EVIDENCE FOR A CHANGE IN THE ACTIVE SITE OF PENICILLINASE CAUSED BY A COMPETITIVE INHIBITOR[1]

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The susceptibility of exopenicillinase of Bacillus cereus to iodination has been found to provide a sensitive indication of structural changes in the molecule of the enzyme (Citri, 1958). Thus, exposure of penicillinase to moderate concentrations of urea which have no effect on the enzymic activity, brings about changes which are accurately reflected in increased sensitivity of the enzyme to iodination. It has been suggested that the effect of urea on the enzyme is reversed by the substrate, and evidence has been presented that penicillinase modified by urea becomes resistant to iodination after contact with benzylpenicillin (Citri and Garber, 1958, 1960).

In the present communication penicillinase is shown to become sensitive to iodination through interaction with a competitive inhibitor. The sensitization of the enzyme is reversed by the substrate.

6(2.6-dimethoxybenzamido) penicillanic acid (DMP)[2] is a structural analogue of benzylpenicillin. It is a competitive inhibitor of penicillinase of Bacillus cereus (Citri, unpublished).

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[2]Kindly supplied by Teva Ltd. (under the trademark Staphcillin Bristol Laboratories).

The effect of varying concentrations of DMP on the susceptibility of the enzyme to iodine, is shown in Fig. 1.

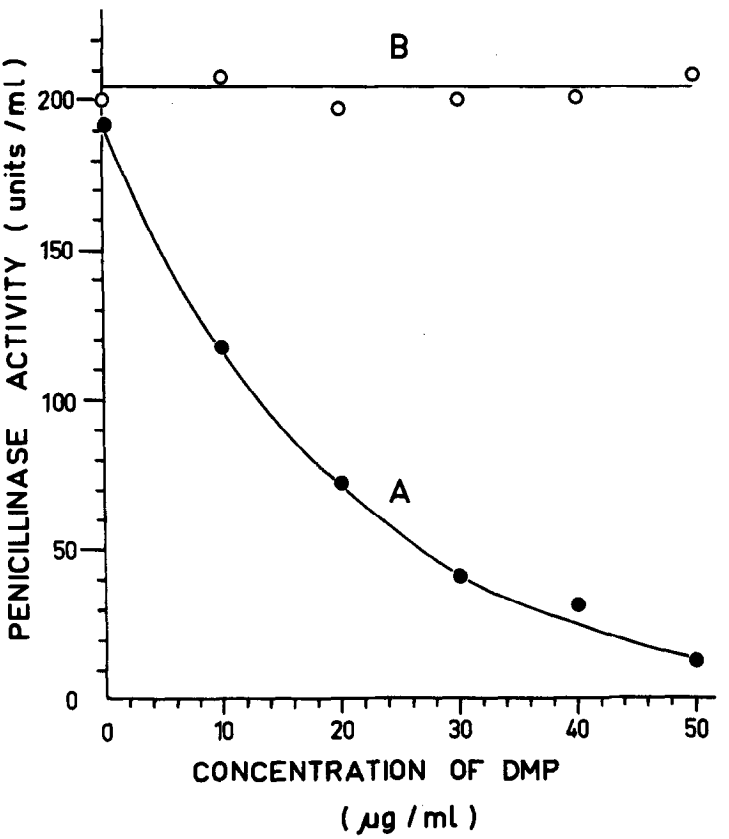

Fig. 1 Survival of penicillinase activity following exposure to DMP. (A) 0.0038M I_2 added; (B) no addition. Aliquots of penicillinase (0.65 µg of pure enzyme) were exposed to varying concentrations of DMP in the presence and in the absence of iodine (0.0038M I_2 in 0.02M KI). The treatment was carried out at 0°C in 1 ml. of gelatin-phosphate medium (0.5% gelatin and 0.03M phosphate buffer pH7). At the end of 5 min. the reactants were diluted with an excess of the substrate (3000 µg benzylpenicillin in 4 ml. of the gelatin-phosphate medium). The rate of hydrolysis of benzylpenicillin by the preparation was immediately assayed (for procedure see Citri, 1958).

