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Cocarboxylase Activity of Thiamine Triphosphate in Yeast Transketolase

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The ability of thiamine tri- and monophosphate to replace thiamine diphosphate (cocarboxylase) in transketolase isolated from baker's yeast has been studied.

Neither the tri- nor the monophosphate showed any remarkable cocarboxylase activity in this system. Only when thiamine triphosphate was transformed into thiamine diphosphate by means of phosphatases cocarboxylase activity was obtained.

Thiamine triphosphate * has been found in rat liver [1] and in baker's yeast [2,3]. Isolated from yeast this compound was shown to be identical with synthetic thiamine triphosphate with regard to the arrangement of the phosphate groups [3].

Very little is known about the action of TTP and its localization. On the basis of the observed action of TTP on the frog's heart Plotka *et al.* [4] put forward the hypothesis that TTP plays a role in the special metabolism related to nerve impulse transmission.

The ability of synthetic TTP to replace TDP in an isolated carboxylase from baker's yeast has been studied by de la Fuente and Diaz-Cadavieco [5], Kiessling [3], and Rossi-Fanelli *et al.*[6] In this system TTP was found to be inactive. This was also the case with TTP isolated from yeast [3]. Cocarboxylase activity was obtained from TTP only after pre-incubation with a potato apyrase at 0°C, when γ-P is split off [3].

An entirely different explanation of the above negative results has been put forward by Bartos [7] who suggests the existence in yeast of two distinct apoenzymes, one combining with TDP, the other with TTP. Thus only the former should have been isolated in the experiments referred to above [5,3,6].

The turnover rate of the different phosphate groups of TTP in baker's yeast has been studied with glucose as a substrate and in the presence of

* Abbreviations: TTP, thiamine triphosphate; TDP, thiamine diphosphate (cocarboxylase); TMP, thiamine monophosphate; EDTA, ethylenediaminetetraacetate (Versene); DPNH, reduced diphosphopyridine nucleotide; AMP, adenosine-5-phosphate.

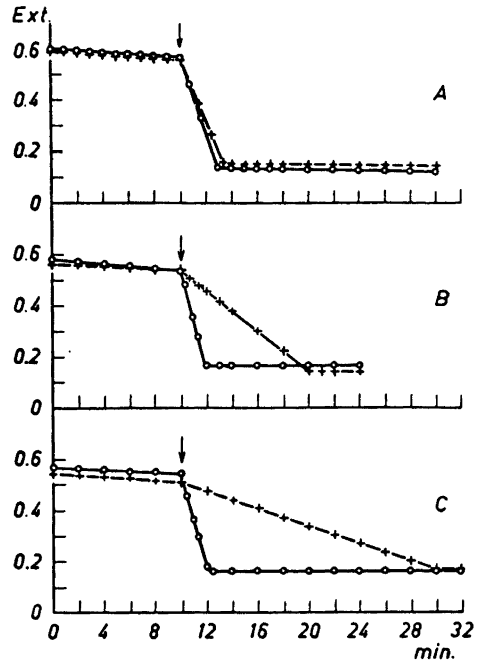

Fig. 1. The necessity of TDP by transketolase after dialysis.
The isolation of the enzyme was performed according to de la Haba *et al.*[12], and carried on to the protamine step.
The arrows indicate the addition of transketolase. For remaining additions, see "Composition of samples".
A, no dialysis; B, 23 h; and C, 43 h dialysis.

+———+ no synthetic TDP present
O———O 0.05 ml 0.1 % TDP present

radioactive inorganic phosphate [8]. The terminal phosphate group became radioactive more rapidly than the β- and α-P. Synthetic TTP added to rat liver mitochondria did, however, not exchange its phosphate groups [9].

In homogenates and in protein fractions from rat liver, however, Greiling and Kiesow [10] found, in the absence of ATP, an enzymatic transfer of phosphate from TTP to glucose.

From yeast an enzyme, named transketolase, has been crystallized [11,12] which transforms ribose-5-phosphate and ribulose-5-phosphate into a mixture of heptulose-7-phosphate and glyceraldehyde-3-phosphate:

Ribose-5-phosphate + ribulose-5-phosphate $\rightleftharpoons$
$\rightleftharpoons$ heptulose-7-phosphate + glyceraldehyde-3-phosphate

This reaction has been shown to be conditioned by TDP and magnesium [11]. As TTP occurs in yeast and the concentration can be considerably increased by suitable pre-incubation of the yeast [2,3], the ability of TTP to replace TDP in a transketolase isolated from baker's yeast has been investigated in this paper.

EXPERIMENTAL

Transketolase. The transketolase has been isolated from baker's yeast * according to de la Haba *et al.*[12] The isolation was carried on to the protamine treatment. At this stage transketolase is stable for weeks if kept at 5°C. The enzyme shows no need of TDP and magnesium (Fig. 1 A). Only after prolonged dialysis against a solution [11] of 0.6 % EDTA-0.9 % KCl, pH 7.4, reduction and eventual loss of transketolase activity was obtained. This was, however, restored by the addition of magnesium and TDP (Figs. 1 B and 1 C).

Ribose-5-phosphate was prepared by boiling adenosine-5-phosphate for ten minutes in 1 N HCl, removal of insoluble barium salts, and precipitation of the barium salt of ribose-5-phosphate with four volumes of ethyl alcohol [13].

