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A new form of baker's yeast transketolase

An enzyme-RNA complex

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Using an immunosorbent, a new form of transketolase, namely, an enzyme-RNA complex, was isolated from a baker's yeast extract. Spontaneous fission of RNA (or its enzymic hydrolysis by RNase) is accompanied by a sharp increase in the catalytic activity of transketolase, which may be directly related to the enzyme's regulation mechanism.

1. INTRODUCTION

The method of transketolase (TK) isolation from baker's yeast was developed by Racker et al. in 1955 [1] and modified by them in 1958 [2]. Until recently, it was employed only with minor modifications [3-5]. Meanwhile, we have suggested a new method of isolating TK by means of TK-specific antibodies, immobilized on a non-soluble matrix [6]. The enzyme yield attained to 90% of its amount in the original yeast extract. The spectral characteristics of the enzyme preparation, obtained at the stage of elution from the immunosorbent (other protein mixtures were not present), demonstrated the presence of a non-protein component.

In the present work we give experimental data showing that this non-protein component is found in a complex with TK; it represents ribonucleic acid and appears to be directly involved in the regulation of TK activity.

2. MATERIALS AND METHODS

TK was isolated from baker's yeast by the method of Racker et al. [2] with some modifications [5], as well as by the method with the use of the immunosorbent [6]. TK activity was measured as in [1]. The amount of protein was estimated by the Bradford method [7]. Chromatography on Sephacryl S-300 (Pharmacia) was performed on a column (1.6 × 82 cm) equilibrated with 0.1 M NaCl, 0.5 mM K-P buffer, pH 7.6. Elution was carried out with the same buffer. The rate of elution was 1 ml/min, the volume of fractions 2-3 ml. The volume of the applied enzyme solution was 2-5 ml. The concentration of TK was 2 mg/ml.

Absorption spectra were taken on a two-line spectrophotometer Hitachi U 3400 (Japan).

To investigate pH-stability, 10-13 μg of TK in 1 ml of 0.1 M K-P buffer at the given pH value was incubated for 15 min at 37°C, then 20-100 μl of this solution was transferred to cuvettes to determine the TK activity in standard conditions at pH 7.6.

To study the effect of ribonuclease A (Worthington) on the TK activity, 20-50 μg of TK with 0.3-0.6 μg of RNase A in 0.43 ml of 10 mM MOPS buffer, 0.5 mM EDTA, pH 7.6, was incubated at 37°C. At fixed intervals aliquots were collected to assay the TK activity.

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Abbreviations: TK, transketolase (EC 2.2.1.1); TK-I, TK isolated using the immunosorbent, but not passed through a column with DEAE-cellulose [6]; TK-R (or free TK), TK isolated according to Racker et al. [2]; RNase, ribonuclease A; MOPS, 3-*N*-morpholinopropanesulfonic acid; EDTA, ethylenediaminetetraacetate

3. RESULTS

The method of TK purification suggested by us in [6], includes the following steps: extraction, immunosorbent chromatography, dialysis, concentration, and chromatography on DEAE-cellulose. The absorption spectrum of the enzyme, obtained by this technique, does not differ from that of TK-R (spectrum 1 and 2 in Fig. 1) and is a typical absorption spectrum of a simple protein.

On the other hand, the absorption spectrum of TK-I before passage through DEAE-cellulose (3 in Fig. 1) differs noticeably from spectra 1 and 2 in Fig. 1 and attests to the presence of a non-protein component in the enzyme preparation. It ought to be observed that on passing through DEAE-cellulose, a portion of TK-I does not bind to an ion-exchange column, while some other portion of the enzyme and the non-protein component to bind tightly enough (data not shown).

3.1. *TK chromatography on Sephacryl S-300*

In TK-I, run through the Sephacryl S-300 column, the main peak of enzyme yield was considerably smaller than that in TK-R (Figs 2A,B). This points to the higher value of TK-I molecular mass when compared with the

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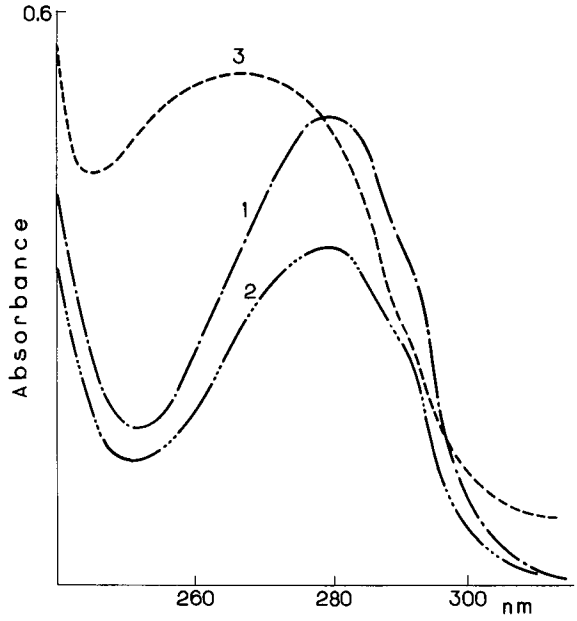

