A COMPLEX OF FUNCTIONALLY-BOUND ENZYMES: TRANSKETOLASE AND GLYCERALDEHYDEPHOSPHATE DEHYDROGENASE

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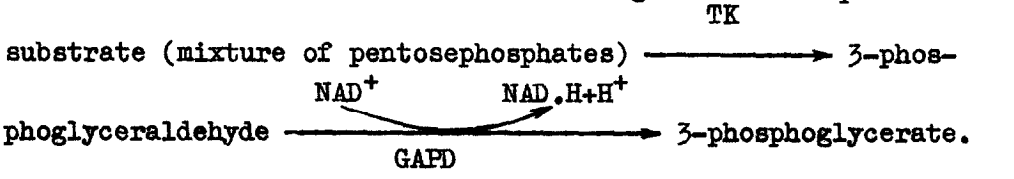
Summary. By the methods of ion exchange chromatography and disc electrophoresis on polyacrylamide gel it has been shown that in the enzyme preparations isolated from baker's yeast there are both free transketolase and glyceraldehydephospate-dehydrogenase and the complex of these functionally bound enzymes. Being stored in alkaline solution of ammonium sulphate this complex dissociates into the above two components.

In the course of the isolation of transketolase (TK; EC: 2.2.1.1) from the baker's yeast, considerable amounts of glyceraldehydephosphate dehydrogenase (GAPD; EC: 1.2.1.12) have been noticed to be present in all preparations of the enzyme at all stages of its purification. TK free from GAPD could only be obtained by several fractionations from an alkaline solution of ammonium sulphate. This fact is not in line with the physicochemical properties of these enzymes (1-5): they should have readily separated, both in the course of DEAE-cellulose chromatography and during fractionation by ammonium sulphate (stages of TK purification). Therefore we suggest that together with the free forms of TK and GAPD in the yeast extract there is also a complex of these enzymes. When the complex is treated with an alkaline solution of ammonium sulphate it breaks down yielding TK free from GAPD. The present paper describes the results of the above investigation.

MATERIALS AND METHODS

Transketolase was isolated from the baker's yeast according to the procedure of Racker et al. (2). Crystalline enzyme and also preparations obtained at intermediate stages of purification were used in this work.

TK activity was determined spectrophotometrically by the rate of reduction of NAD in the following reaction sequence:



GAPD isolated from the rabbit muscle (6) was used as a coupling enzyme. The mixture of pentosephosphates which was used as a TK substrate was isolated from a ribose-5-phosphate preparation (1). Transketolase activity was determined in the following system (system 1, final concentrations): 1.5×10^{-4}M Tris buffer, pH 7.6; 8×10^{-3}M pentosephosphates mixture; 4.4×10^{-5}M sodium arsenate; 5×10^{-7}M NAD; 3 units of GAPD from rabbit muscle; 2×10^{-3}M $MgCl_2$; 2×10^{-4}M thiamine pyrophosphate (total volume - 2 ml).

The activity of GAPD was measured spectrophotometrically by the rate of formation of reduced NAD with 3-phosphoglyceraldehyde (PGA) as a substrate in the following system (system 2; final concentrations): 1.5×10^{-4}M Tris buffer, pH 7.8; 10^{-3}M PGA, 5×10^{-5}M sodium arsenate; 10^{-6}M NAD; 1.2×10^{-2}M β-mercaptoethanol (total volume - 2 ml).

Ion exchange chromatography of the TK preparations was done on CM-Sephadex C-50. Disc electrophoresis was carried out on 6% polyacrylamide gel in the anion system for 2.5 hours at a current of 2 ma per tube. Tris buffer (0.041M, pH 8.9) containing 0.0063M boric acid and 0.0017M EDTA was used in the electrode vessels.

After electrophoretic separation the enzymatic activity of the protein fractions obtained was visualized by means of tetrazolium staining (7). Gel tubes were incubated at 37°C in system 1* (to reveal TK activity) or in system 2* (to reveal GAPD activity).

RESULTS AND DISCUSSION

Figure 1a presents the data of chromatographic separation of the TK preparation containing GAPD. Three active fractions could be detected. Fractions II and III are, respectively, GAPD and TK. Fraction I has the activities of both enzymes. Attempts to separate fraction I by means of other types of ion exchange Sephadexes in pH range from 5.5 to 9.5 and under different chromatographic conditions have also failed.

At 60% ammonium sulphate saturation fractions I and III precipitate; fraction II remains in the solution. This can be seen in figure 1b showing the results of chromatographic separation of the protein precipitate. It should be noted that if the enzymatic preparation is submitted to chromatography not at once (as in the case shown in Fig. 1b), but after being kept in alkaline solution of ammonium sulphate (pH 7.7), fraction II reappears (Fig. 1c). The above experimental results may be explained by the presence in the initial enzyme preparation of the TK-GAPD complex which breaks up in the course of treatment with alkaline solution of ammonium sulphate.

The existence of TK-GAPD complex is also confirmed by disc electrophoresis evidence. Fig. 2 shows the electrophoretic separation of the partially purified TK preparation containing

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* Each system contained also 0.3 mg/ml of nitroblue tetrazolium and 0.03 mg/ml phenazine methosulphate; in system 2 β-mercaptoethanol was omitted.

