Penicillins Activate Autolysins Extracted from Both
Escherichia coli and Klebsiella pneumoniae Envelopes

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Ampicillin at a 50-μg/ml concentration effects a doubling of the autolysis rate of cell envelopes isolated from Klebsiella pneumoniae Mir A12. This antibiotic increases also by two- to threefold the depolymerizing activity of both K. pneumoniae and Escherichia coli crude extracts on a labeled peptidoglycan-lipoprotein complex. Several other penicillins also activate autolysins.

Lysis of baterial cells treated with penicillins has been considered by many authors as a consequence of peptidoglycan cross-linking inhibition (1, 3). However, several in vitro studies, using membrane preparations and isolated enzymes, have shown a considerable difference between the concentrations that inhibit cross-linking formation and those that kill the cell (1, 7).

The alternative possibility, that penicillins directly activate autolysis by destabilizing the endogenous complex of an autolysin inhibitor (lipoteicic acid) and the autolytic enzymes, has been emphasized quite recently (2, 11). However, such a suggestion has so far been based on, and supported only by, studies of gram-positive organisms. Moreover, direct activation by penicillins of extracted autolysins has never been described as yet.

We will show, in this paper, that several different penicillins raise, by two- to threefold, both the rate of autolysis of isolated envelopes and the peptidoglycan hydrolytic activity of Klebsiella pneumoniae Mir A12 (9, 10) and Escherichia coli B/r envelope extracts.

When K. pneumoniae envelopes were suspended again in an adequate buffer at 37°C, a gradual decrease in the optical density (OD) of the suspension was observed. The OD decrease was rapid during the first few minutes and declined afterwards at a reduced rate for at least 20 min. When a dose of ampicillin (Sigma) corresponding to the minimal bactericidal concentration for this strain was added to the envelope suspension, the rate of OD decrease was amplified. Such a rise was dramatic during the initial minutes of incubation. After 20 min, the OD decrease of the sample containing ampicillin was double that of the control sample (Fig. 1).

To prove that the faster OD decrease observed in the presence of the antibiotic was dependent on autolysin activation, envelopes of K. pneumoniae and E. coli were extracted, and the depolymerizing activity of such extracts on a radioactively labeled peptidoglycan-lipoprotein complex was evaluated, both in the presence and in the absence of ampicillin.

As shown in Fig. 2, radioactive substrate degradation (measured as liberation of trichloroacetic acid-soluble radioactive fragments) was intensely stimulated by the antibiotic.

The effect of different concentrations of ampicillin has been further tested, and results are shown in Fig. 3.

To determine if stimulation of autolytic activity was a specific property of ampicillin, other β-lactam antibiotics, such as penicillin G (Squibb), 6-aminopenicillanic acid (Sigma Chemical Co.), cephaloridine (Glaxo Laboratories Ltd.), and mecillinam (FL 1060; Leo Pharmaceuticals Co.) were tested. All the above antibiotics stimulated the autolysins extracted from K. pneumoniae envelopes, with ampicillin and mecillinam the most and the least active, respectively.

This report presents the first description of the stimulating effect of penicillins on isolated autolytic enzymes and may be important in explaining the killing mechanism of these antibiotics. In fact, the data presented here not only give a stronger and more direct indication that penicillin compounds activate autolysins, but also demonstrate that gram-negative bacteria are prone to such an effect. Therefore, among the effects of penicillin varieties on
sensitive bacteria, stimulation of lytic activity appears to be the one common to a larger variety of species.

It is important to note that mecillinam, which was previously described as uninhibitory for either D-alanine carboxypeptidase or transpeptidase and ineffective in causing detectable alterations in peptidoglycan chemical composition (6, 8), is, on the contrary, capable of activating autolysins.

It is likely that complete elucidation of the mechanism by which autolysin stimulation occurs may lead to a clearer explanation of this killing mechanism by penicillin compounds.

The results presented in this paper appear to contrast with others, who have shown that partially purified autolytic enzymes are not stimulated, but inhibited, by penicillin G (5). However, conditions in our experiments were rather different as compared to those of this previous work. It may be possible that the amount of antibiotic that we used per autolysin content was low. In fact, at concentrations exceeding 500 µg/ml we have obtained inhibition of autolytic activity.

While this manuscript was in preparation, Wolf-Waltz and Normark (12) reported that

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**Fig. 1.** Time course of autolysis of K. pneumoniae Mir AI2 envelopes in absence and in presence of ampicillin. Envelopes from cells exponentially growing in PLGS medium (containing per liter: peptone, 10 g; lactose, 1 g; glucose, 1 g; sucrose, 0.5 g; Na₂HPO₄·2H₂O, 6.8 g; NaH₂PO₄, 2.1 g) were prepared essentially as described by Hartmann et al. (4), with the only exception that cells were broken by sonic treatment with a Hoefer sonicator. Cell envelopes were then suspended in 10 mM tris(hydroxymethyl)aminomethane-maleate buffer (pH 6.8) and in the same buffer containing 50 µg of ampicillin per ml (■). Incubation was carried out at 37°C. Turbidity was monitored with a Beckman DB spectrophotometer.

**Fig. 2.** Degradation of radioactive peptidoglycan-lipoprotein complex by K. pneumoniae (a) and E. coli B/r (b) envelope extracts in presence and in absence of ampicillin. To solubilize autolytic activity, the envelopes were suspended in 10 mM tris(hydroxymethyl)aminomethane-maleate buffer (pH 6.8) containing 5% Triton X-100 and 0.5 M NaCl at a protein concentration of 20 mg/ml and kept for 20 min at room temperature. After the insolubilized material was removed, 1 ml of envelope extract was added to 10 ml of radioactive peptidoglycan-lipoprotein complex (0.25 mg/ml; 2.5 × 10⁶ cpm/ml) prepared as described by Hartmann et al. Ampicillin (50 µg/ml) was added to one half of the suspension at zero time (●); the other part served as a control (▲). Incubation was performed at 37°C. At intervals, 0.5-ml samples were taken to determine the solubilized radioactivity as described by Hartmann et al. (4).
the lytic activity of the crude extract of a chain-forming mutant increased significantly if the cells were grown in the presence of ampicillin before preparing the extracts. This appears as another confirmation that penicillins activate autolysins.

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LITERATURE CITED


