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Enzymatic synthesis of D-xylulose 5-phosphate from hydroxypyruvate and D-glyceraldehyde-3-phosphate

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Abstract

An enzymatic method for obtaining D-xylulose 5-phosphate has been developed, based on the irreversible reaction catalyzed by transketolase: hydroxypyruvate + D-glyceraldehyde-3-phosphate → D-xylulose 5-phosphate. The preparations of sodium D-xylulose 5-phosphate, obtained using this approach, were 88% pure and contained no aldehyde admixtures.

Keywords: Enzymatic synthesis; D-Xylulose 5-phosphate; Transketolase

1. Introduction

Transketolase (EC 2.2.1.1) catalyzes the reversible transfer of a two-carbon fragment, a glycol aldehyde residue, from ketose (donor substrate) to aldose (acceptor substrate). If hydroxypyruvate is used as a donor substrate, the reaction becomes irreversible, yielding, among other products, CO_2.

The most common method for measurement of transketolase activity is spectrophotometric measurement based on the use of D-xylulose 5-phosphate as a donor substrate and D-ribose 5-phosphate as an acceptor substrate [1]. The first product of the transketolase reaction is D-glyceraldehyde-3-phosphate, which is formed as a result of D-xylulose 5-phosphate cleavage. The reaction is monitored spectrophotometrically, by measuring the rate of NAD reduction in the course of D-glyceraldehyde-3-phosphate oxidation; glyceraldehyde-3-phosphate dehydrogenase is used as an accessory enzyme.

D-Xylulose 5-phosphate is currently available as a commercial preparation: Sigma Chemical Company has just started again the production of D-xylulose 5-phosphate, but (on our data) it contains 6–10% of D-ribose 5-phosphate. Besides, it is very expensive. For this reason, everybody working with transketolase or other enzymes using D-xylulose 5-phosphate as a substrate (e.g., D-ribulose-5-phosphate epimerase, EC 5.1.3.1) are forced to synthesize it themselves.

The synthetic use of transketolase with hydroxypyruvate as the donor substrate and with a series of different aldehydes has been well studied [2–6]. The reactions go to completion due to the release of carbon dioxide. The use of D-glyceraldehyde-3-phosphate as acceptor substrate allows to synthesise D-xylulose 5-phosphate.

Several enzymatic methods for obtaining D-xylulose 5-phosphate have been reported in the literature. Racker and co-workers [7,8] used as a source of D-glyceraldehyde-3-phosphate the system D-fructose diphosphate + aldolase. Wood added glyceraldehyde-3-phosphate (as a mixture of D- and L-isomers) directly into the reaction mixture [9]. The method proposed by Zimmermann et al. [10] is in essence similar to that of Racker and co-workers, the only difference being that triose-phosphate isomerase was additionally introduced into the reaction mixture, making it possible to utilize both triose phosphate equivalents formed as a result of aldolase-catalyzed fructose diphosphate cleavage.

These methods give no indications of the purity of D-xylulose 5-phosphate preparations and the presence therein of aldehydes, which are potential acceptor substrates for transketolase. Of note, the amount of aldehydes present in D-xylulose 5-phosphate preparations that were available previously from Sigma was as high as 6% [11]. Specifically, aldehyde admixtures make it extremely complicated to study the one-substrate transketolase

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reaction described recently (in this reaction, only a donor substrate is utilized, whereas an acceptor substrate is not required) [12].

In this work, we describe a method that makes it possible to obtain highly pure aldehyde-free preparations of D-xylulose 5-phosphate with high yields.

2. Experimental

2.1. Materials

Thiamine diphosphate, glycylglycine, and dithiothreitol were obtained from Serva; hydroxypyruvic acid lithium salt, $CaCl_2$, D-fructose 6-phosphate sodium salt, glyceraldehyde-3-phosphate dehydrogenase, and lactate dehydrogenase were from Sigma; other chemicals were of the highest quality commercially available.

