NON-PENICILLIN-LIKE CYCLIC PEPTIDES AS INDUCERS OF AND SUBSTRATES FOR BACILLUS CEREUS 569 PENICILLINASE

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The metabolic function and significance of the enzyme penicillinase, which hydrolyzes the β-lactam ring of various sensitive penicillins to the antibiotically inactive penicilloic acids, is not clear. Possession of such an enzyme seemingly does not afford competitive advantage to the producing cells since despite numerous efforts, the production of penicillin under natural ecological conditions has not been demonstrated. Yet, in certain strains of inducible Bacillus cereus, penicillinase accounts for as much as 2% or more of the total protein produced by the cell. Accordingly, the hypothesis has been entertained that penicillin serves as a fortuitous inducer of, and substrate for, a relatively non-specific enzyme. The observation that certain specialized peptides inhibited the activity of staphylococcal penicillinase, was in accord with this hypothesis (Saz et al, 1961). Recently it has been reported that two synthetic cyclic peptides were capable of acting both as inducers of andsubstrates for staphylococcal penicillinase (Saz and Lowery, 1964).

In this communication, we shall report on the effects of these compounds as well as other naturally occurring cyclic

peptides[1] on induction of penicillinase in B. cereus 569. In addition, preliminary data indicating hydrolysis of certain of these peptides by purified penicillinase extracted from B. cereus 569, will be presented.

Induction was measured by inoculating 0.05 ml of a spore suspension of B. cereus 569 into 60 ml of Difco Nutrient Broth. The cultures were shaken at 35 C for 5 hours, centrifuged and the pellet was then resuspended in an amount of casein hydrolysate-citrate medium (Kogut et al 1956) sufficient to yield a cell density of 200-300 Klett units. 0.8 ml of the cells and 0.1 ml of the appropriate concentration of inducer were mixed together and the volume was made up to 1.0 ml by addition of distilled water. The mixture was then shaken at 35 C for 1-2 hours. After this period, penicillinase was measured by the iodometric method described by Citri (1958). A unit of penicillinase is defined as that amount which hydrolyzes 1.0 μM of the Na salt of benzyl

Footnote 1. We wish to thank Dr. M. Winitz, Dept. of Biochemistry, City of Hope Hospital, Duarte, California for gifts of the synthetic peptides Homogramicidin S (HGS) = cyc L-valyl L-arginyl L-leucyl D-phenylalanyl L-prolyl L-valyl L-arginyl L-leucyl D-phenylalanyl L-prolyl and a cyclic hexapeptide (CHP), = cyc valyl phenylalanyl lysyl valyl phenylalanyl lysyl (configuration of amino acids unknown); Dr. J. G Neilands, Dept. of Biochemistry, Univ. of California, Berkeley, Calif., for crystalline samples of two naturally occurring cyclic peptides isolated from Ustilago sphaerogena, Ferrichrome A (FCA) = cyc diseryl-glycyl-tri-δ-N-hydroxyornithyl and Ferrichrome (FC) = cyc triglycyl-tri-δ-N-hydroxyornithyl; Dr. H. Koffler, Purdue Univ., Lafayette, Ind. for circulin; Dr. C. deFiebre, Wilson Lab., Chicago, Ill. for oxytocin and vasopressin.

penicillin/hr. Hydrolysis of inducers was determined by treating the compounds for 5 minutes at 35 C with 0.05 ml of a purified penicillinase preparation derived from B. cereus 569/4 (Citri et al, 1960). The penicillinase-treated inducers were then tested as described above for capacity to induce.

