Biochemical Pharmacology, 1959, Vol. 3, pp. 38–41. Pergamon Press Ltd., Printed in Great Britain.

METABOLISM *in vitro* OF THE SULPHHYDRYL AMINO ACIDS, L- AND D-PENICILLAMINE*

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(*Received* 14 *May* 1959; *revised* 12 *June* 1959)

Abstract—L-Penicillamine is not active as a substrate of L-amino acid oxidase. Its enantiomorph, however, is slowly oxidized by D-amino acid oxidase. Although neither isomer of penicillamine is desulphhydrated by L-cysteine desulphhydrase, the L-isomer inhibits the action of this enzyme. These findings are correlated with the enzyme-inhibiting and therapeutic activities of penicillamine.

ALTHOUGH the inhibitory activity of L-penicillamine (the $\beta : \beta$-dimethyl analogue of cysteine) has been studied in various biological systems[1–8], and although the therapeutic effectiveness of the enantiomorphs of penicillamine in patients with Wilson's disease and with heavy metal intoxication has received recent attention,[9, 10, 11, 12] the activity of this amino acid as a substrate of the enzymes associated with cysteine catabolism has not been reported. In the experiments of the present communication, the activity of penicillamine as a substrate of L-amino acid oxidase, D-amino acid oxidase and L-cysteine desulphhydrase was investigated to obtain information regarding the metabolism of this amino acid.

EXPERIMENTAL

The oxidases of D-amino acid and L-amino acid were assayed by manometric methods described by Burton[14] and Ratner[14], respectively. *Agkistroden p. piscivorus* (cotton-mouth moccasin) venom was used as the source of L-amino acid oxidase. The venom was obtained from Ross Allen Reptile Institute, Silver Springs, Florida. Flavin adenine dinucleotide (FAD) and crude hog kidney D-amino acid oxidase were purchased from the Sigma Chemical Company. Crude beef liver catalase was purchased from the Nutritional Biochemicals Corp.

Desulphhydrase activity was measured by the method described by Smythe.[14] The enzyme was prepared by making a saline extract of fresh rat liver. The saline extract was extracted with chloroform and the enzyme precipitated by adding acetone. A detailed procedure has been described[14]. The physical constants and sources of the penicillamine have been reported.[9, 10]

*This work was supported in part by a grant from the Surgeon General, Department of the Army, by grant no. CY 2572 from the National Cancer Institute and by an institutional grant from the American Cancer Society to Vanderbilt University.

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RESULTS AND DISCUSSION

L-Penicillamine is not oxidatively deaminated by L-amino acid oxidase of moccasin venom (Fig. 1,A). The small amount of oxygen consumed (curve A) is the same as that noted when the enzyme is incubated without a substrate. Although D-penicillamine is metabolized by D-amino acid oxidase (Fig. 1,C) the rate of oxygen consumption is

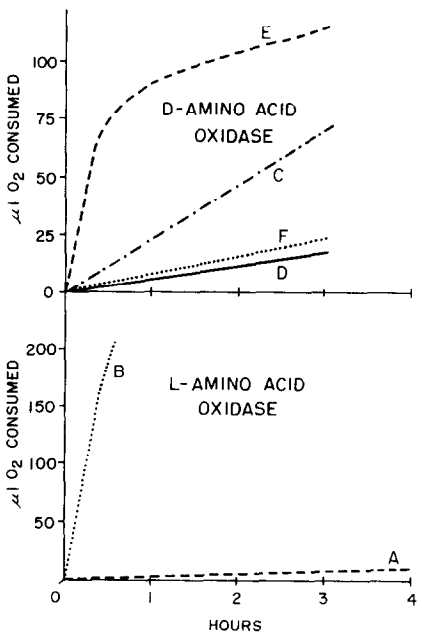

FIG. 1. Activity of penicillamine as a substrate for amino acid oxidase. *Curve A*: main compartment of Warburg vessel contained 5 mg *Agkistrodon p. piscivorus* venom dissolved in 0·5 ml 0·1 M KCl, 1·0 ml 0·2 M tris (hydroxymethyl) aminomethane buffer, pH 7·2; side bulb contained 100 μmoles L-penicillamine dissolved in 0·5 ml H_2O; 1·0 ml water was added; centre well contained 0·2 ml 20% NaOH. *Curve B*: conditions the same as above except 20 μmoles of L-cysteine substituted for L-penicillamine. The oxygen consumptions of the reaction mixtures without the venom have been substracted from the appropriate curves. *Curve C*: main compartment of vessel contained 8 mg crude hog kidney D-amino acid oxidase dissolved in 1 ml of buffer; 1·0 ml 0·1 M sodium pyrophosphate buffer, pH 8·3; 0·1 ml 10^{-4}M FAD, 40 μg crude beef liver catalase; side bulb contained 60 μmoles D-penicillamine dissolved in 0·2 ml water, pH 8·3; 0·2 ml water was added. Centre well conta ined 0·2 ml 20% NaOH. *Curve D*: same as those of curve C except D-amino acid oxidase omitted. *Curve E*: same as those of curve C except 10 μmoles D-cysteine substituted for D-penicillamine. *Curve F*: same as those of curve E except D-amino acid oxidase omitted. The gas phase in all experiments was air, the temperature 37 °C. Manometer readings made every 5 min.

