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Two Methods for Determination of Transketolase Activity

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Abstract—Two new optical methods for transketolase activity assay using only one substrate, xylulose 5-phosphate or glycol aldehyde, have been developed. For transketolase activity assay in the first method, it is necessary to add auxiliary enzyme, glyceraldehyde phosphate dehydrogenase. It is not needed in the second method. The range of transketolase concentration in the activity assay is 0.036-0.144 U/ml for the first method and 1.8-6.8 U/ml for the second one.

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Transketolase (EC 2.2.1.1) is a key enzyme in the nonoxidative branch of the pentose phosphate pathway that transfers a two-carbon glycol aldehyde unit from ketose (donor substrate) to aldose (acceptor substrate) [1-4] (Fig. 1). Thiamin diphosphate and bivalent cations such as Ca^{2+}, Mg^{2+}, and some others are enzyme cofactors. Transketolase can utilize as donor substrates such sugars as xylulose 5-phosphate, sedoheptulose 7-phosphate, fructose 6-phosphate, and erythrose 4-phosphate, and also such compounds as dihydroxyacetone phosphate and hydroxypyruvate. Ribose 5-phosphate, glyceraldehyde 3-phosphate, erythrose 4-phosphate, and glycol aldehyde can serve as acceptor substrates for transketolase. The transketolase reaction intermediate is dihydroxyethyl-thiamin diphosphate, the glycol aldehyde residue bound to the coenzyme.

MATERIALS AND METHODS

There are several methods for measuring the activity of transketolase. In one, the activity can be determined chemically by the quantity of the product, sedoheptulose 7-phosphate, formed in the transketolase reaction (Fig. 1) if ribose 5-phosphate is used as acceptor substrate [1]. Another method is based on the ability of dihydroxyethyl-thiamin diphosphate to be readily oxidized to glycolate in the presence of $K_3[Fe(CN)_6]$. The reaction can be followed spectrophotometrically, by the optical density decrease at 420 nm [5]. There is yet another method that requires auxiliary enzymes; transketolase activity is determined by measuring the rate of glyceraldehyde 3-phosphate formation by transketolase from xylulose 5-phosphate (Fig. 1). The amount of glyceraldehyde 3-phosphate can be measured by the increase in absorbance at 340 nm due to the formation of NADH by coupled glyceraldehyde phosphate dehydrogenase [1].

Each of these methods has its advantages and drawbacks, and the choice of each particular method depends on the objective of the research. For instance, in routine experiments the most convenient is the method employing auxiliary enzymes [6-9]. However, the determination of affinity of donor substrates to transketolase is best achieved by use of one more, simple and convenient, method employing the artificial electron acceptor $K_3[Fe(CN)_6]$ because this method enables, among other things, to work with a wide variety of donor substrates [10, 11].

In the study presented herein, we propose two more methods for determination of transketolase activity using only one substrate: either donor substrate (xylulose 5-phosphate) or acceptor substrate (glycol aldehyde). In this case, use of an artificial electron acceptor is not required.

Materials. In this work we used thiamin diphosphate, glycyl-glycine, and $CaCl_2$ from MP Biomedicals (Germany); glyceraldehyde-3-phosphate dehydrogenase, xylulose 5-phosphate, and glycol aldehyde were from Sigma (USA); other chemicals were domestic products of the highest quality commercially available. Transketolase