2.2. Purification of transketolase

Transketolase (as an apoenzyme) was purified from Saccharomyces cerevisiae as described previously [13] and had a specific activity of 28 U/mg. To prepare the holoenzyme, the apoenzyme was incubated with 0.5 mM $CaCl_2$ and 40 μM thiamine diphosphate at room temperature for 15 min.

2.3. Preparation of D-glyceraldehyde-3-phosphate

The calcium salt of D-glyceraldehyde-3-phosphate was obtained by periodate oxidation of D-fructose 6-phosphate [14]. It was converted into the potassium salt prior to use.

2.4. Determination of D-glyceraldehyde-3-phosphate and hydroxypyruvate

The concentrations of D-glyceraldehyde-3-phosphate and the initial concentration of hydroxypyruvate were determined enzymatically using glyceraldehyde-3-phosphate dehydrogenase [15] and lactate dehydrogenase [16], respectively.

2.5. Determination of D-xylulose 5-phosphate

The concentration of D-xylulose 5-phosphate was measured spectrophotometrically, by the amount of NADH formed as a result of oxidation of D-glyceraldehyde-3-phosphate, the product of transketolase-catalyzed D-xylulose 5-phosphate cleavage (two-substrate transketolase reaction). Glyceraldehyde-3-phosphate dehydrogenase was used as an accessory enzyme. The reaction mixture (2 ml) had the following composition: 50 mM glycylglycine, 10 mM sodium arsenate; 3.2 mM dithiothreitol, 0.37 mM NAD^+; 3 U/ml glyceraldehyde-3-phosphate dehydrogenase; 0.5 mM D-ribose 5-phosphate; 0.7 U/ml holotransketolase; pH 7.6. The reaction was initiated by addition of an aliquot of the sample under study, which contained 0.05–0.2 μmol D-xylulose 5-phosphate. The process was followed until the completion of the reaction (i.e., until complete exhaustion of D-xylulose 5-phosphate) by measuring changes in the optical density of the reaction mixture at 340 nm. The amount of D-xylulose 5-phosphate in the sample under study was calculated considering the molar extinction coefficient for NADH to be equal to $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

2.6. Aldehyde assay

Aldehyde admixtures possibly present in the preparation of D-xylulose 5-phosphate were assessed enzymatically using the system for measuring D-xylulose 5-phosphate concentration (see above), which was modified by (1) omitting D-ribose 5-phosphate and (2) increasing 50 times the amount of the sample under study. The amount of aldehydes capable of serving the function the acceptor substrate in the two-substrate transketolase reaction will be equal to the amount of the converted donor substrate, D-xylulose 5-phosphate.

3. Results

3.1. Principle

When acted upon by transketolase, the residue of glycol aldehyde is transferred from hydroxypyruvate to D-glyceraldehyde-3-phosphate; as a result, D-xylulose 5-phosphate is formed:

$$\text{Hydroxypyruvate} + \text{d-glyceraldehyde-3-phosphate} \rightarrow \text{d-xylulose5-phosphate}.$$

The reaction is irreversible, and, if one of the substrates (e.g., hydroxypyruvate) is taken in excess, the other one (D-glyceraldehyde-3-phosphate) will undergo complete conversion into the reaction product, D-xylulose 5-phosphate.

3.2. Obtaining of barium D-xylulose 5-phosphate

The reaction mixture for the enzymatic synthesis of D-xylulose 5-phosphate (final volume, 100 ml) contained 2 mmol glycylglycine, 2 mmol D-glyceraldehyde-3-phosphate, 10 μmol thiamine diphosphate, 3 mmol hydroxypyruvate, and 600 U holotransketolase (pH 7.3). The incubation was carried out at room temperature. Aliquots of 100 μl were collected at 5–20 min intervals during 120 min. Thereafter, 12 μl 50% $HClO_4$ were added into each aliquot, and the mixtures were centrifuged. D-Xylulose 5-phosphate and D-glyceraldehyde-3-phosphate were measured in supernatants.

