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A Direct Interconversion: D-Fructose 6-Phosphate ⇌ Sedoheptulose 7-Phosphate and D-Xylulose 5-Phosphate Catalyzed by the Enzymes Transketolase and Transaldolase*

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The synthesis of pentose phosphate and heptulose phosphate from hexose monophosphate is known to result from the operation of the oxidative pathway via D-gluconate 6-phosphate, or from the series of nonoxidative transfer reactions catalyzed by transketolase and transaldolase. These transfer reactions require the presence not only of the common substrate, D-fructose 6-phosphate, but also of an acceptor such as D-glyceraldehyde 3-phosphate (1).

The first indication that D-fructose 6-phosphate alone might be converted to heptulose phosphate came from the work of Bonsignore *et al.* (2–4), who found that a thoroughly dialyzed soluble fraction from rat liver would catalyze the synthesis of sedoheptulose 7-phosphate from hexose monophosphate in the absence of any detectable amount of acceptor. This observation has been made independently by other investigators (5).[1]

The synthesis of heptulose in this system was tentatively explained according to three different hypotheses. The first was based on the presence of residual catalytic quantities of acceptor in the enzyme preparation; the second postulated the transfer of glycolaldehyde to D-glucose 6-phosphate to form octulose 8-phosphate, thus generating D-erythrose 4-phosphate; and the third suggested a coupled attack by transketolase and transaldolase upon 2 molecules of D-fructose 6-phosphate, each enzyme cleavage reaction producing the acceptor for the other enzyme (3).

The first hypothesis appeared to be excluded since it was shown that the conversion of hexose monophosphate to sedoheptulose 7-phosphate could be duplicated quantitatively by corresponding mixtures of purified transketolase and transaldolase with D-fructose 6-phosphate as the sole substrate (7, 8). The second hypothesis was based on the report of Racker and Schroeder (9) and more recently of Datta and Racker (10) that D-glucose 6-phosphate can act as an acceptor of "active glycolaldehyde" in the reaction catalyzed by transketolase. This was considered to be unlikely since D-glucose 6-phosphate did not serve as acceptor for the cleavage of D-fructose 6-phosphate with the concentration of transketolase present in these experiments.

However, despite this evidence to indicate that glucose 6-phosphate was not involved in the reaction under our conditions, the presence of hexose phosphate isomerase as an impurity in our earlier enzyme preparations (7, 8) led us to reexamine the reaction with highly purified enzymes. The results presented in this paper demonstrate that the process proceeds as well in the complete absence of hexose phosphate isomerase and support the third hypothesis, namely that of a coupled reaction catalyzed by transketolase and transaldolase. We have observed that D-fructose 6-phosphate, completely free of D-glucose 6-phosphate, is converted to sedoheptulose 7-phosphate and D-xylulose 5-phosphate with preparations of transaldolase and transketolase which are highly purified and completely free of hexose phosphate isomerase and D-xylulose 5-phosphate 3-epimerase activity.

The reverse reaction has also been observed: sedoheptulose 7-phosphate and D-xylulose 5-phosphate are converted to D-fructose 6-phosphate in the presence of transketolase and transaldolase. The reaction can thus be represented by the equilibrium

D-Fructose-6-P ⇌ sedoheptulose-7-P + D-xylulose-5-P

EXPERIMENTAL PROCEDURE

Materials—*Candida utilis* dried at low temperature was kindly provided by the Lake States Yeast Corporation, Rhinelander, Wisconsin.

