse in the presence of teicoplanin even at a concentration of 250 µg ml⁻¹. Only minor growth inhibition was observed (data not shown).

B. pumilus CCM 1697 was extremely sensitive to teicoplanin: it lysed rapidly at teicoplanin concentrations of 0.1 to 1 µg ml⁻¹ (Fig. 1b). A concentration of 0.05 µg ml⁻¹ caused only partial inhibition of growth followed by regrowth after 75 min. After 24 h incubation in the presence of 0.1 µg teicoplanin ml⁻¹, *B. pumilus*

showed some regrowth, but not exceeding 25% of the growth of a control culture without the antibiotic. Teicoplanin at $2 \mu\text{g ml}^{-1}$ caused total lysis of *B. pumilus* with no regrowth, even after 48 h of incubation.

Effect of growth phase and growth rate on lysis

Teicoplanin-induced lysis was dependent on the growth phase of *B. pumilus* at the time of addition of the antibiotic. Up to 15 min after the transfer of overnight culture to fresh medium, addition of $2 \mu\text{g teicoplanin ml}^{-1}$ caused only growth inhibition (data not shown). This phenomenon, i.e. lack of lysis of non-growing cells, seems to be a feature common to all bacteria and was described by Tuomanen *et al.* (1986) as phenotypic tolerance.

During the early and late exponential growth phase, teicoplanin, as expected, induced rapid lysis. However, addition of the antibiotic towards the end of exponential growth resulted in an increased delay (45 min) before lysis started. When the growth rate was clearly declining, addition of the antibiotic caused only partial inhibition of growth instead of lysis (data not shown).

In their experiments on the lysis of *Escherichia coli* by ampicillin, Boman & Erikson (1963) showed that the time of lysis was strongly dependent on the growth rate. As shown in Fig. 2, teicoplanin induced lysis after a longer lag phase with slowly growing *Strep. faecalis* CCM 1875 and lysis was not very extensive.

Our results confirm the finding of Tuomanen *et al.* (1986) that the rate of bacterial lysis following exposure to cell-wall-inhibitory antibiotics appears to be a direct function of bacterial growth prior to the addition of the antibiotic.

Effect of teicoplanin on growth in hypertonic medium.

It is of interest that in hypertonic media, teicoplanin at concentrations of $1 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, respectively, induced lysis of *B. pumilus* CCM 1697 and *Strep. faecalis* CCM 1875 (Fig. 3). In contrast only a bacteriostatic effect was observed with vancomycin and methicillin. This observation suggests that vancomycin and teicoplanin, although closely related, differ in their mode of action. The mechanism of teicoplanin-induced lysis in hypertonic media is unknown. It is possible that teicoplanin, as well as inhibiting peptidoglycan synthesis, exerts some effect on the cytoplasmic membrane.

Reynolds (1971), studying the effect of vancomycin at concentrations of 10 and $50 \mu\text{g ml}^{-1}$ on growth of reconditioned *B. megaterium* KM protoplasts, and Rosenthal *et al.* (1975), carrying out similar experiments with *Strep. faecalis* ATCC 9790 protoplasts, did not observe any lytic effects.

Fig. 2. Action of teicoplanin on slowly growing bacteria. To a culture (10 ml) of early exponential phase cells (approx. $2 \times 10^8 \text{ ml}^{-1}$) of *Strep. faecalis* CCM 1875 growing in MBD LYC 'poor' medium, teicoplanin (100 μl of an aqueous solution of appropriate concentration) was added at time zero. ○, Untreated cells; □, $0.1 \mu\text{g teicoplanin ml}^{-1}$; ■, $1.0 \mu\text{g teicoplanin ml}^{-1}$; △, $10.0 \mu\text{g teicoplanin ml}^{-1}$.

Influence of Mg^{2+} on lysis induced by teicoplanin

Best & Durham (1964) reported that inhibition of peptidoglycan synthesis and growth in *B. subtilis* W23 by a low concentration of vancomycin was depressed by Mg^{2+} (1.66 mM).

We demonstrated that Mg^{2+} , even at a concentration of 50 mM, did not inhibit teicoplanin-induced lysis of *B. pumilus* CCM 1697 and *Strep. faecalis* CCM 1875 when the antibiotic was used at concentrations of $2 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, respectively. The only detectable effect was a prolongation by about 15 min, of the lag phase before lysis. The slope of the lysis curve was identical with that obtained in experiments with teicoplanin in the absence of Mg^{2+} (data not shown).

