THE PROGRESSIVE REACTION OF *ISO*NICOTINYL HYDRAZIDE WITH TWO BACTERIAL AMINO ACID DECARBOXYLASES

by

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1-*iso*Nicotinyl hydrazide (isoniazid) inhibits a number of enzyme systems, some of which are known to require vitamin B_6 as co-enzyme[1,2]. The recent studies of DAVISON[3] on some vitamin B_6 enzymes from mammalian tissues have shown that isoniazid acts as an inhibitor by combining with the co-enzyme, the reaction between inhibitor and co-enzyme being slow and progressive. A preliminary investigation on the isoniazid inhibition of two partially purified vitamin B_6 enzymes from bacteria was carried out with the object of demonstrating that a similar progressive inhibition took place in these systems.

EXPERIMENTAL

Substrates. Meso-diaminopimelic acid was prepared from the culture filtrate of the lysine-auxotrophe *Escherichia coli 26–26* according to the procedure of WORK AND DENMAN[4] modified by HOARE AND WORK[5].

*Iso*nicotinyl hydrazide and 1-*iso*nicotinyl-2-*iso*propyl hydrazide were kindly provided by Dr. A. L. Morrison, Roche Products, Welwyn Garden City, England. Pyridoxal phosphate was provided by Dr. W. W. Umbreit.

Enzyme preparations. Diaminopimelic acid decarboxylase was prepared and partially purified from cell-free extracts of *Aerobacter aerogenes* as described by HOARE AND WORK[5]. Lysine decarboxylase was prepared from *Escherichia coli* strain B (American Collection) by the following procedure:

The organism was grown in large bottles containing nutrient broth with 2% (w/v) glucose, moderate aeration being achieved by shaking the bottles on inclined rollers. Growth was continued for 12 h at 37°. The cells were centrifuged off, washed, and acetone-dried. Cell-free extracts were obtained under the same conditions as employed for *Aerobacter aerogenes* (above) except that *M*/10 potassium phosphate buffer pH 6.0 was used. The cell-free extract was dialysed overnight at +2° against glass-distilled water. The dialysed extract was fractionated with acetone at −5°, the lysine decarboxylase being carried down between 30 and 40% (v/v) acetone.

Enzyme assays. Diaminopimelic acid decarboxylase was assayed manometrically at pH 6.8 by the method of DEWEY, HOARE AND WORK[6] using nitrogen as the gas phase, and meso-dimianopimelic acid (10^{-2} *M*) as substrate. Lysine decarboxylase was also assayed manometrically, the reaction being carried out at pH 6.0 with L-lysine ($1.3 \cdot 10^{-2}$ *M* final concn.) as substrate. Pyridoxal phosphate (10^{-5} *M* final concn.) was present unless otherwise stated. Isoniazid ($5 \cdot 10^{-4}$ *M* final concn.) in a solution of the appropriate buffer was tipped in from one side-bulb of a Warburg flask and was pre-incubated with the enzyme preparation for various times prior to the addition of the substrate, dissolved in the appropriate buffer and added from a second side-bulb.

RESULTS

Both the partially purified decarboxylase preparations are largely undissociated from the co-enzyme (pyridoxal phosphate). The percentage activation of the enzymes by

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pyridoxal phosphate (10^{-5} *M*) was: lysine decarboxylase 66% activation; diaminopimelic acid decarboxylase 118% activation.

The progressive inhibition of lysine and of diaminopimelic acid decarboxylase by isoniazid is illustrated in Figs. 1 and 2 respectively. In both cases, a comparison of the curves for the control (*i.e.* without isoniazid) with those for the enzyme with isoniazid added at time 0, shows that although the *initial* rates are approximately the same, in the presence of inhibitor the reaction rate falls off as the substrate is used up.