D-Glucose 6-phosphate, D-ribose 5-phosphate, D-fructose 1,6-diphosphate (Na salts), TPN, DPN, DPNH, and acid phosphatase from wheat germ were obtained from the Sigma Chemical Company. Hexose phosphate isomerase, aldolase, D-glucose 6-phosphate dehydrogenase, glycerophosphate dehydrogenase with triose phosphate isomerase, and D-lactic dehydrogenase were purchased from Boehringer and Soehne. D-Fructose 6-phosphate was also obtained from Boehringer and Soehne; D-glucose 6-phosphate present as an impurity was removed either by treatment with bromine or by oxidation to D-gluconate 6-phosphate in the presence of D-glucose 6-phosphate dehydrogenase, catalytic amounts of TPN, and a system (pyruvate + D-lactic dehydrogenase) capable of regenerating the oxidized coenzyme. In both cases D-fructose 6-phosphate was subsequently purified by chromatography on Dowex 1-formate columns (11), precipitated as the Li salt, and utilized as such. This preparation was completely free of D-glucose 6-phosphate on the basis of enzymatic assay with TPN and D-glucose 6-phosphate dehydrogenase. Sedoheptulose 7-phosphate was prepared following the procedure of Horecker, Smyrniotis, and Klenow (12). Sedoheptulose 1,7-diphosphate was prepared according to Smyrniotis and Horecker (13). D-Xylulose 5-phosphate was prepared as described by Srere *et al.* (14). D-Xylulose was prepared from D-fructose 6-phosphate and D-glyceraldehyde in the

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[1] G. de la Haba, unpublished observations, cited by A. G. Datta and E. Racker (6).

TABLE I

Purification of spinach transketolase

Step	Total units	Specific activity
Extract	3.000	0.3
Ammonium sulfate Fraction I	2.600	3.5
Calcium phosphate fraction	2.200	7.0
Acetone fraction	1.400	19.0
Ammonium sulfate Fraction II	1.130	51
DEAE-cellulose eluate		
Fractions 82 to 83	91	120
Fractions 84 to 85	294	128
Fractions 86 to 89	240	112
Fractions 90 to 91	108	105

presence of transketolase by the following procedure. To a final volume of 3.0 ml the following reagents were added: 250 μmoles of glycylglycine buffer, pH 7.4; 102 μmoles of D-fructose 6-phosphate, purified as previously described; 100 μmoles of D-glyceraldehyde; 20 μmoles of $MgCl_2$; 4 μmoles of thiamine pyrophosphate, and 2.5 units of transketolase. Incubation was carried out at 25° for 4 hours. The reaction was stopped by the addition of 0.5 ml of 50% trichloroacetic acid and the contents of the test tube were centrifuged. As measured by the cysteine-carbazole and by the orcinol reactions, 35 μmoles of ketopentose were present in the supernatant solution. This was adjusted to pH 6.5 and treated with bromine to oxidize the remaining D-glyceraldehyde. The incubation mixture was then treated with mixed bed resin to remove D-fructose 6-phosphate, D-erythrose 4-phosphate, and D-glyceric-acid. The solution was then concentrated to a small volume and stored at −16°. A sample of D-ribulose *O*-nitrophenylhydrazone was kindly supplied by Dr. B. L. Horecker of New York University; this was converted to free D-ribulose by decomposition with benzaldehyde (15). Sedoheptulose was obtained from sedoheptulose 7-phosphate by the action of the acid phosphatase. The liberated phosphate and the remaining phosphate esters were removed by treatment with mixed bed resin. Sedoheptulosan employed as standard for the colorimetric procedures was a gift of Dr. N. K. Richtmyer of the National Institutes of Health. Calcium phosphate gel was prepared according to Keilin and Hartree (16). Transaldolase was a crystalline preparation from *Candida utilis*, free of phosphohexose isomerase and D-xylulose 5-phosphate 3-epimerase (17). D-Xylulose 5-phosphate 3-epimerase and D-ribose 5-phosphate isomerase were prepared following the procedure of Ashwell and Hickman (18). Transketolase was purified from spinach and obtained free of hexose phosphate isomerase.