Effect of teicoplanin on growth of an autolysis-defective mutant

Bacteriolysis is not a direct effect of the primary action of cell-wall antibiotics which inhibit the biosynthesis of peptidoglycan at various stages. It has been shown that a secondary effect is a deregulation of bacterial autolysins, enzymes which hydrolyse the existing peptidoglycan, thereby leading to bacterial lysis (Rogers & Forsberg,

Fig. 3. Effect of teicoplanin on the growth of (a) *B. pumilus* CCM 1697 and (b) *Strep. faecalis* CCM 1875 in hypertonic medium. To a culture (10 ml) of early exponential phase cells (2×10^8 ml⁻¹) growing in MBD LYC medium, supplemented with 500 mM-sucrose and 50 mM-Mg²⁺, teicoplanin, vancomycin or methicillin was added at time zero. (a) ○, Untreated cells; ●, 0.2 µg teicoplanin ml⁻¹; ■, 1 µg teicoplanin ml⁻¹; □, 2 µg vancomycin ml⁻¹; △, 2 µg methicillin ml⁻¹. (b) ○, Untreated cells; ●, 1 µg teicoplanin ml⁻¹; ■, 10 µg teicoplanin ml⁻¹; □, 10 µg vancomycin ml⁻¹; △, 10 µg methicillin ml⁻¹.

1971; Best *et al.*, 1974; Tomasz & Höltje, 1977). Subsequently it was shown (Rogers & Forsberg, 1971; Ayusawa *et al.*, 1975; Fein & Rogers, 1976; Rogers *et al.*, 1983) that strains of various micro-organisms deprived of autolysins are lysed less rapidly by antibiotics that inhibit cell wall synthesis than are the parent organisms. The term 'tolerant' was used to describe such strains.

In our studies we used *B. subtilis* 168 and the autolysis defective mutant FJ6. Compared to the corresponding wild-type strain, the autolysis defective mutant FJ6 exhibited much slower rates of lysis in the presence of 1 or 10 µg teicoplanin ml⁻¹ (data not shown). The lysis was not fully inhibited because the mutant is not completely deprived of autolysins but retains some activity (Rogers *et al.*, 1983). The main lytic enzyme of *B. subtilis* 168 is an *N*-acetylmuramoyl-L-alanine amidase (Herbold & Glaser, 1975). Similar results were obtained when the teicoplanin-induced lysis of *Strep. faecium* ATCC 9790 and its autolysis-defective mutant LYT 14 were compared. The mutant showed much slower rates of lysis in the presence of teicoplanin (1 and 10 µg ml⁻¹).

Effect of chloramphenicol on lysis

Further evidence supporting the essential role of autolysins in teicoplanin-induced bacteriolysis was obtained by studying the effect of protein synthesis inhibition by chloramphenicol.

As can be seen from Fig. 4, the addition of 1 µg teicoplanin ml⁻¹ caused rapid lysis of *B. pumilus* CCM 1697 cells. Chloramphenicol at a concentration of 100 µg

Fig. 4. Effect of chloramphenicol on teicoplanin-induced lysis of *B. pumilus* CCM 1697. To a culture (10 ml) of early exponential phase cells growing in MBD LYC medium, teicoplanin (1 µg ml⁻¹) and chloramphenicol (100 µg ml⁻¹) were added at different time points. ○, Untreated cells; ■, teicoplanin added at 0 min; □, chloramphenicol added at 0 min; △, chloramphenicol added with teicoplanin at 0 min; ▲, ●, chloramphenicol added at 0 min with teicoplanin at 15 min (▲) and 30 min (●).

ml⁻¹ inhibited growth, although not completely. When chloramphenicol was added together with teicoplanin the bacteria lysed, but not so extensively as with

teicoplanin alone. A complete inhibition of lysis was observed when chloramphenicol was added 30 min prior to the addition of teicoplanin. The effect of lysis inhibition lasted for at least 4 h. Quite similar effects were described by Rogers & Forsberg (1971), who recorded complete inhibition of lysis of *B. licheniformis* by vancomycin ($10 \mu\text{g ml}^{-1}$) when chloramphenicol ($50 \mu\text{g ml}^{-1}$) was added at least 60 min before the addition of vancomycin.

