Isoforms of Baker's Yeast Transketolase

Some data obtained in our laboratory have pointed indirectly to the heterogeneity of crystalline transketolase (EC 2.2.1.1) isolated from baker's yeast by the procedure of RACKER et al.[1]. Ion exchange chromatography and disc electrophoresis on polyacrylamide gel have revealed the existence of 2 isoforms of transketolase. The present paper reports the results of the above investigation.

Crystalline transketolase was isolated by the method of RACKER et al.[1]. An equilibrated mixture of enzymatically prepared pentosephosphates was used as a substrate for measurements of activity[2]. The transketolase activity was assayed spectrophotometrically by the rate of reduction of NAD at the expense of oxidation of glyceraldehydephosphate in the coupled reaction with glyceraldehydephosphate dehydrogenase[3]. The same system was used to visualize the activity in polyacrylamide gel in the presence of nitroblue tetrazolium and phenazine methosulphate. The protein content was determined by the method of LOWRY et al.[4] and by means of direct measurement of optical density at 280 nm. Protein regions on disc electrophoregrams were stained with amido black 10B.

Ion exchange chromatography was carried out with CM-Sephadex C-50. The columns (0.5×10 cm) were packed with ion exchanger equilibrated with $0.05\,M$ potassium-phosphate buffer, pH 5.75. A sample of the enzyme was passed through Sephadex G-50 in the initial buffer solution prior to applying it to the column. Gradient elution was carried out with 50 ml of $0.7\,M$ potassium phosphate buffer, pH 5.75, against 50 ml of $0.05\,M$ phosphate buffer. A constant rate of flow of 20 ml/cm²/h was ensured by a peristaltic micropump. Eluted protein was recorded by means of a highly sensitive flow densitometer at 253 nm, and the increase in salt concentration was followed electrometrically. Fractions (2 ml) were collected and assayed for enzymatic activity and protein. All the experiments were carried out at 3 °C.

For purposes of rechromatography active fractions were pooled, concentrated by means of dry Sephadex G-50, and excessive salt was removed by a Sephadex column prior to reapplication on an ion exchanger. Disc electrophoresis was carried out on 6% polyacrylamide gel. In the electrophoretic separations various buffer systems, ensuring different pH values, were used.

Two isoforms of transketolase have been detected in a highly purified crystalline preparation by means of chromatography with ion exchange Sephadexes. The best way to separate the isoforms is to pass transketolase in $0.05\,M$ potassium phosphate buffer, pH 5.6–5.8, through a column filled with CM-Sephadex. The enzyme becomes bound to with the negatively charged groups of the ion exchanger and, as the concentration of phosphate buffer is gradually increased, the enzyme is eluted from the column as 2 separate peaks, each having transketolase activity (Figure 1, a). Each peak of activity has a clear-cut corresponding protein peak. Isoform II is more

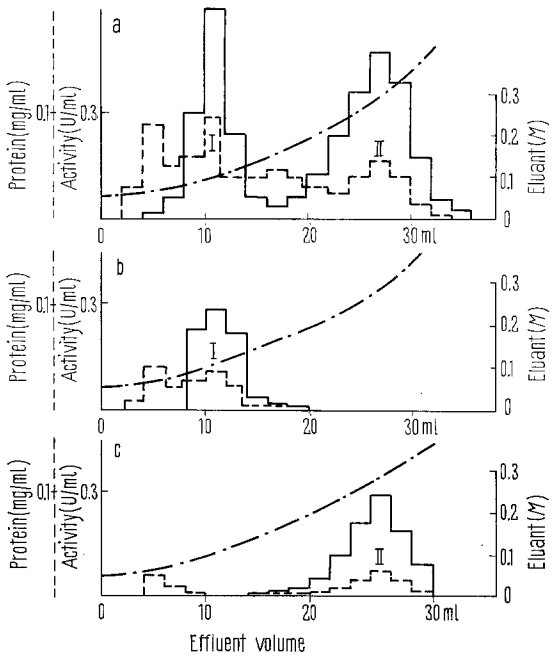

Fig. 1. Separation of isoforms of transketolase apoenzyme on CM-Sephadex and subsequent rechromatography of each isoform under the same conditions. Substances applied were: a) 1.5 mg of transketolase; b) 0.4 mg of isoform I and c) 0.3 mg of isoform II. —·—, increasing concentration of the eluting buffer; —, transketolase activity; – – –, concentration of protein.

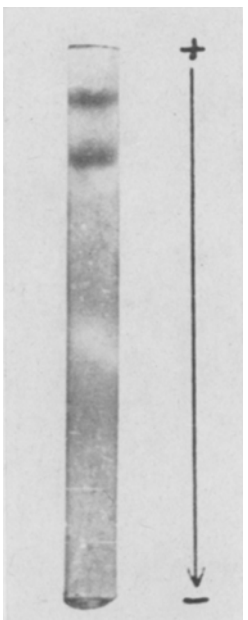

Fig. 2. Electrophoretic separation of transketolase apoenzyme 40 µg in the cation system. Pyridine-CH_3COOH ($0.04\,M$, pH 6.5) buffer was used in the electrode vessels. Electrophoresis was carried out for 2.5 h at a current of 2 mA per tube. Protein fractions were stained with amido black.

[1] J. R. COOPER, P. A. SRERE, M. TABACHNICK and E. RACKER, Arch. Biochem. Biophys. *74*, 306 (1958).
[2] G. DE LA HABA, J. G. LEDER and E. RACKER, J. biol. Chem. *214*, 409 (1955).
[3] G. A. KOCHETOV and P. P. PHILIPPOV, Biochimija, *35*, 422 (1970).
[4] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

strongly bound to the cation exchanger and, consequently, must have a higher positive charge.