Methods—D-Xylulose 5-phosphate 3-epimerase activity was evaluated with purified transketolase according to the procedure reported by Horecker, Smyrniotis, and Hurwitz (19). D-Ribose 5-phosphate isomerase was determined essentially as described by Axelrod and Jang (20). Triose phosphates were determined enzymatically by the method of Racker (21). D-Glucose 6-phosphate and D-fructose 6-phosphate were determined with D-glucose 6-phosphate dehydrogenase with or without phosphohexose isomerase, respectively. The cysteine-sulfuric acid reaction of Diche (22) was used for the determination of heptuloses. Pentoses were analyzed by the cysteine-carbazole reaction of Dische and Borenfreund (23) and by the orcinol reaction following the procedures suggested by Dickens and Williamson (24). Total pentose phosphate was also measured by the transketolase reaction as described by Cooper *et al.* (25). Transaldolase and transketolase were determined as reported elsewhere (17, 19). Hexose phosphate isomerase was assayed with D-fructose 6-phosphate, TPN, and D-glucose 6-phosphate dehydrogenase. Protein was determined by the turbidimetric procedure of Bücher (26).

RESULTS

Purification of Transketolase

The procedure is a modification of that reported by Horecker, Smyrniotis, and Hurwitz (19).

Extract—All operations were carried out at 2° except when otherwise indicated. Fresh spinach, 600 g, freed of coarse stems, was homogenized for 3 minutes in a large Waring Blendor, model CB-2, with 1000 ml of 0.01 M K_2HPO_4. The suspension was filtered with Schleicher and Schuell No. 588 filter paper (extract, 1200 ml).

First Ammonium Sulfate Fractionation—The dark green extract was treated with 197 g of ammonium sulfate and the precipitate discarded by centrifugation. To the supernatant solution were added 140 g of ammonium sulfate and the precipitate was again removed. The supernatant solution was treated with 72 g of ammonium sulfate and the precipitate collected and dissolved in 17 ml of water. A final fraction was obtained by the addition of 102 g of ammonium sulfate. The precipitate was collected and dissolved in 20 ml of water. The last two fractions were tested separately and combined when necessary (ammonium sulfate Fraction I, 36 ml).

Calcium Phosphate Gel—To the ammonium sulfate fraction were added 137 ml of calcium phosphate gel containing 4.24 mg of solids per ml. The suspension was kept at 0° for 10 minutes and then centrifuged at 12,000 × *g* for 8 minutes. The supernatant solution (165 ml) was treated with 85 g of ammonium sulfate and the precipitate collected by centrifugation and dissolved in 15 ml of water (gel fraction, 15 ml).

Acetone Fractionation—The gel fraction was dialyzed overnight against cold flowing Na acetate, 0.1 M, adjusted to pH 7.5. The dialysate (14.5 ml) was diluted with 82 ml of 0.1 M Na acetate to bring the protein concentration to 4.5 mg per ml. Three fractions were collected by the successive addition of 58.2 ml, 16.6 ml, and 20.6 ml of cold (−14°) acetone, which was added dropwise in approximately 10 minutes for each fraction. The second and third precipitates were dissolved in 0.01 M glycylglycine buffer, pH 7.5, tested separately, and combined when necessary. Usually the bulk of activity was in the third fraction (acetone fraction, 5.2 ml).

Second Ammonium Sulfate Fractionation—The acetone fraction was treated with equal volume of an ammonium sulfate solution saturated at 0°, pH 7.2. The precipitate was removed by centrifugation and the supernatant solution brought to 60% saturation with the same ammonium sulfate solution. The precipitate was collected by centrifugation and dissolved in 0.005 M phosphate buffer, pH 7.6. To the supernatant solution was added solid ammonium sulfate to a saturation of 70%. After 10 minutes at 2°, the precipitate was collected and dissolved in 0.005 M phosphate buffer, pH 7.6. These two fractions were tested separately and combined when necessary. In this particular preparation the bulk of activity was in the last fraction (ammonium sulfate Fraction II, 5 ml).