The mechanism of chloramphenicol protection is not yet understood. As reported by Tomasz & Waks (1975) several experiments strongly suggest that this phenomenon is not caused by a deficiency in the number of hydrolase molecules at the cell surface, but rather by a disturbance of the metabolic regulation of the activity of the autolysins.

Induction of lysis of bacterial cells growing in media at different pH

The response of *B. pumilus* CCM 1697 and *Strep. faecalis* CCM 1875 to teicoplanin was dependent upon the pH of the growth medium. At pH 7.0 or 8.0 the bacteria lysed rapidly after a short lag phase, but only slow lysis could be observed for *B. pumilus* at pH 6.2 and no lysis was detected at pH 5.3. In the case of *Strep. faecalis*, rapid lysis was observed at pH 7.0 or 8.0 but no lysis was detected at pH 6.2. Additional bacterial strains tested (*Staphylococcus aureus*, *Micrococcus luteus*, *Corynebacterium* sp.) exhibited a similar response to the pH of the medium. These results (data not shown) indicate that appropriate modulation of the pH of the growth medium produces the typical features of antibiotic tolerance. The cellular response changes from lysis to bacteriostasis. It should be noted that the inhibition of lysis was not associated with slower cell growth in media of low pH. All bacterial strains examined grew equally well at both pH 6.2 and 7.0. Slower growth was observed only in the medium of pH 5.3; however, the growth was only about 30% slower as compared to growth at pH 7.0. The tolerance of cells for teicoplanin in media of low pH was fully reversible. After 90 min exposure to $1.0 \mu\text{g ml}^{-1}$ *B. pumilus* cells growing at pH 5.3 started to lyse rapidly after a lag phase of 30 min when washed and resuspended in medium of pH 7.0. This was also found with *Strep. faecalis* pretreated with $10 \mu\text{g ml}^{-1}$ teicoplanin at pH 6.2.

This phenomenon of bacterial tolerance for teicoplanin at low pH corroborates the results of Goodell *et al.* (1976) and Lopez *et al.* (1976), who found that cell-wall antibiotics, irrespective of their site(s) of action in the biosynthetic pathway of peptidoglycan, exhibit suppressed lytic activity on bacteria growing in media of low pH.

Fig. 5. Reversal by the peptide Ac-L-Lys(Ac)-D-Ala-D-Ala of teicoplanin-induced lysis of *B. pumilus* CCM 1697. To a culture (10 ml) of exponential phase cells growing in MBD LYC medium, teicoplanin ($2 \mu\text{g ml}^{-1}$) was added at 0 min, followed by the addition of a 250 molar excess of the peptide at different time points (arrows): ■, 15 min; △, 30 min; ▲, 60 min; ▽, 120 min; ○, untreated cells; ●, teicoplanin without peptide; □, teicoplanin and peptide added at 0 min.

Inhibition of teicoplanin-induced lysis by the peptide Ac-L-Lys(Ac)-D-Ala-D-Ala

Somma *et al.* (1984) reported that teicoplanin, like vancomycin (Best & Durham, 1965; Nieto & Perkins, 1971), binds to cell walls and forms a complex with peptides related to bacterial peptidoglycan, such as Ac-L-Lys(Ac)-D-Ala-D-Ala. This peptide when used at a 250-fold molar excess with respect to teicoplanin ($2 \mu\text{g ml}^{-1}$) and when added simultaneously with the antibiotic to cultures of *B. pumilus* CCM 1687 (Fig. 5) not only protected the cells from lysis but allowed bacterial growth indistinguishable from the control. Broadly similar results were obtained with *M. luteus* (data not shown).

The peptides Ac-L-Lys(Ac)-D-Ala-L-Ala or Ac-L-Lys(Ac)-L-Ala-D-Ala in a 250-fold molar excess relative antibiotic did not exert any inhibitory influence on teicoplanin-induced lysis of *B. pumilus* and *Strep. faecalis* (data not shown). On the other hand the peptides Ac-L-Lys(Ac)-D-Ala-Gly and Ac-L-Lys(Ac)-D-Ala-D-Leu, tested under the same conditions, protected the cells from lysis but allowed cell growth to reach only 30–40% of a control culture.

