The phosphotransacetylase of *E. coli* is quite similar to that described by STADTMAN[2,4] and its characteristics will therefore not be described in detail. The enzyme shows a complete requirement for CoA and sulfhydryl source[4]. Our phosphotransacetylase of specific activity 30 would have a specific activity of about 900 in STADTMAN's arsenolysis assay[5].

Reaction (1) can, under certain conditions, be carried out by glyceraldehyde phosphate dehydrogenase[9,10]. The possibility that the reaction followed in our assay system is actually catalyzed by this dehydrogenase rather than by phosphotransacetylase is considered remote since the dehydrogenase-catalyzed transacylation is quite slow and requires enzyme concentrations considerably higher than those routinely employed in the phosphotransacetylase assay. This question must, however, remain open until the glyceraldehyde phosphate dehydrogenase of *E. coli* is purified and then used in the phosphotransacetylase assay system.

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1 D. S. GOLDMAN, *Biochim. Biophys. Acta*, 27 (1957) 503.
2 E. R. STADTMAN AND H. A. BARKER, *J. Biol. Chem.*, 180 (1949) 1085.
3 E. R. STADTMAN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, Inc., New York, 1955, p. 518.
4 E. R. STADTMAN, *J. Biol. Chem.*, 196 (1952) 527.
5 E. R. STADTMAN, G. D. NOVELLI AND F. LIPMANN, *J. Biol. Chem.*, 191 (1951) 365.
6 D. S. GOLDMAN, *J. Bacteriol.*, 72 (1956) 401.
7 D. S. GOLDMAN, *J. Bacteriol.*, 73 (1957) 602.
8 B. L. HORECKER AND A. KORNBERG, *J. Biol. Chem.*, 175 (1948) 385.
9 E. RACKER, in W. D. MCELROY AND B. GLASS, *The Mechanism of Enzyme Action*, The Johns Hopkins Press, Baltimore, 1954, p. 467.
10 S. F. VELICK, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, Inc., New York, 1955, p. 405.

Received January 7th, 1958

* With the technical assistance of Mrs. PATRICIA ANN KOCH.

Oxidative deamination of cysteinesulfinic acid

In a previous paper on the metabolism of D-cysteine, *in vivo*, we have obtained some results, namely the excretion of taurine after oral administration of D-cysteinesulfinic acid (CSA), which could be tentatively explained by a deamination and reamination of this compound[1]. The object of the present investigation was to test if CSA was deaminated by D-amino acid oxidase preparations.

CSA was prepared from D-cysteine (Fluka) according to LAVINE[2]. D-Aspartic acid was a commercial product (Fluka). Crystalline catalase was a commercial product (Boehringer). FAD was prepared according to COLOWICK AND KAPLAN[3]. D-Amino acid oxidase was prepared from sheep and hog kidney[3]. D-Aspartic oxidase was prepared according to STILL *et al.*[4].

The oxygen uptake was measured in the classical Warburg apparatus. Keto acids were determined chromatographically and spectrophotometrically identified by the method of C. VALLINI *et al.*[5].

Fig. 1 shows that D-CSA is a substrate for a crude preparation of D-amino acid oxidase (step 1[3]), prepared either from sheep or hog kidney (acetone powder dissolved in pyrophosphate buffer). Compared to DL-alanine, CSA is oxidized more slowly and the oxygen uptake reaches values above the theoretical amount of 0.5 mole O_2/mole substrate. Chromatographic analysis of the keto acids formed in the reaction showed the formation of pyruvic acid.

During further purifications of D-amino acid oxidase, a separation between the alanine oxidase and the CSA oxidase was achieved. The activity towards CSA was retained for only the first 3–4 stages of the purification. (Catalase and FAD were added after the third stage of the purification, and we moreover obtained preparations purified up to 7th stage, active towards alanine.)

Fig. 2 shows that the D-aspartic oxidase from rabbit kidney also oxidizes CSA. In the presence of ethanol the oxygen consumption was doubled with both substrates, showing that both reactions follow the classical pathway of oxidative deamination with production of H_2O_2. The D-aspartic oxidase is completely inactive toward alanine.

These results suggested that CSA is deaminated by D-aspartic oxidase present in incompletely purified preparations of D-amino acid oxidase. This was tested by incubation of D-amino acid oxidase from hog kidney with alanine and CSA in presence of $3 \cdot 10^{-3}$ *M* sodium benzoate, an

inhibitor of this enzyme[6]. Fig. 1 shows that alanine oxidation was inhibited, while the oxidation of CSA was unaffected; it was found that the oxidation of aspartic acid by these preparations was also unaffected by benzoate. It was concluded that crude preparations of D-amino acid oxidase from hog kidney contain an aspartic oxidase which is able to deaminate the D-CSA.