Chromatography on DEAE-cellulose—The preceding fraction was dialyzed for 3 hours against flowing phosphate buffer 0.005 M, pH 7.6, and then placed on a DEAE-cellulose column (11 × 170 mm) that had previously been equilibrated with the same phosphate buffer. The column was washed with the equilibrating solution and elution was started with a linear gradient of increasing phosphate buffer, concentration up to 0.05 M. The enzyme elution was then completed with a linear gradient of increasing NaCl and phosphate buffer the reservoir containing 1 M NaCl in 0.08 M phosphate buffer, pH 7.6, whereas the mixing bottle contained 150 ml of 0.05 M phosphate buffer at the same pH. The rate of elution was approximately 30 ml per hour and fractions of about 7 ml each were collected. The fractions were tested for protein and enzymatic activity. The fractions containing transketolase activity were collected and treated, separately or combined, with equal volumes of ammonium sulfate-saturated solution at 0°, pH 7.2, and with solid ammonium sulfate to reach a final concentration of about 80%. The precipitates were collected and each dissolved in 0.25 M glycylglycine buffer, pH 7.4 (DEAE fractions).

The preparations were stored at −10°, with practically no loss of activity, for 1 or 2 months. D-Xylulose 5-phosphate 3-epimerase and phosphohexose isomerase activities were tested according to the procedures reported under "Methods," and found to be completely absent at this stage of the purification. However some D-ribose 5-phosphate isomerase was present in the enzyme preparation.

Formation of Heptulose from D-Fructose 6-Phosphate

Heptulose formation has been followed in the presence of transketolase and transaldolase[2] with D-glucose 6-phosphate and D-fructose 6-phosphate as substrates (Fig. 1).

With D-glucose 6-phosphate alone, in the absence of hexose phosphate isomerase, neither disappearance of aldohexose nor synthesis of ketoheptose could be detected even after prolonged incubation. With D-fructose 6-phosphate, on the other hand, the rate and quantity of heptulose synthesis is greater in the absence than in the presence of hexose phosphate isomerase. Maximal rate and yield of heptulose synthesis are observed when D-xylulose 5-phosphate 3-epimerase is present and D-fructose 6-phosphate is the substrate.

Stoichiometry of Reactions—It is evident from the results in Fig. 1 that the equilibrium can readily be shifted by the addition of other enzymes. These stoichiometric relations are presented in Table II. In the absence of D-xylulose 5-phosphate 3-epimerase or hexose phosphate isomerase, each mole of fructose phosphate utilized gives rise to 0.5 mole of heptulose phosphate and an equivalent quantity of ketopentose phosphate, accounting for all of the hexose carbon atoms. Similar stoichiometry is observed if hexose phosphate isomerase is added to the incubation mixture containing either D-fructose 6-phosphate or D-glucose 6-phosphate. In this case, however, less heptulose phosphate is formed (Fig. 1).

If D-xylulose 5-phosphate 3-epimerase is added, with or without hexose phosphate isomerase, the stoichiometry of the reaction changes significantly. In this case each mole of hexose monophosphate utilized gives rise to 0.75 mole of heptulose phosphate formed together with 0.2 mole of triose phosphate. Only trace amounts of pentose phosphate accumulate.

[2] The quantities of transketolase and transaldolase used in all our experiments were exactly equal to those found by analysis to be present in aliquots of liver preparations shown to catalyze the conversion of hexose monophosphate to heptulose phosphate (8).