Fig. 1 shows a typical result of the analysis of the reaction mixture. It is evident that the loss of D-glyceraldehyde-3-phosphate (curve 1) and the formation of D-xylulose 5-phosphate (curve 2) cease in 90 min, although approximately 0.7% of the original amount of D-glyceraldehyde-3-phosphate remains unreacted (the initial molar ratio hydroxypyruvate/D-glyceraldehyde-3-phosphate is equal to 1.5). Increasing the duration of the incubation did not affect the picture, as Fig. 1 demonstrates.

Following completion of D-xylulose 5-phosphate synthesis, the incubation mixture was cooled in ice (all subsequent stages of the procedure were also carried out in the cold), 15 ml 50%

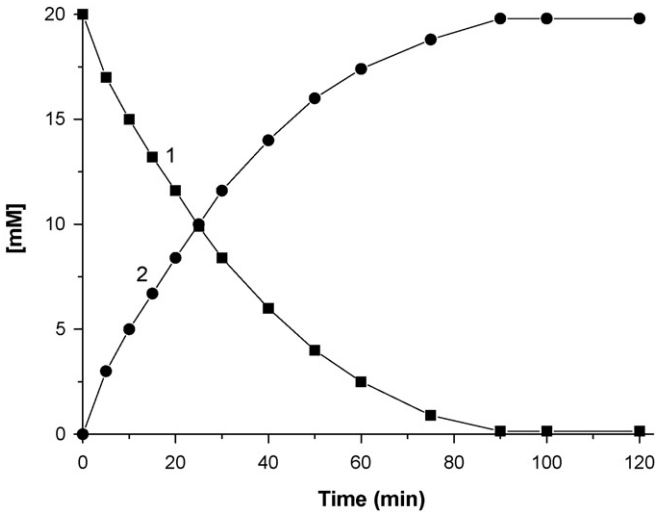

Fig. 1. The decrease in D-glyceraldehyde-3-phosphate (curve 1) and the formation of D-xylulose 5-phosphate (curve 2) in the course of enzymatic synthesis of D-xylulose 5-phosphate from hydroxypyruvate and D-glyceraldehyde-3-phosphate. The ordinate shows the concentrations of D-glyceraldehyde-3-phosphate (■) and D-xylulose 5-phosphate (●), respectively.

$HClO_4$ were added, and the mixture was stirred, followed by centrifugation after 15 min. The precipitate was discarded and the supernatant collected. Dry barium hydroxide (6 mmol) was introduced under stirring into the supernatant until the salt was completely dissolved; pH was adjusted to 6.0 using 2 M KOH. Following 15 min of incubation on ice, the precipitate formed was removed by centrifugation. A fourfold volume of ice-cold ethanol was added to the supernatant, followed by centrifugation after 30 min. The residue was washed twice with ethanol, once with acetone, and air-dried. The preparation was stored frozen.

The preparation of barium D-xylulose 5-phosphate thus obtained contained neither hydroxypyruvate nor D-glyceraldehyde-3-phosphate or other aldehydes. The yield of the product was 2.03 g (30% purity). The preparation was contaminated by barium hydroxide (70%, data not presented).

3.3. Obtaining of sodium D-xylulose 5-phosphate

The part of a preparation of barium D-xylulose 5-phosphate (214 mg), having a purity of 30%, was converted into sodium salt on a Dowex 50 × 8 column, and pH was adjusted to 6.0 using 1 M NaOH, followed by lyophilization. Yield, 41 mg (62% calculated for the initial amount of D-glyceraldehyde-3-phosphate). The enzymatic measurement (see Section 2.5) has shown that the purity of sodium D-xylulose 5-phosphate preparation is 88%.

4. Conclusion

Several enzymatic methods for preparation of D-xylulose 5-phosphate have been reported in the literature. But, these methods give no indications of the purity of D-xylulose 5-phosphate preparations and the presence therein of aldehydes. Specifically, aldehyde admixtures make it extremely complicated to study, for example, one-substrate transketolase reactions described recently [12].

In our work, we describe a method that makes it possible to obtain highly pure (88%) aldehyde-free preparation of D-xylulose 5-phosphate with high yields. What is important is that preparations of D-xylulose 5-phosphate do not contain any admixture of aldehydes.

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