Fig. 6. Effect of a washing step on the lysis of *B. pumilus* CCM 1697 cells pretreated with teicoplanin. To a culture (40 ml) of exponential phase cells growing in MBD LYC medium, teicoplanin ($2 \mu\text{g ml}^{-1}$) was added at the time indicated by the arrow. After 15 min, the cells were harvested, rapidly washed and resuspended in 10 ml of: ■, fresh medium; ●, fresh medium (pH 7.0) containing Ac-L-Lys(Ac)-D-Ala-D-Ala in a 250-fold molar excess; △, ▲, 100 mM-Bistris/HCl buffer, adjusted to the indicated pH values.

Nieto *et al.* (1972) have similarly observed that the inhibition of growth of *B. megaterium* KM, *Staph. aureus* and *M. lysodeicticus* NCTC 2665 by iodovancomycin could be reversed by diacetyl- α,γ -diaminobutyryl-D-alanyl-D-alanine added in a 38-fold molar excess relative to the antibiotic.

Effect of teicoplanin pretreatment of bacterial cells on lysis

A consequence of the binding of teicoplanin to the bacterial cell wall (Somma *et al.*, 1984) is that simple washing of cells pretreated with the antibiotic is not sufficient to prevent lysis. *B. pumilus* CCM 1697 pretreated with $2 \mu\text{g}$ teicoplanin ml^{-1} for 15 min, washed and transferred to fresh medium or buffers behaved as if the antibiotic was still present (Fig. 6). Cells suspended in Bistris/HCl buffer of pH 7.5 lysed rapidly, but when the buffer was slightly acidic, no extensive lysis was observed. For *B. pumilus* a pH value of 6.0 was enough to prevent lysis. On the other hand, cells resuspended in fresh medium at pH 7.0 lysed to the same degree as if the antibiotic was still present. Cells transferred to fresh

medium containing Ac-L-Lys(Ac)-D-Ala-D-Ala in a 250-fold molar excess relative to the antibiotic did not undergo lysis and, moreover, immediately started to grow. Untreated cells of *B. pumilus*, when suspended in buffer at pH 7.5, exhibited only slight lysis, not exceeding 15–20% of the initial optical density. Peptides such as Ac-L-Lys(Ac)-L-Ala-D-Ala and Ac-L-Lys(Ac)-D-Ala-L-Ala, when added to teicoplanin-pretreated and washed cells, did not protect the cells from lysis (data not shown) indicating that the D-D-sequence is indispensable for a reversal of teicoplanin-induced lysis.

The reversal of teicoplanin-induced bacteriolysis by Ac-L-Lys(Ac)-D-Ala-D-Ala occurred only with growing cells. The peptide, at a 250-fold molar excess, did not confer protection from lysis in buffer (pH 7.5) (data not shown).

Specific effects of teicoplanin

The results of our studies on the response of bacterial cells to the action of teicoplanin largely coincide with the generally known effects of cell-wall-inhibitory antibiotics including phenomena such as the dependence of the lysis rate on the growth rate and lack of lysis of non-growing cells.

We have made, in addition, the more unusual observation that teicoplanin, unlike vancomycin and methicillin, induces some lysis in hypertonic medium. This suggests that the antibiotic, as well as inhibiting cell wall biosynthesis, also exhibits some membrane-damaging activity. The molecule of the antibiotic contains acyl residues of C_9 – C_{10} fatty acids (Borghi *et al.*, 1984). This structural feature, making teicoplanin more lipophilic than vancomycin, may favour penetration into the bacterial cytoplasm membrane, thus causing membrane-damaging effects.

Another interesting phenomenon is the rather high tolerance of *Strep. faecalis* to teicoplanin in slightly acidic media. A similar effect of tolerance to penicillin in acidic medium was earlier described by Goodell *et al.* (1976). Correspondent with this phenomenon is the tolerance of autolysis-defective strains to teicoplanin. The tolerance of autolysis-defective bacteria to penicillin has been reported many times (Rogers & Forsberg, 1971; Rogers *et al.*, 1983). The clinical importance of these aspects of penicillin action has been underlined by a recent report describing the isolation of penicillin-tolerant autolysis-defective pathogenic bacteria from patients with recurring infections (Horne & Tomasz, 1977). The teicoplanin tolerance of bacteria in a slightly acidic environment may have comparable clinical implications.

The presence of cell-bound teicoplanin is indispensable for its lytic action in growing cells, as shown by the experiments with the specific peptide Ac-L-Lys(Ac)-D-

Ala-D-Ala. In buffer alone (with permissive pH) the peptide did not protect the cells pretreated with teicoplanin from progressive lysis. The elucidation of this phenomenon requires further studies.

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