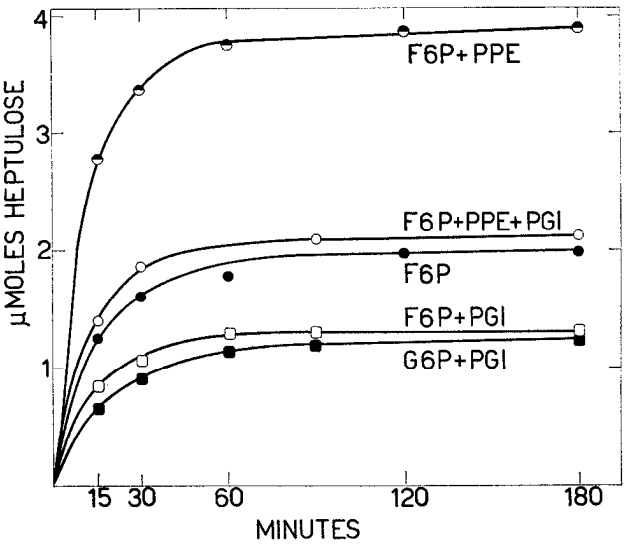

FIG. 1. Heptulose formation from hexose monophosphate with purified transketolase and transaldolase. The reaction mixtures (2.5 ml) contained 10 μmoles of D-fructose 6-phosphate or D-glucose 6-phosphate as indicated, 1.2 μmoles of thiamine pyrophosphate, 4 μmoles of $MgCl_2$, 12.5 μg of transketolase (specific activity, 123 units per mg) 2.5 μg of transaldolase (specific activity, 280 units per mg), and 125 μmoles of glycylglycine buffer, pH 7.4. Phosphohexose isomerase (PGI) (0.01 ml) and phosphopentose epimerase (PPE) (5.2 μg, specific activity 200 units per mg) were added as indicated in the figure. Incubation was at 37°. Aliquots (0.1 ml) for heptulose assay were treated with equal volume of trichloroacetic acid 10% and centrifuged. PPE, D-xylulose 5-phosphate-3-epimerase; PGI, hexose phosphate isomerase; F6P, D-fructose 6-phosphate; G6P, glucose 6-phosphate.

Reversibility of Reaction—The reverse reaction is shown in Table III. When a mixture of sedoheptulose 7-phosphate and D-xylulose 5-phosphate is incubated with transketolase and transaldolase, D-fructose 6-phosphate is formed as the sole reaction product. The stoichiometry observed corresponds closely to that obtained with D-fructose 6-phosphate as the initial substrate. When either transketolase or transaldolase is omitted, or when only one of the two substrates is added, no fructose phosphate is formed.

Identification of Reaction Products—In the reaction with fructose 6-phosphate as substrate, the products of the reaction were identified as sedoheptulose 7-phosphate and D-xylulose 5-phosphate. For the isolation of these products, 50 μmoles of D-fructose 6-phosphate were utilized in a 12.5-ml. reaction mixture containing 6.0 μmoles of thiamine pyrophosphate, 20 μmoles of $MgCl_2$, 60 μg of transketolase (specific activity 123 units per mg) and 12.5 μg of transaldolase (specific activity 280 units per mg), in 0.05 M glycylglycine buffer, pH 7.4. At 30 and 60 minutes aliquots, 1.0 ml, were removed, treated with 0.1 ml of 60% perchloric acid, centrifuged, and neutralized. After 4 hours the reaction mixture containing 8.7 μmoles of heptulose phosphate and 9.3 μmoles of ketopentose phosphate was treated with 1.0 ml of perchloric acid, centrifuged, and neutralized. In the two aliquots and in the final mixture ketopentose phosphate was identified enzymatically as follows. D-Fructose 6-phosphate remaining in the incubation mixture, was converted to D-gluconate

TABLE II

Stoichiometry in the reaction

The reaction conditions were as described in the legend to Fig. 1. Aliquots (0.5 ml) were collected, treated with 0.05 ml of 70% perchloric acid, and neutralized with 0.08 ml of 10 N KOH. Potassium perchlorate was removed by centrifugation and suitable aliquots of the supernatant solution were assayed for the several components as described in "Methods."