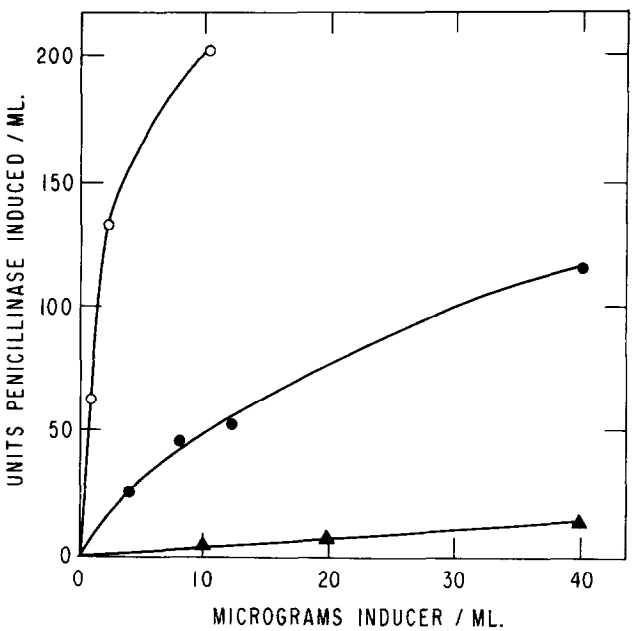

Figure 1 - Induction of penicillinase in B. cereus 569 by various cyclic peptides. The endogenous values have been subtracted. Open circles = HGS; closed circles = FCA; closed triangles = FC

Figure 1 indicates that HGS at low concentrations and Ferrichrome A at higher levels are both potent inducers of penicillinase formation. Indeed, in other experiments (See Table 1), the former compound has been shown to be as potent in this respect as various penicillins tested, including methicillin and oxacillin as well as benzyl penicillin. Ferrichrome, a cyclic hexapeptide differing from FCA in the substitution of a diglycyl residue in

lieu of a diseryl residue, is much less active than either of the other two cyclic peptides. However, the probability that the induction mediated by FC is real is strengthened by the data plotted in Fig. 2, where a good concentration response curve for FC is noted. This figure also indicates that at higher concentrations both the antibiotic circulin and the hormone

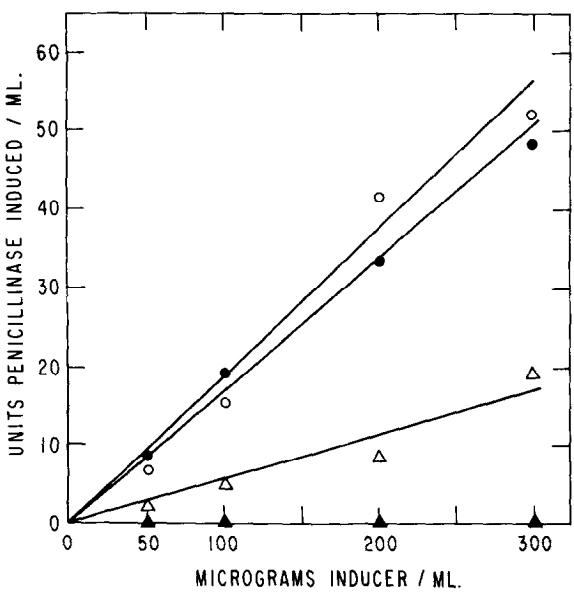

Figure 2 - Induction of penicillinase in B. cereus 569 by various cyclic peptides. The endogenous values have been substracted Open circles = FC; closed circles = circulin; open triangles = vasopressin; closed triangles = oxytocin.

vasopressin induce activity of the enzyme. The hormone oxytocin has no effect. It should be noted that neither the hormones nor circulin were crystalline preparations. Table 1 compares the capacity of various cyclic peptides to induce penicillinase activity.

Table 1

INDUCTION OF PENICILLINASE BY CYCLIC PEPTIDES

Peptide	Concentration (μg/ml.)	Penicillinase (u/ml.)
HGS	2.0	352.0
	4.0	305.0
CHP	2.0	402.0
	4.0	450.0
FCA	20.0	119.0
	40.0	77.6
BP	1.0	376.0
-	-	22.5

HGS = Homogramicidin S; CHP = cyclic hexapeptide
FCA = Ferrichrome A; BP = Benzyl penicillin

It is evident that at very low concentration both HGS and CHP are extremely active, indeed as potent as Pen G. The activity of FCA shown in Figure 1 is confirmed in the data presented in this table. It is interesting that Gramicidin S and Gramicidin D do not induce activity.

Preliminary experiments indicate that prior treatment of HGS, CHP, FCA, and FC with a purified penicillinase derived from B. cereus 569/4 completely abolishes the capacity of these cyclic peptides to induce penicillinase. Further HGS and CHP do not sensitise penicillinase to iodine.

It is thus evident that so-called penicillinase is a non-specific enzyme both in its inducibility and activity. There is no readily apparent similarity in structure between the various penicillins on the one hand and the active cyclic peptides on the other. Yet, these peptides are both inducers and substrates for the enzyme. Obviously, an understanding of the metabolic role of penicillinase in producing cells must take into account the wider parameters of inducibility and activity reported in this communication.

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