low. The amount (8 mg) of the D-amino acid oxidase preparation used in the experiments described in Fig. 1 was twice that which is routinely used in this laboratory for D-amino acid oxidase studies. If 4 mg of the enzyme preparation are used, the rate of oxygen consumption is barely detectable when D-penicillamine is the substrate. Oxygen is consumed rapidly, however, if D-cysteine is the substrate in the presence of 4 mg of the enzyme preparation. This indicates that D-penicillamine is much less active than D-cysteine as a substrate of this enzyme.

When the action of a partially purified preparation of rat liver L-cysteine desulphhydrase on penicillamine is studied, neither enantiomorph is active as a substrate (Table 1). In fact, L-penicillamine inhibits the desulphhydration of L-cysteine (Table 1).

TABLE 1. ACTIVITY OF PENICILLAMINE AS A SUBSTRATE OF L-CYSTEINE DESULPHHYDRASE

Substrate (μmoles)	H_2S produced (μmoles)	Activity (%)
5·0 L-cysteine	3·01	100
5·0 L-penicillamine	0·0	0
5·0 D-penicillamine	0·0	0
5·0 L-cysteine + 5·0 L-penicillamine	0·53	18
5·0 L-cysteine + 5·0 D-penicillamine	2·07	69

Main compartment of Warburg vessel contained 33 mg partially purified rat liver cysteine desulphhydrase dissolved in 2·0 ml pH 7·4 phosphate buffer; side bulb contained 5·0 μmoles of each of the amino acids studied; total reaction volume 2·2 ml. Gas phase, nitrogen; incubated 2 hr at 37 °C. Centre well contained 0·1 ml of 1·0 M cadmium acetate.

Inhibition by the D-isomer is slight. Since pyridoxal-5-phosphate appears to be the coenzyme of cysteine desulphhydrase,[17, 18, 19] the inhibitory action of L-penicillamine is possibly the result of this amino acid's forming a thiazolidine carboxylic acid with the coenzyme. Such a reaction has been demonstrated between penicillamine and pyridoxal-5-phosphate in a non-enzymic preparation *in vitro*, and has been suggested to occur with pyridoxal-5-phosphate enzymes by du Vigneaud and coworkers.[6, 7, 8]

There are many reactions by which cysteine is catabolized.[15] The ineffectiveness of cysteine in the treatment of heavy metal intoxication[9, 10] is probably the result of its rapid catabolism. The effectiveness of penicillamine[9, 10, 11, 12] as a metal-binding agent* appears to be due, in part, to its relative stability to metabolic degradation. This is substantiated by the present experiments with amino acid oxidases and cysteine desulphhydrase, and also by the observation that N-acetyl-DL-penicillamine is superior to DL-penicillamine in protecting rats against the lethal effects of mercuric chloride.[10] As would be expected, the N-acetyl derivative is not active as a substrate of either amino acid oxidase since the addition of the acetyl group has changed the amino group to an amide group which is not degraded by amino acid oxidase.[16]

It has been suggested that the growth inhibitory activity of L-penicillamine in the rat may be the result of the combination of this amino acid with pyridoxal-5-phosphate.[6, 7, 8] This anti-vitamin B_6 activity has been shown *in vitro* to depend on the presence of a free amino and a free sulphhydryl group.[7, 8] For this to be true *in vivo*, L-penicillamine should be inactive as a substrate of L-amino acid oxidase. This is substantiated by the present experiments.

Thus, oxidative deamination of D-penicillamine, as shown in the present experiments, and thiazolidine formation of L-penicillamine with pyridoxal-5-phosphate, as

*The combination of a metal ion with an electron donor results in a substance called a complex or co-ordination compound. If the substance which combines with the metal contains two or more donor groups so that one or more rings are formed the resulting structure is said to be a chelate.[13] If the metal combines with the electron donor and a ring structure is not formed, the resulting structure is called a complex. Although penicillamine has been shown to increase the excretion of many metals, it is not known whether it chelates or complexes.

shown by du Vigneaud and coworkers,[7, 8] appear to be two of the routes for the metabolic inactivation of penicillamine. The inactivation of penicillamine by the amino acid oxidases, however, is considerably less than that of cysteine.

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