Separate rechromatography of both active fractions of transketolase revealed that each isoform was eluted as an individual protein peak displaying enzymatic activity (Figures 1, b and c). Isoform I is always removed from the column at 0.1 *M* concentration of the eluting phosphate buffer and isoform II at 0.2 *M*. The specific activity of transketolase across the protein peaks is constant in all fractions, indicating that both isoforms are free from protein contamination. A nonactive peak of protein which is weakly bound to the cation exchanger appears to be an inactivated form of transketolase. It is also seen in the course of rechromatography of individual isoforms as a result of partial inactivation of the enzyme during its concentration, desalting and storage at pH lower than 6.0.

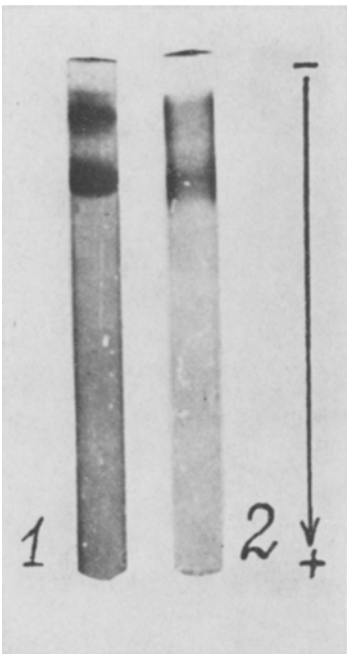

Fig. 3. Electrophoretic separation of transketolase apoenzyme (100 μg) in the anion system with subsequent determination of transketolase activity. *Tris* buffer (0.041 *M*, pH 8.9) containing 0.0063 *M* boric acid and 0.0017 *M* EDTA was used in the electrode vessels. 1. Protein stained with amido black. 2. Transketolase activity.

Phosphate is most important for chromatographic separation of the isoforms. Adequate separation was not observed when phosphate was substituted by acetate or when the concentration of phosphate buffer was decreased 15-fold (the ionic strength of the solution was kept constant by addition of KCl).

The existence of isoforms of transketolase first demonstrated by ion exchange chromatography was confirmed by electrophoresis (Figures 2 and 3). It can be seen in the figures that in both acidic and alkaline ranges the enzyme has 2 protein fractions.

Выводы. Методом ионообменной хроматографии с использованием ионообменных сефадексов показано существование двух изоформ транскетолазы пекарских дрожжей. Наличие двух активных изоформ фермента было подтверждено также при электрофоретическом разделении высокоочищенного препарата апотранскетолазы в полиакриламидном геле.

G. A. KOCHETOV and N. N. CHERNOV

Laboratory of Bioorganic Chemistry, Moscow State University, Moskwa W234 (USSR), 1 April 1970.

Absence of Dehydroxylation of Caffeic Acid in Germ-Free Rats

The dehydroxylation of caffeic acid (3,4-dihydroxycinnamic acid) to *m*-hydroxy derivatives following administration to animals was first reported by DEEDS et al.[1,2] *m*-Hydroxyphenylpropionic acid was shown to be the major metabolite of caffeic acid in rats and the finding that this metabolite was also excreted following the i.p. administration of caffeic acid suggested that dehydroxylation was not a result of the action of intestinal microorganisms. However, several subsequent studies with caffeic acid and related compounds have definitely implicated the intestinal microflora as the site of the dehydroxylation reaction[3–6]. The initial finding that dehydroxylation of caffeic acid occurred also after its i.p. injection has been shown to be dependent upon the biliary excretion of caffeic acid and/or its metabolites which can then undergo dehydroxylation in the intestine[7]. These findings in animals, which point to the intestine as the site of dehydroxylation, have been further substantiated in studies of the metabolism of caffeic acid by intestinal microorganisms[7–9]. The evidence now available thus indicates that the dehydroxylation of caffeic acid occurs in the intestine as a result of bacterial metabolism rather than in the tissues of the animal. However, unequivocal evidence in this regard is dependent upon studies in germ-free animals. No such studies with caffeic acid have hitherto been published and we therefore thought it would be of interest to report our findings on the metabolism of caffeic acid in germ-free rats.

The germ-free rats used were of the CDF strain and were reared according to techniques described previously[10]. The conventional rats were of the same strain and were the fourth generation after conventionalization.

[1] F. DEEDS, A. N. BOOTH and F. T. JONES, J. biol. Chem. *225*, 615 (1957).
[2] A. N. BOOTH, O. H. EMERSON, F. T. JONES and F. DEEDS, J. biol. Chem. *229*, 51 (1957).
[3] K. N. F. SHAW, M. GUTENSTEIN and J. B. JEPSON, Int. Congr. Biochem. (Ed. N. M. SISSAKIAN; Pergamon Press, Oxford 1963), vol. 9, p. 427.
[4] J. C. DACRE, R. R. SCHELINE and R. T. WILLIAMS, J. Pharm. Pharmac. *20*, 619 (1968).
[5] J. C. DACRE and R. T. WILLIAMS, J. Pharm. Pharmac. *20*, 610 (1968).
[6] J. DAYMAN and J. B. JEPSON, Biochem. J. *113*, 11P (1969).
[7] R. R. SCHELINE, Acta pharmac. tox. *26*, 189 (1968).
[8] A. N. BOOTH and R. T. WILLIAMS, Nature *198*, 684 (1963).
[9] A. N. BOOTH and R. T. WILLIAMS, Biochem. J. *88*, 66P (1963).
[10] T. MIDTVEDT and A. TRIPPESTAD, Acta path. microbiol. scand., in press.