Isomerase, free of transketolase, was isolated from baker's yeast as described by de la Haba *et al.*[12]

Isomerase product. The equilibrium mixture of ribose-5-phosphate and ribulose-5-phosphate (isomerase product) was prepared from ribose-5-phosphate by incubation for 3 h with dialyzed isomerase [12]. The resulting product was kept in the form of the barium salt at 2°C, and transformed into the ammonium salt before use [12].

α-Glycerophosphate dehydrogenase from rabbit muscle was prepared according to Racker [13].

TTP, TDP, TMP. A mixture of thiamine phosphates was prepared according to Viscontini *et al.*[14], and TTP, TDP and TMP were isolated according to Kiessling [15]. As, however, the samples obtained in this way were poisonous when added to the transketolase, the solvent impurities were removed by re-chromatography of the isolated compounds in a second non-poisonous solvent (*n*-propanol-H_2O-1 M acetate buffer pH 5, 70:20:10) described by Siliprandi and Siliprandi [16].

DPNH and crystalline *AMP*, both from yeast, were obtained from Sigma Chemical Company. The extinction was measured in a Beckman spectrophotometer at 340 mμ.

Composition of samples. The incubation was performed mainly according to de la Haba *et al.*[12] Into a quartz cell (holding 4 ml) were pipetted: 0.05 ml of 0.5 M glycylglycine buffer, pH 7.6, 0.05 ml of 0.002 M DPNH, 0.05 ml of crude glycerophosphate dehydrogenase, 0.05 ml of the ammonium salt of isomerase product solution, 0.05 ml of 0.06 M $MgCl_2$, 0.1 ml of a transketolase solution containing about 1 000 units per ml **, samples of TTP, TDP or TMP (see below), and water to make 4 ml.

RESULTS

Dialysis of the transketolase preparation for 48 h gives an enzyme which, without addition of TDP, is inactive (Fig. 2A). Addition of TDP rapidly gives rise to an oxidation of DPNH. The same amount of TTP is less active (Fig. 2A). That TTP is active at all may be explained in different ways. Either TTP, to a certain degree, can replace TDP, or it is decomposed to TDP by phosphatases. The possibility that the added TTP is initially contaminated with TDP is excluded by the paper chromatographic controls. In order to find out if TDP is formed from TTP by phosphatases, samples were taken after different incubation intervals, and analyzed by paper chromatography according to Kiessling[15]. The chromatograms showed that TTP was rapidly transformed into TDP and TMP. After 15 min only about 30 % remained as TTP.

When, on the other hand, TTP and TDP are added later during the incubation (Fig. 2B), the oxidation of DPNH in the TTP incubation is considerably retarded, whereas in the TDP incubation the oxidation starts almost immedia-

* The baker's yeast was from Uppsala Ångkvarn Ltd., Sweden.

** A unit of enzyme is defined according to de la Haba *et al.*[12] as the amount of enzyme which gives an extinction change of 0.001 per min under the conditions of the assay.

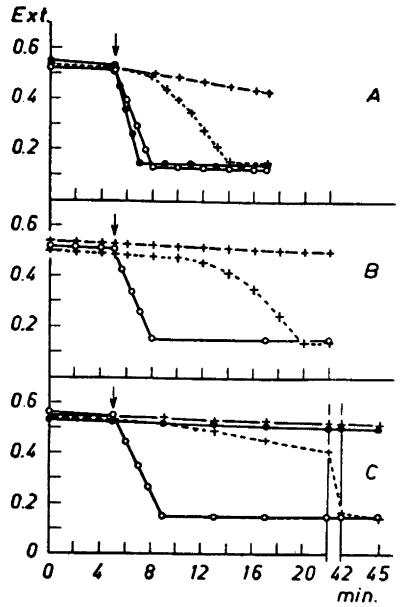

Fig. 2A. Effect of TDP and TTP on transketolase dialyzed for 48 h. The arrow indicates the addition of transketolase. TDP and TTP have been purified by paper chromatography as described in "Experimental".

+————+ no thiamine phosphate added
+– – – –+ 0.36 ml TTP (100 μg)
○————○ 0.11 ml TDP (100 μg)
●————● 0.36 ml TDP (327 μg)

B. Effect of the later addition of TTP and TDP. Transketolase is present from the beginning. The arrow indicates the point, when the thiamine phosphates are added. The designations of the curves are the same as in Fig. 2 A.

C. Effect of TTP, TDP and TMP on transketolase in the presence of thiamine. The arrow indicates the addition of the thiamine phosphates. 300 μg thiamine were added to each sample at the beginning of the incubation.

+————+ no thiamine phosphate added
+– – – –+ 0.36 ml TTP (100 μg)
●————● 0.30 ml TMP (100 μg)
○————○ 0.11 ml TDP (100 μg)

tely after the addition of TDP. This result gives further evidence for TTP being inactive as coenzyme in transketolase, and becoming active only after phosphatases have transformed it into TDP.

Westenbrink and van Dorp [17] have shown that yeast phosphatases acting on thiamine phosphates are strongly inhibited by thiamine. Thus addition of thiamine to the incubation mixture might prevent the decomposition of TTP to TDP. The results from an experiment are shown in Fig. 2C. It is seen that the complete oxidation of DPNH is effected at a much slower rate in

the presence of thiamine than in its absence when phosphatase activity is not inhibited. The oxidation rate in the presence of TDP is not noticeably affected by thiamine.

Fig. 2C also shows that TMP has no ability to replace TDP in this system.

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