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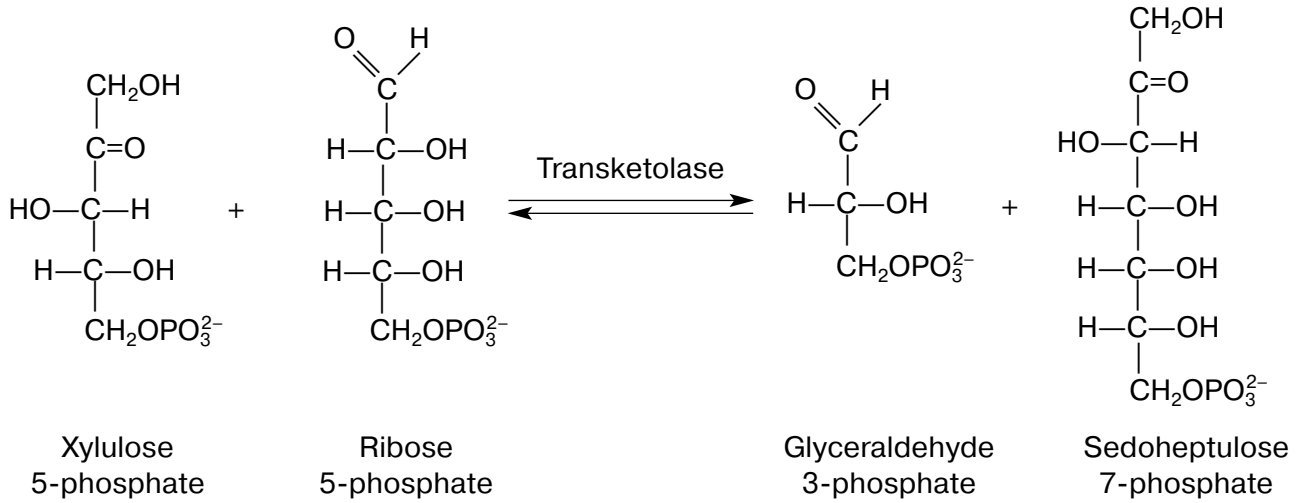

Fig. 1. Scheme of the transketolase reaction. Here xylulose 5-phosphate and ribose 5-phosphate are used as substrates for the transketolase reaction.

was isolated from *Saccharomyces cerevisiae* according to the method described earlier [12].

RESULTS AND DISCUSSION

Determination of transketolase activity using a ketose (xylulose 5-phosphate) as substrate. Following the splitting of the ketose (donor substrate) under the action of transketolase, the first product of the transketolase reaction, aldose, is formed (Fig. 1), along with formation of dihydroxyethyl-thiamin diphosphate, a glycol aldehyde residue covalently bound to the coenzyme within the holoenzyme. In the typical two-substrate reaction, the glycol aldehyde residue is then transferred to the aldose, the acceptor substrate. As a result, the second product of the transketolase reaction, a new ketose, is formed; at the same time, the initial (not containing the glycol aldehyde residue) form of the holoenzyme is restored. The splitting of the glycol aldehyde residue from the coenzyme can occur in the absence of acceptor substrate as well. In other words, transketolase is able to catalyze not only the two-substrate but also the one-substrate reaction (in the absence of aldose, with only ketose as substrate) [13]. If xylulose 5-phosphate is used as substrate, the first product of the reaction will be glyceraldehyde 3-phosphate. By using glyceraldehyde phosphate dehydrogenase as an auxiliary enzyme it is possible to monitor the one-substrate reaction course by the increase in absorbance at 340 nm caused by NAD reduction due to glyceraldehyde 3-phosphate oxidation.

As seen from Fig. 2, the reaction rate is constant for at least 14 min, and there is a well defined linear dependence between the reaction rate and the quantity of the transketolase added (inset in Fig. 2). The range of transketolase concentration in the activity assay is 0.036-0.144 U/ml.

Determination of transketolase activity using glycol aldehyde as substrate. The transketolase reaction intermediate, dihydroxyethyl-thiamin diphosphate (glycol