Fig. 1. Absorption spectra of TK. (1) TK-R (0.33 mg/ml); (2) TK-I after chromatography on DEAE-cellulose and concentration (0.24 mg/ml); (3) TK-I (0.12 mg/ml).

free enzyme one. During TK-I re-chromatography (Fig. 2C), the protein's yield (and its activity) increased considerably to become equal to the yield of the TK-R from the column. The absorption spectrum also changed; whereas before re-chromatography it had been analogous to the spectrum of initial TK-I, after re-chromatography it became essentially the same as that of the free enzyme (cf. 1,2 in Fig. 3 and 1,3 in Fig. 1). During re-chromatography the non-protein component was eluted from the column as well (Fig. 2C): its spectrum is given in Fig. 3 (3).

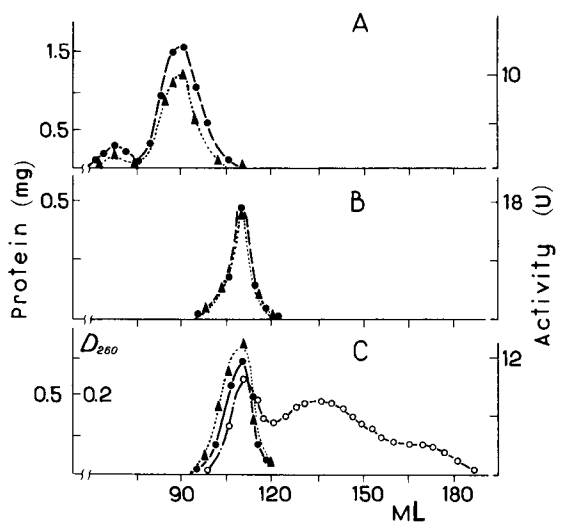

Fig. 2. Chromatography of TK-I (A) and TK-R (B) and re-chromatography of TK-I (C) on Sephacryl S-300; (●—●) protein; (▲—▲) activity of TK; (○—○) optical density at 260 nm.

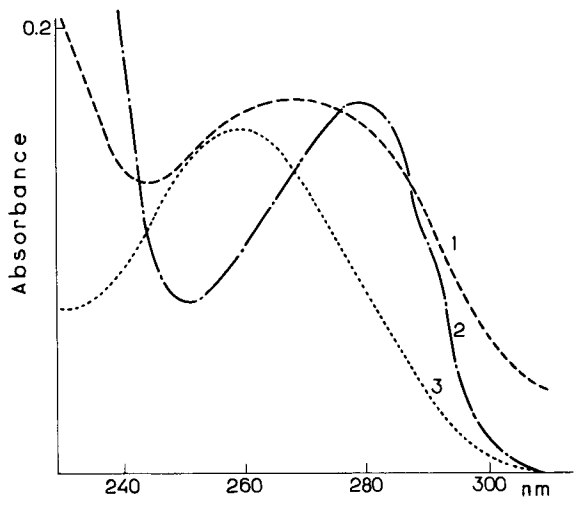

Fig. 3. Absorption spectra of TK-I (after chromatography (1) and re-chromatography (2) on Sephacryl S-300) and a non-protein component (3).

3.2. *pH-stability of TK*

The curve of pH-stability of TK-I has a sufficiently sharp peak at pH 9.0 (1 in Fig. 4) and differs from the curve of pH-stability of TK-R (2 in Fig. 4). The TK-R curve does not change if a free non-protein component, isolated from TK-I, is added to the enzyme (3 in Fig. 4). In case a stored preparation of TK-I was used (4 and 5 in Fig. 4), the curve of pH-stability became similar to that of pH-stability of the free enzyme (2 in Fig. 4).