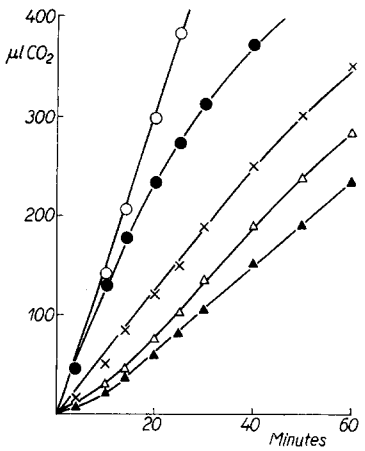

Fig. 1. Inhibition of lysine decarboxylase by isoniazid. Reaction mixture of total volume 2.5 ml in 0.1 *M* phosphate buffer pH 6.0 containing 2 mg enzyme, pyridoxal phosphate (10^{-5} *M*), L-lysine monohydrochloride ($1.3 \cdot 10^{-2}$ *M*), and isoniazid ($5 \cdot 10^{-4}$ *M*). Reaction in nitrogen at 37°. O—O Control (no isoniazid); ●—● Isoniazid at time 0; ×—× Isoniazid pre-incubated 10 min; △—△ 20 min pre-incubation; ▲—▲ 40 min pre-incubation.

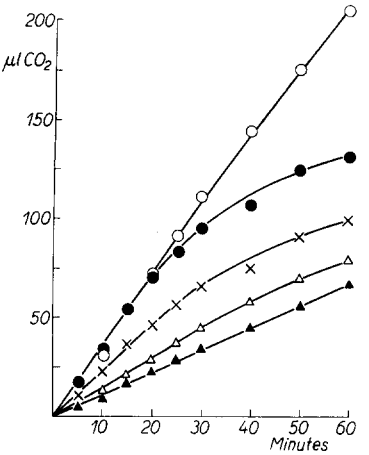

Fig. 2. Inhibition of diaminopimelic acid decarboxylase by isoniazid. Reaction mixt re of total volume 2.5 ml in 0.1 *M* phosphate buffer pH 6.8 containing 5 mg enzyme, pyridoxal phosphate (10^{-5} *M*), meso-diaminopimelic acid (10^{-2} *M*), and isoniazid ($5 \cdot 10^{-4}$ *M*). Reaction in nitrogen at 37°. Key as in Fig. 1.

Similar curves were obtained for lysine decarboxylase in the absence of pyridoxal phosphate.

No significant inhibition of lysine or of diaminopimelic acid decarboxylase was observed in the presence of 1-*iso*nicotinyl-2-*iso*propyl hydrazide ($5 \cdot 10^{-4}$ *M*) under the same conditions.

Diaminopimelic acid decarboxylase was completely inhibited by hydroxylamine (10^{-4} *M*), no pre-incubation being necessary.

DISCUSSION

The inhibition of lysine decarboxylase and of diaminopimelic acid decarboxylase by isoniazid increases with the pre-incubation time of the enzyme with isoniazid. This is similar to the effect observed with some amino acid decarboxylases of mammalian tissues (DAVISON[3]). YONEDA *et al.*[1] had previously observed that the complete inter-

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action of isoniazid and pyridoxine in the tryptophanase of *Escherichia coli* required a 30 minute period of pre-incubation at 38°. Again in their studies on the inhibition of arginine decarboxylase[2] in *Escherichia coli* by isoniazid, the inhibitor was always pre-incubated with the enzyme for 30 minutes. It may be found that all vitamin B_6 enzymes are progressively inhibited by isoniazid. In contrast, the reaction between hydroxylamine and vitamin B_6 enzymes is very rapid. This has been examined in some detail with the glutamic acid decarboxylase of *Escherichia coli* (ROBERTS[7]).

SUMMARY

In the absence of substrate, lysine and diaminopimelic acid decarboxylases are inhibited progressively with isoniazid. This effect is similar to that found with some mammalian amino acid decarboxylases.

RÉSUMÉ

En absence de substrate les décarboxylases de lysine et d'acide diaminopimélique sont inhibées progressivement par l'isoniazide. Cet effet est semblable à celui qui avait été trouvé pour certaines décarboxylases d'acides aminés de mammifères.

ZUSAMMENFASSUNG

In Abwesenheit eines Substrats werden die Lysin- und Diaminopimelinsäure-Decarboxylasen progressif durch Isoniazid gehemmt. Dieser Effekt ist demjenigen ähnlich, welcher für einige Säugetier-Aminosäuredecarboxylasen gefunden wurden.

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