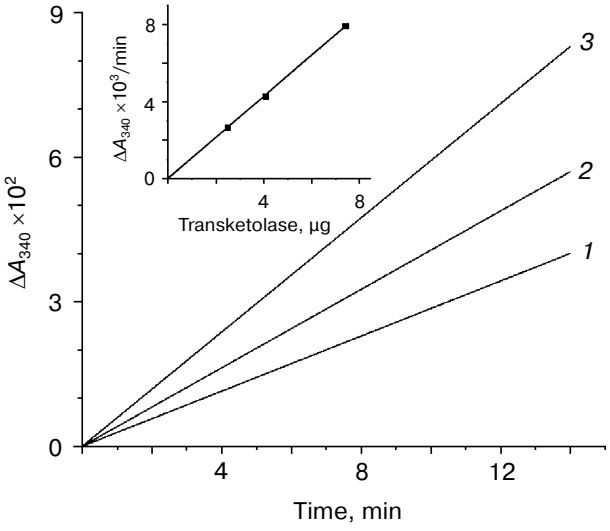

Fig. 2. Changes in optical density at 340 nm during one-substrate transketolase-catalyzed reaction. The reaction mixture (final volume 1 ml) contained 50 mM glycyl-glycine, 1 mM sodium arsenate, 0.37 mM NAD, 3 U glyceraldehyde phosphate dehydrogenase, 3.2 mM dithiothreitol, 2.5 mM $CaCl_2$, 80 μM thiamin diphosphate, 70 μM xylulose-5-phosphate, and 2.5, 4, or 7.5 μg (curves *1*, *2*, and *3*, respectively) transketolase (12 U/mg), pH 7.6. The reaction was initiated by transketolase and was monitored via the change in absorbance at 340 nm (Aminco DW 2000 spectrophotometer (USA); path length 1 cm). Inset: dependence of the one-substrate reaction rate on transketolase concentration.

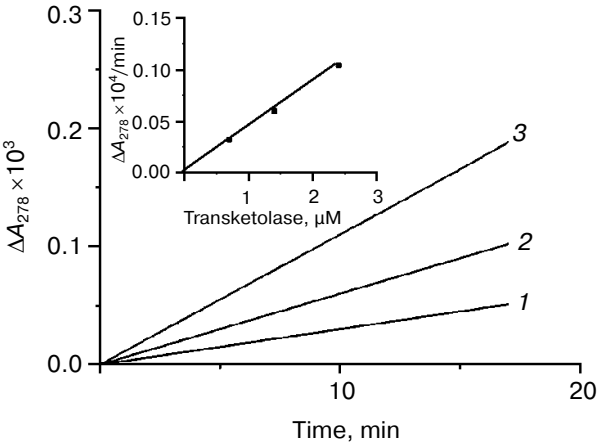

Fig. 3. Kinetics of erythrulose formation by transketolase at different enzyme concentrations. Reaction mixture (final volume 1 ml) contained 50 mM glycyl-glycine, 2.5 mM $CaCl_2$, 60 μM thiamin diphosphate, and 0.7, 1.4, or 2.4 μM (curves *1*, *2*, and *3*, respectively) transketolase (12 U/mg), pH 7.6. The reaction was started by addition of 400 mM glycol aldehyde and was monitored via the change in CD signal at 278 nm (Mark V spectropolarimeter (Jobin Yvon, France), path length 1 cm). The optical density of the reaction mixture at 278 nm was not above $0.25 \cdot 10^{-3}$. Inset: the enzyme concentration dependence of the transketolase reaction rate.

aldehyde residue covalently bound to the coenzyme), can be formed not only through ketose (donor substrate) splitting but also upon direct interaction of the holoenzyme with free glycol aldehyde. The glycol aldehyde residue is then transferred from dihydroxyethyl-thiamin diphosphate to the second molecule of free glycol aldehyde, as a result of which the optically active compound erythrulose is formed [14]. Its amount can be determined by the ellipticity value in the 260-290 nm range. The above events provided the foundation for the proposed method of transketolase activity determination in the one-substrate reaction with glycol aldehyde as substrate. The developed method does not require the use of auxiliary enzymes. The reaction rate remains constant for a sufficiently long time period (Fig. 3) and appears to be proportional to the transketolase concentration (inset in Fig. 3). The range of transketolase concentration in the activity assay is 1.8-6.8 U/ml.

To summarize, we present herein two newly developed, simple, and reproducible optical methods for determination of transketolase activity by using, respectively, xylulose 5-phosphate or glycol aldehyde as substrates. These methods can be of particular assistance in studying the kinetic mechanisms of the transketolase function and the individual stages of the transketolase reaction. Besides, the second method is specific and the reaction product can be readily detected in a composite mixture of substances with low optical activity.

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