Purification of phosphotransacetylase from *Escherichia coli*, K-12

Phosphotransacetylase was required as a reagent in a series of experiments which were part of a study of a soluble pyruvic dehydrogenase of the H37Ra strain of *Mycobacterium tuberculosis var. hominis*[1]. The large-scale preparation of *Clostridium kluyveri*[2,3] and the preparation of a highly purified phosphotransacetylase[4] from cell-free extracts of this anaerobe have been described. Owing to the difficulties of growing *Cl. kluyveri* on a large scale, a more convenient source of this enzyme was sought among common aerobic bacteria. When cell-free extracts of several strains of *Escherichia coli* were analyzed for their phosphotransacetylase content, extracts of the K-12 strain were found to have the highest activity. STADTMAN *et al.*[5] have reported the presence of phosphotransacetylase in several aerobic organisms.

E. coli, K-12 was grown for 24 h at 25° in 14 l of medium containing peptone (1 %), yeast extract (0.1 %), K_2HPO_4 (0.25 %) and glucose (1 %). Vigorous aeration was maintained throughout the growth period. The cells were harvested by centrifugation and washed and resuspended three times in 0.1 *M* Tris* buffer, pH 7.5. The cell paste after the last centrifugation was recovered from the centrifuge tubes, dried in a vacuum desiccator and stored at —20°. Cells prepared from 14 l of culture medium were placed in a cold mortar with 5 g Al_2O_3. The thick mixture was ground thoroughly for about 10 min. The mixture was gradually diluted with 0.10 *M* Tris buffer, pH 7.5, and centrifuged at 140,000 × *g* for 30 min. The supernatant solution was set aside; the residue was re-extracted twice by grinding and diluting as before. The three supernatant solutions were combined and fractionated at 2° with ammonium sulfate (pH 7.5). The fractions precipitating between the limits of 34 and 53 % saturation were recovered by centrifugation, combined, and refractionated with neutral ammonium sulfate. The fraction precipitating between the limits of 37 and 47 % saturation was recovered by centrifugation and retained.

The phosphotransacetylase prepared in this manner is stable in concentrated solution in the presence of ammonium sulfate. Dilution to about 0.1 mg protein/ml in 0.1 *M* Tris buffer, pH 7.5, leads to inactivation of the enzyme within a few minutes at 0°. Enzyme solutions are, therefore, diluted with a buffer mixture containing 0.10 *M* Tris (pH 7.6), 0.003 *M* reduced glutathione and 0.1 % bovine serum albumin. Protein dilutions containing 0.05 mg/ml are stable for several hours in this solution at 0°.

The assay system for the phosphotransacetylase is based on the following linked reactions;

$$\text{acetyl phosphate} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + P_i \quad (1)$$

$$\text{L-malate} + DPN^+ \rightleftharpoons \text{oxaloacetate} + DPNH + H^+ \quad (2)$$

$$\text{Sum reaction: } \frac{\text{acetyl CoA} + \text{oxaloacetate} \rightleftharpoons \text{citrate} + \text{CoA}}{\text{acetyl phosphate} + \text{L-malate} + DPN^+ \rightleftharpoons \text{citrate} + P_i + DPNH + H^+} \quad (3)$$

The assay mixture contains (in μmoles): Tris buffer, pH 7.6 (60), L-malate (5.0), L-cysteine (6.0) CoA (0.15), acetyl phosphate (4.0), DPN (1.0), malic dehydrogenase[6] (0.45 unit), condensing enzyme[7] (0.08 unit) and phosphotransacetylase (0.2 to 3.0 μg). The final volume is 1.0 ml. The reaction is started by the addition of the DPN and carried out at 22°. The reduction of DPN is followed by measuring the change in extinction per min at 340 mμ over a 5-min period. The molecular extinction coefficient of DPNH at 340 mμ[8] is taken as $6.22 \cdot 10^6$ cm² × mole^{-1}. The rate of DPN reduction is linear over the 5-min period under the above conditions providing the total extent of DPN reduction does not exceed 0.05 μmole. One unit of phosphotransacetylase activity is defined as that amount which catalyzes the formation of 1.0 μmole acetyl CoA (hence DPNH) in 1 min under the above assay conditions. Specific activity is defined as units/mg protein. The results of a typical purification of the *E. coli* phosphotransacetylase are shown in Table I.

TABLE I

PURIFICATION OF THE PHOSPHOTRANSACETYLASE OF *E. coli*, K-12

Fraction	*Volume (ml)*	*Protein*			*Units*		
		Concentration (mg/ml)	*Total (mg)*	*% recovered*	*Specific activity (units/mg)*	*Total (units)*	*% recovered*
Crude extract	39	20.8	811	100	2.55	2070	100
1st $(NH_4)_2SO_4$	8.1	22.0	178	22	8.55	1520	73
2nd $(NH_4)_2SO_4$	2.2	18.5	41	5.0	30.6	1250	61

* The following abbreviations will be used: Tris, tris(hydroxymethyl)aminomethane; CoA, coenzyme A; DPN and DPNH, oxidized and reduced forms of diphosphopyridine nucleotide; P_i, inorganic phosphate.

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.

DEXTER S. GOLDMAN*

Tuberculosis Research Laboratory, Veterans Administration Hospital and The Institute for Enzyme Research, University of Wisconsin, Madison, Wisc. (U.S.A.)

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