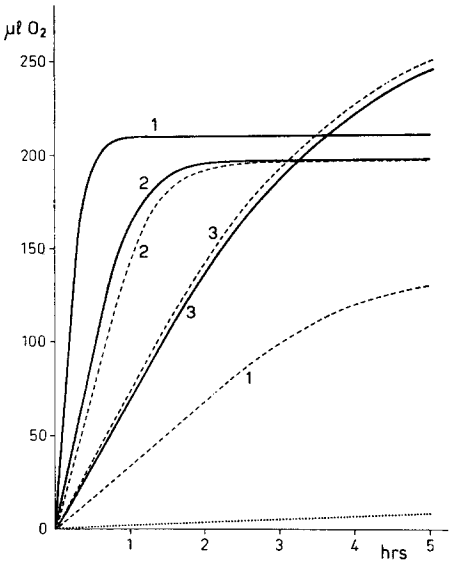

Fig. 1. D-amino acid oxidase from hog kidney. Acetone powder (1:10) in pyrophosphate buffer (0.017 *M*; pH, 8.3), 1 ml; pyrophosphate buffer (0.05 *M*, pH 8.3), 2 ml. Substrates: 1, 40 μmoles DL-alanine; 2, 20 μmoles D-aspartic acid; 3, 20 μmoles D-cysteinesulfinic acid. Dotted line, 20 μmoles L-cysteinesulfinic acid; broken line, in presence of $3 \cdot 10^{-3}$ *M* sodium benzoate. Gas, air. Temp., 38°.

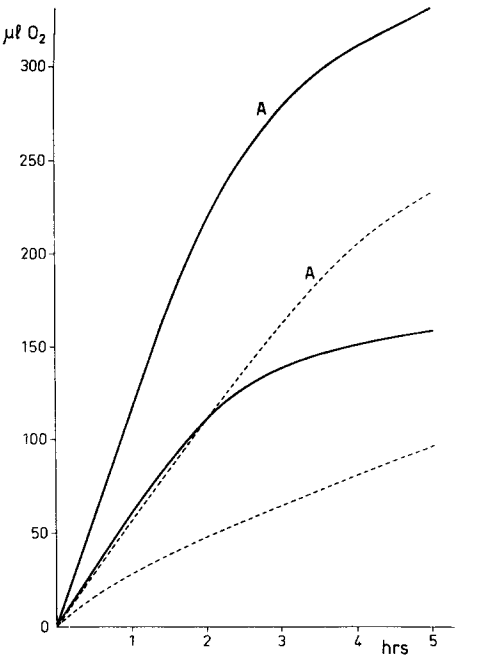

Fig. 2. D-aspartic oxidase from rabbit kidney. Acetone powder (1:10) in phosphate buffer (0.1 *M*; pH, 7.2), 1.5 ml; phosphate buffer (0.067 *M*, pH 7.2), 1.5 ml. Substrates: 20 μmoles D-aspartic acid (full line); 20 μmoles D-cysteinesulfinic acid (broken line) A, in presence of 0.4 ml 4% ethanol. Gas, air. Temp., 38°.

All these preparations were inactive on L-cysteinesulfinic acids.

Studies are in progress in our laboratory to separate the D-aspartic oxidase activity from D-amino acid oxidase activity in hog-kidney preparations. We have obtained a partial resolution of the D-aspartic oxidase, which still acts on CSA.

The fact that the oxygen uptake exceeds the theoretical amount for a simple oxidative deamination and the fate of sulfur deserves further investigation. In connection with the observation that pyruvic acid has been identified as product of the reaction, the instability of sulfinylpyruvic acid should be kept in mind.

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1 D. CAVALLINI, C. DE MARCO AND B. MONDOVÌ, *J. Biol. Chem.*, in the press.
2 T. F. LAVINE, *J. Biol. Chem.*, 113 (1936) 583.
3 S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, Inc., New York, 1955, p. 199.
4 J. L. STILL, M. V. BUELL, W. E. KNOX AND D. E. GREEN, *J. Biol. Chem.*, 179 (1949) 831.
5 D. CAVALLINI AND N. FRONTALI, *Biochim. Biophys. Acta*, 13 (1954) 439.
6 J. R. KLEIN AND H. KAMIN, *J. Biol. Chem.*, 138 (1941) 507.

Received January 27th, 1958