STAPHYLOCOCCAL PENICILLINASE

II. NON-PENICILLIN-LIKE CYCLIC PEPTIDES AS INDUCERS OF, AND SUBSTRATES FOR, THE ENZYME

Arthur K. Saz and Dolores L. Lowery

Section on Medical and Physiological Bacteriology, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland.

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Under natural ecological conditions, the possession of an enzyme such as penicillinase which hydrolyzes the β-lactam ring of various penicillins to the antibiotically inactive penicilloic acids would not seem to afford competitive advantage or to be of survival value to those organisms exhibiting this activity since, despite numerous attempts, efforts to demonstrate the production of penicillin *in situ* and *au naturel* have been uniformly unsuccessful. Yet, in induced strains of bacteria and in particular in some strains of *B. cereus*, penicillinase activity can account, on occasion, for as much as 2% or more of the total protein produced by the cell.

These observations, as well as the fact that various special peptides can inhibit the activity of staphylococcal penicillinase (Saz, Lowery, Jackson, 1961), are in accord with the hypothesis that penicillin serves as a fortuitous inducer of, and substrate for, a relatively non-specific enzyme.

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[2]HGS = cyc L-valyl L-arginyl L-leucyl L-phenylalanyl L-prolyl L-valyl L-arginyl L-leucyl D-phenylalanyl L-prolyl.

CHP = cyc valyl phenylalanyl lysyl valyl phenylalanyl lysyl (configuration of amino acids unknown)

This communication will report on the activity of two synthetic cyclic peptides,[1] Homogramicidin S (HGS),[2] and a cyclic hexapeptide (CHP),[2] which are structurally unrelated to penicillin and which act as both inducers of, and substrates for, penicillinase derived from Staphylococcus aureus S 1, a strain which produces the enzyme both constitutively and inducibly.

Staphylococcal penicillinase activity was measured in the Metrohm pH stat and is expressed as microliters of 0.006 N NaOH necessary to neutralize the carboxyl group of the penicilloic acid formed as a result of hydrolysis of the β-lactam ring of penicillin by penicillinase. S. aureus S 1 was grown for 5 hours with shaking at 37 C in 10 ml of nutrient broth, dispensed in 50 ml Erlenmeyer flasks. Inducer was added and shaking was continued for an additional two hours. The organisms were then centrifuged in the cold at 27,000 G, the pellet washed once with cold distilled water and resuspended in 1.0 ml of distiled water. 0.2 ml of 0.024 M PO_4 buffer, pH 7.0 was added and the reaction started by the addition of 0.2 ml of the sodium salt of benzyl penicillin (Penicillin G) at 6 mg/ml to give a final concentration of 0.86 mg/ml. The effect of the peptides on growth was determined by inoculating nutrient broth containing the peptides with 0.05 ml of 18 hour nutrient broth culture. Stock cultures of penicillin-sensitive S. aureus S 10, the penicillinase-producing penicillin-resistant S. aureus S 1 and the moderately resistant Escherichia coli E 26 were carried in nutrient broth.

Results: Table I indicates that the effects of HGS and CHP on the growth of several penicillin-sensitive and -resistant bacteria were similar to those which were obtained with penicillin. The penicillin-resistant S. aureus S 1 and E. coli E 26 were resistant to concentrations of HGS and CHP of at least 100 μg/ml of all three compounds.

These observations suggested that HGS and CHP were susceptible to hydrolysis (and consequent inactivation) in a manner analogous to the hydrolysis of penicillin mediated by penicillinase. Accordingly, the possibility of hydrolysis by whole cells of S. aureus S 1 was determined mano-

Table I

Effect of Cyclic Peptides on Growth

(See Methods for details)

Peptide	S. aureus S_1	S. aureus S_{10}	E. coli E_{26}
Penicillin			
100 μg/ml	Growth	No growth	Growth (Partial)
10 μg/ml	Growth	No growth	Growth
Homogramicidin S			
100 μg/ml	Growth	No growth	Growth (Partial)
10 μg/ml	Growth	No growth	Growth
C-Hexapeptide			
100 μg/ml	Growth	No growth	Growth
10 μg/ml	Growth	No growth	Growth

metrically by the technique of Henry & Housewright (1947). HGS appeared to be hydrolyzed with approximately 2 moles of carboxyl group formed per mole of HS utilized. This result is consistent with the symmetrical structure of the cyclic-decapeptide. Similar results were obtained using the colorimetric technique previously employed (Saz, Lowery, Jackson, 1961). Cell-free preparations derived from S. aureus S 1 also seemingly hydrolyzed both HGS and CHP. The possibility remained, however, that the enzyme hydrolyzing the cyclic decapeptide was not penicillinase, but rather an accompanying cyclic peptidase. Attempts to purify the particulate staphylococcal penicillinase have been uniformly unsuccessful, six to twelve fold purification being the maximum attained (Saz, Lowery, Jackson, 1961). Accordingly, it was decided to test the ability of the cyclic peptides to induce the formation of staphylococcal penicillinase. The ability of a compound to cause induction is highly specific. Induction of penicillinase activity in inducible strains of organisms has been limited to the various

penicillins and to the closely related Cephalosporin C and 6-aminopenicillanic acid. Fig. 1 shows that both CHP and HGS cause induction of penicillinase activity. The Δ in the figure represents the microliters of 0.006 N NaOH utilized per minute above that of the control. Indeed, HGS is actually a more potent inducer than Penicillin G. These data offer further indication that HGS and CHP are hydrolyzed by penicillinase as is Penicillin G since all these compounds are inferior to Staphcillin (dimethoxyphenyl) as inducers. The fact that Staphcillin is a more potent inducer than Penicillin G in staphylococci has been ascribed to the observation that it is considerably more resistant to hydrolysis by penicillinase than Penicillin G. Presumably, in staphylococci the continuous presence of unaltered inducer is essential for maximal induction. A later communication will show that both cyclic peptides are approximately as active inducers as the penicillinase-resistant penicillin Bacillus cereus where the commitment to induction as a result of the presence of an inducer is extremely rapid.

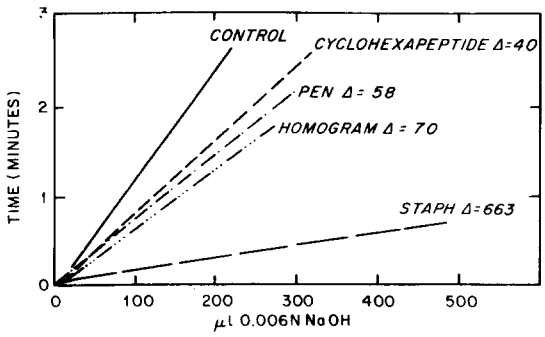

Figure 1

Penicillinase Induction in S. aureus S 1

(See text for details)

Pen = Na salt of Penicillin G: Homogram = HGS;

Staph = Staphcillin (dimethoxyphenyl penicillin).

Final concentration of inducers: Pen, CHP, HGS all at 10 μg/ml;

Staph at 1.0 μg/ml

It would thus appear that "penicillinase is not a specific enzyme either in its inducibility or activity. There is no apparent structural relationship between the various penicillins on the one hand and the cyclic peptides HGS and CHP on the other, yet both can induce penicillinase and presumably both can act as substrates for the activity of the induced enzyme.

References

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