It will be noted that pretreatment with the analogue has no effect on the subsequent hydrolysis of the substrate (Fig.1, B). However, if the pretreatment is carried out in the presence of iodine, the susceptibility of the enzyme to iodination is found to increase as a function of the concentration of the analogue in the medium (Fig.1, A).

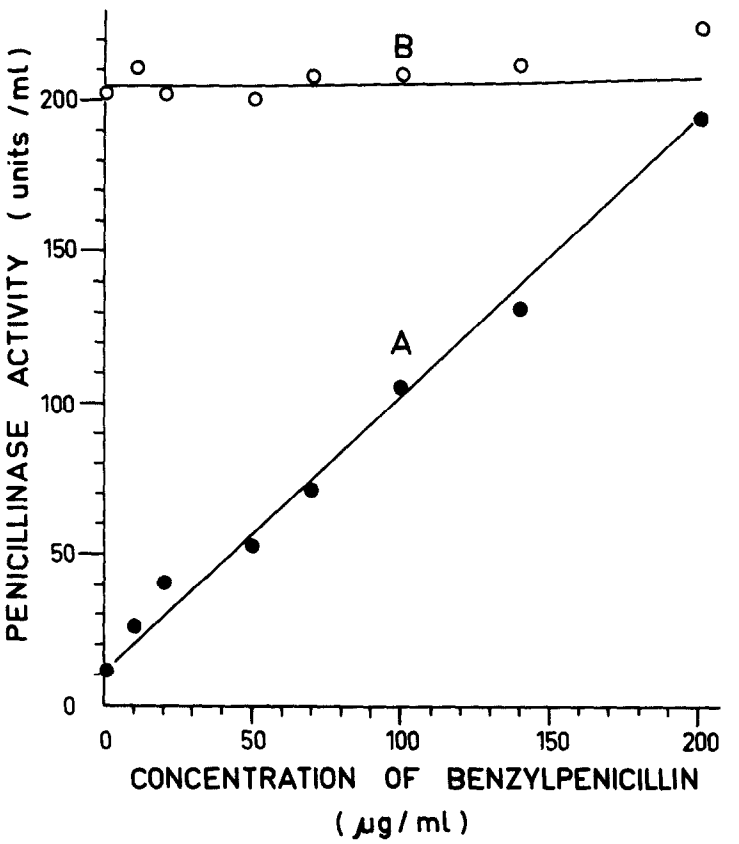

Fig. 2 Survival of penicillinase activity following exposure to DMP and benzylpenicillin. (A) 0.0038M I_2 added; (B) no addition. Aliquots of penicillinase (0.65 µg of pure enzyme) were exposed to DMP (50 µg/ml) and varying concentrations of benzylpenicillin in the presence and in the absence of iodine (0.0038 M I_2 in 0.02M KI). For experimental conditions see Legend of Fig. 1. The extent of hydrolysis of benzylpenicillin under these conditions was negligible.

The susceptibility induced by the analogue is competitively reversed by the substrate, as shown in Fig. 2.

The conclusions can be summarized as follows:

1. DMP being a competitive inhibitor of penicillinase obviously combines with the active site of the enzyme.

2. By combining with DMP the enzyme becomes sensitive to iodination.

3. Change in the susceptibility to iodination reflects a definite conformational change in penicillinase (Citri et al., 1960). It follows that by interaction with DMP the conformation of the active site is changed.

4. The effect of the competitive inhibitor on the conformation of the active site is reversed by the substrate.

References

Citri, N., Biochim. Biophys. Acta, 27, 277 (1958).

Citri, N., and Garber, N., Biochim. Biophys. Acta, 30, 664 (1958).

Citri, N., and Garber, N., Biochim. Biophys. Acta, 38, 50 (1960).

Citri, N., Garber, N., and Sela, M., J. Biol. Chem., 235, 3454 (1960).