3.3. *The ribonuclease A effect on the transketolase activity*

The incubation of TK-I with RNase A results in a dramatic increase in the catalytic activity of the enzyme

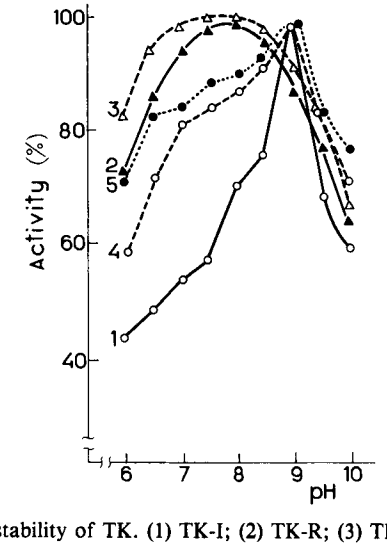

Fig. 4. pH-stability of TK. (1) TK-I; (2) TK-R; (3) TK-R + a non-protein component, isolated from TK-I (see Fig. 2C); (4) and (5) TK-I was stored for 3 and 4 days in 0.1 M K-P buffer, pH 7.6 at 4°C, respectively. 100% activity corresponds to the activity in the maximum of pH stability of each enzymic preparation.

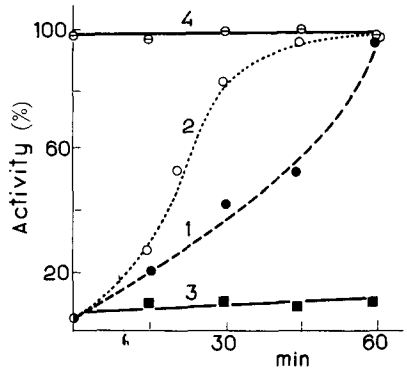

Fig. 5. RNase effect on the activity of TK. (1) and (2) TK-I in the presence of 3 and 6 μg of RNase A, respectively; (3) TK-I in the absence of RNase A; (4) TK-R in the presence of 6 μg of RNase. 100% activity corresponds to maximum activity TK-I in the presence of 6 μg of RNase (curves 1,2,3) and for TK-R (curve 4) to the activity before incubation.

(1 and 2 in Fig. 5). In the absence of RNase, this increase is not so pronounced (3 in Fig. 5). RNase did not affect the activity of free TK (4 in Fig. 5).

4. DISCUSSION

The absorption spectrum of TK, isolated from the yeast extract with the aid of an immunosorbent (TK-I), is not typical of absorption spectra of simple proteins and indicates the presence of a non-protein component in the enzyme preparation. This non-protein component and TK form a complex. The following experimental data support this statement.

Sephacryl S-300 chromatography revealed no separation of the protein and the non-protein component. This is confirmed, first, by the protein peak absorption spectrum, taken from the column (1 in Fig. 3) - it does not essentially differ from that of the initial enzyme before passage through Sephacryl S-300 (3 in Fig. 1). Secondly, the TK-I yield from the column is considerably lower than that of TK-R (Figs 2A and B), which indicates a greater value of TK-I molecular mass compared with free TK.

TK-I is not a stable complex, it dissociates into the protein and the non-protein component by rechromatography (Fig. 2C).

The sharp difference of pH-stabilities of TK-I and TK-R points to the presence of the complex. The addition of the free non-protein component to TK-R has no effect on its pH-stability (Fig. 4). The absorption spectra of TK-I (3 in Fig. 1 and 1 in Fig. 3) and of its non-protein component (3 in Fig. 3) show the latter to be a nucleic acid. The TK-I positive reactions in both orcinol and ethidium bromide tests and identifiction of A, G, T(U) and C in the hydrolyzate of the free non-protein component (data not shown) support this statement as well. And finally, the RNase effect on the enzymic activity of TK-I, but not of the free TK (Fig. 5), testifies that RNA is a non-protein component of TK-I. The increase in the TK-I specific activity during its storage (data not shown), together with the decrease in the differences between pH-stabilities of TK-I and the free TK (Fig. 4), shows that the TK-RNA complex is not stable.

Thus, a new form of TK, that is an enzyme-RNA complex (bound TK) was isolated from the yeast extract using an immunosorbent. Although the existence of the thiamine diphosphate-enzyme complexes with RNA was not shown before, other enzymes were known to form complexes with RNA [8-11]. The relative proportion of TK in the free and in the bound forms of the enzyme preparation varies from experiment to experiment. It is difficult to say at present whether this proportion is inherent to the initial yeast extract or even to the yeast cell itself. Since the TK-RNA complex is sufficiently labile, we cannot exclude the possibility of its partial dissociation into RNA and free TK during its extraction from yeast and subsequent purification. The degree of this process differs in different isolations. A pronounced increase in TK activity is due to the spontaneous liberation of RNA from the complex with TK or its cleavage by RNase. Thus, the possibility that RNA is involved in the enzyme's activity regulation in the living cell cannot be excluded.

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