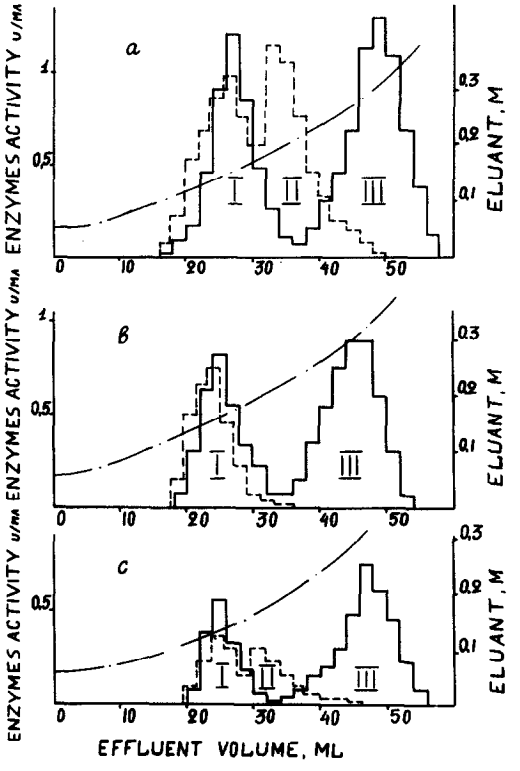

Fig. 1. Chromatographic separation of TK preparation containing GAPD activity.

A solution of the enzyme was applied to a column (0.5 x 30 cm) filled with CM-Sephadex C-50 equilibrated with 0.05 M phosphate buffer, pH 5.7. Elution was carried out by increasing concentration of phosphate buffer, pH 5.7. —— TK activity; — — — — GAPD activity —— · —— concentration of the eluting phosphate buffer.

a. Initial TK preparation. 7 mg of protein were applied.

b. Protein fraction obtained by treatment of the initial TK preparation by ammonium sulphate (60% saturation). 4 mg were applied.

c. Same as in "b" but prior to applying to the column the preparation was kept in alkaline solution of ammonium sulphate. 2 mg of protein were applied.

GAPD activity on polyacrylamide gel. Transketolase and glyceraldehydephosphate dehydrogenase activities were subsequently determined by tetrazolium staining. Figs. 2a and 2c show respectively the activity in systems 1 and 2 (see "Materials and methods"). Fig. 2b shows the activity in system 1 without the

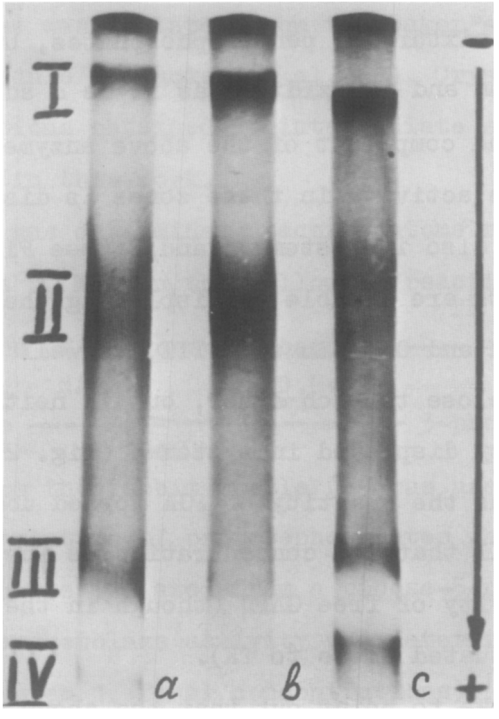

Fig. 2. Electrophoretic separation of TK preparation (one of the intermediate stages of enzyme purification); TK and GAPD activities was visualized by tetrazolium staining. 100 μg of protein per tube was applied.

a. Enzymatic activity in system 1.

b. Enzymatic activity in system 3.

c. Enzymatic activity in system 2.

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coupling enzyme (GAPD from muscle) (system 3). Activities revealed in systems 1 and 2 belong respectively to TK and GAPD. As to system 3, no activity should have been displayed as it contains no substrate for GAPD, besides there is no coupling enzyme (GAPD from muscle) for TK in whose absence transketolase reaction does not appear in electrophoretogram (see "Materials and Methods"). Nevertheless, this system displays activity (Fig. 2b). This phenomenon can be explained only by the presence of a complex of the two enzymes - TK and GAPD in

zones I and II*. Due to the action of transketolase, PGA is formed from the mixture of pentosephosphates, but it does not get into solution and is oxidized as it is a substrate for GAPD - the second component of the above enzyme complex. It is natural that the activity in these zones is displayed not only in system 3 but also in systems 1 and 2 (see Figs. 2a and 2c), as the components are capable of displaying their activity individually. TK and GAPD in zone III (as well as zone IV) are localized very close to each other, but in neither of these zones is activity displayed in system 3 (Fig. 2b). This is understandable as the quantity of PGA formed under the action of TK is so small that its concentration is insufficient to reveal the activity of free GAPD (though in the electrophoretogram it is situated close to TK).

We would like to point out that the above work may be interpreted to mean that combinations of functionally related enzymes occur not only in organelles of the cell but also in the cytoplasm.

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* It should be noted that in the recent paper (8) the authors also suggest the existence of the complex between two functionally bound enzymes - TK and transaldolase.

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