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Imidazole and penicilloylation in penicillin allergy

Recently Bundgaard (1972) discussed some aspects of an imidazole-catalysed penicilloylation and suggested that *N*-penicilloyl imidazole is a reactive intermediate capable of transferring penicilloyl groups to acceptor amino-groups and other nucleophiles. This reaction is thought to be of potential significance in penicillin allergy since it could be involved in the efficient formation of penicilloyl antigenic determinants *in vivo*. Direct penicilloylation of primary amino-groups is quite slow in neutral aqueous solution and its significance as an antigen forming step *in vivo* is therefore not self-evident. For this reason we had studied (Schneider & de Weck, 1968, 1969) the direct neutral penicilloylation of a number of functional groups occurring on proteins hoping to find (inter alia) accelerated penicilloylations. In these investigations, the penamaldate stability test (Schneider & de Weck, 1966), which enables the formation of stable penicilloyl derivatives in the presence of penicilloic acid to be detected, played a key role. With regard to the imidazole function, our screening program showed that no penicilloyl derivatives detectable by the penamaldate technique would form after incubation of benzylpenicillin with either N^{α}-Z-DL-histidine or N^{α}-Z-L-histidylglycine at pH 9 and 10·5 respectively. On the other hand these incubations revealed a catalysed hydrolysis of penicillin to penicilloic acid which was also reported.

These results are only in apparent conflict with the report of Bundgaard (1972) on the formation of unstable and intermediary penicilloyl imidazoles. In order to clarify this point we present here some additional data on penicilloylation and penicilloic acid formation in the presence of imidazole compounds which were either not included in the communications of 1968 and 1969 or have accumulated since.

With the incubation and measurement technique described in detail before (Schneider & de Weck, 1968), we found at pH 8·5 that 0·36 M imidazole accelerates

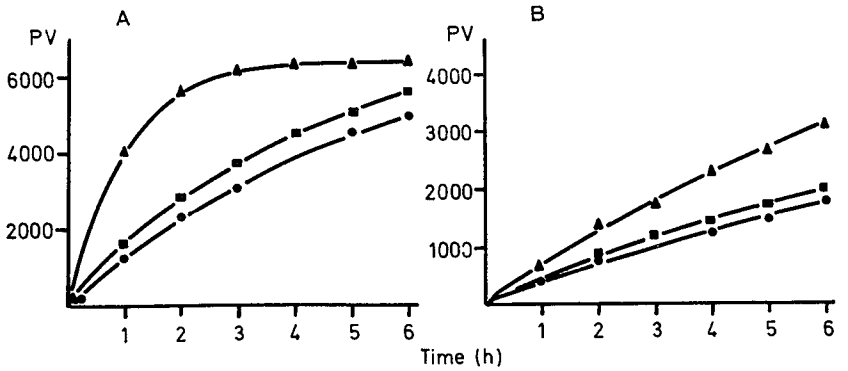

FIG. 1. Measurements of the PV in reaction solutions of benzylpenicillin (0·36M) and EACA (1·45M) at pH 8·5 or pH 7·4 and 37° in the presence of imidazole (▲—▲), N^{α}-Z-DL-histidine (■—■) and without additive (●—●). The pH 8·5 incubations (A) contained 0·36M, the pH 7·4 incubations (B) 0·1M of the additives.

penicilloylation of ϵ-aminocaproic acid (EACA) whereas N^{α}-Z-DL-histidine effects only a marginal acceleration, the penamaldate value (PV) after 6 h being only 10% higher than observed in an incubation solution containing benzylpenicillin and EACA without a catalyst (Fig. 1A). At pH 7·4 the imidazole compounds were used at 0·1 M concentration due to the low solubility of N^{α}-Z-DL-histidine. According to Fig. 1B imidazole slightly accelerates penicilloylation of EACA at this pH whereas N^{α}-Z-DL-histidine again has only a marginal effect. Thus the PV after 6 h is about 3200 and 2000 in the incubations containing imidazole and N^{α}-Z-DL-histidine respectively whereas the uncatalysed reaction shows a PV of 1800. Imidazole (0·1 M) at pH 6·5 shows also a marginal acceleration of penicilloylation, the PV being 2100 after 6 h (not included in the Fig.). The reaction solutions were subjected to the penamaldate stability test after 6 h. The penamaldate stability (PS_{10}) was in all cases around 90% which shows that penicilloic acid was formed in only small amounts during the incubations.

Benzylpenicillin was also incubated with imidazole and N^{α}-Z-DL-histidine in the absence of EACA. In these solutions penamaldate values with low PS_{10} characteristic for penicilloic acid were obtained. The curves of Fig. 2 show that penicillin hydrolysis in the presence of 0·1M N^{α}-Z-DL-histidine is about 5 times lower than with 0·1M

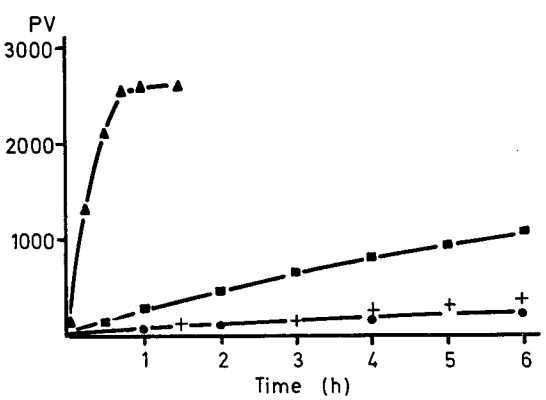

FIG. 2. Measurement of the PV in solutions of 0·36M benzylpenicillin and 0·72M imidazole (▲—▲) or 0·1M imidazole (■—■) or 0·1M N^{α}-Z-DL-histidine (●—●) at pH 7·4 and 37°. In one run with the histidine derivative KCl (0·3M) was added (+—+).

imidazole. It can also be seen that imidazole at 0·1M requires 240 min to hydrolyse penicillin to a PV of 800 whereas the same extent of hydrolysis is achieved by 0·72M imidazole in only 9 min. This indicates a non-linear relation between catalyst concentration and hydrolysis rate, the catalysis being relatively more efficient at high catalyst concentration.

These experiments show a significant difference between the catalytic efficiency of imidazole and N^{α}-Z-DL-histidine. The substituted imidazole is 2 to 5 times less effective than imidazole in accelerating penicilloylation and penicillin hydrolysis under slightly alkaline conditions. It is also apparent that acceleration of penicilloylation at low concentration of catalyst is not very effective. N^{α}-Z-DL-histidine at 0·1M or 0·36M achieved only a slight acceleration of penicilloylation of 1·45M EACA at pH 7·4 and 8·5.

In discussing accelerated penicilloylation as an antigen-forming step in penicillin allergy, the possible catalytic effect of high concentrations of free imidazole is probably not relevant. The question is rather whether some carrier proteins accessible to penicillin *in vivo* may be preferentially penicilloylated thanks to a suitable arrangement of nucleophilic groups and histidine residues which would allow the imidazole catalysis to become efficient. It might be desirable to study a number of suitable histidine- and at the same time lysine-containing peptides in order to establish the possibility of preferential penicilloylation due to histidine catalysis since it seems difficult from the present data to attribute to this mechanism a significant role in facilitating protein penicilloylation. A possible point of preferential penicilloylation could be *N*-terminal histidine where a free primary amino-group is adjacent to imidazole. We did not include studies on penicilloylation of free histidine since with this compound, preliminary studies indicate that reliable end points are not reached in the penamaldate assay.

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