		Minutes					Equilibrium ratio: product formed/hexose phosphate utilized
		15	30	60	120	180	
		μmoles					
Fructose-6-P	Hexose phosphate utilized	2.80	3.15	3.70	4.35	4.35	
	Heptulose phosphate formed	1.36	1.68	1.88	2.15	2.15	0.49
	Pentose phosphate formed	1.30	1.57	1.75	2.20	2.20	0.51
	Triose phosphate formed						
Fructose-6-P + PPE*	Hexose phosphate utilized	3.70	4.65	5.18	5.44	5.41	
	Heptulose phosphate formed	2.91	3.55	3.89	4.06	4.10	0.76
	Pentose phosphate formed	0.07	0.08	0.10	0.16	0.17	0.03
	Triose phosphate formed	0.81	0.93	1.01	1.1	1.06	0.20
Fructose-6-P + PPE + PGI	Hexose phosphate utilized	1.68	2.35	2.57	2.73	2.72	
	Heptulose phosphate formed	1.34	1.81	1.96	2.04	2.03	0.75
	Pentose phosphate formed	0.03	0.05	0.05	0.06	0.06	0.02
	Triose phosphate formed	0.32	0.43	0.49	0.53	0.51	0.19
Glucose-6-P + PPE + PGI	Hexose phosphate utilized	1.55	2.26	2.56	2.75	2.70	
	Heptulose phosphate formed	1.28	1.75	1.91	2.02	2.02	0.74
	Pentose phosphate formed	0.03	0.04	0.06	0.09	0.08	0.03
	Triose phosphate formed	0.29	0.40	0.48	0.60	0.57	0.21

* PPE, D-xylulose 5-phosphate-3-epimerase; PGI, hexose phosphate isomerase.

TABLE III

Reversibility of the reaction

The reaction mixtures (2.5 ml) contained 4.92 μmoles of sedoheptulose 7-phosphate, 2.10 μmoles of xylulose 5-phosphate, 1.2 μmoles of thiamine pyrophosphate, 4 μmoles of $MgCl_2$, 11.5 μg of transketolase (specific activity 118 units per mg), 2.5 μg of transaldolase (specific activity 280 units per mg), and 125 μmoles of glycylglycine buffer, pH 7.4. Incubation was at 37°.

	Minutes			
	0	30	60	Ratio: compound utilized/ fructose-6-P formed
D-Fructose-6-P	0	1 61	2.39	
Sedoheptulose-7-P	4.92	4.14	3.81	0.47
D-Xylulose-5-P	2.10	1.38	0.82	0.53

6-phosphate with TPN, hexose phosphate isomerase, and D-glucose 6-phosphate dehydrogenase. When the reaction was completed proteins were again removed with perchloric acid. D-Ribose 5-phosphate and DPNH were then added followed by glycerophosphate dehydrogenase with triose phosphate isomerase. No DPNH was oxidized until transketolase was added. The presence of D-xylulose 5-phosphate was established by the rapid oxidation of DPNH under these conditions (18), accounting for approximately the 100% of the ketopentose phosphate formed. D-Ribulose 5-phosphate will not react.

The presence of D-xylulose was confirmed by paper chromatography.

An aliquot of the incubation mixture at equilibrium (2 ml containing 1.76 μmoles of ketopentose phosphate and 1.81 μmoles of heptulose phosphate) was brought to pH 4.7 with 0.2 M acetate buffer, pH 4.7, and incubated for 120 minutes with 5 mg of wheat germ acid phosphatase. The reaction mixture was then passed through a column of mixed bed resin. The effluents and washings were combined, concentrated to a small volume, and analyzed. Heptulose, fructose, and ketopentose were found to correspond to approximately 88% of the original amount. Aliquots (0.06 ml containing 0.38 μmoles of ketopentose) were applied to Whatman No. 1 paper and chromatographed with phenol-water as the moving solvent. The spots were developed by spraying with water-saturated *n*-butanol containing 0.5% orcinol and 15% trichloroacetic acid (27) followed by anilinephthalate (28). Three spots were present with R_F values of 0.38, 0.48, and 0.56 corresponding in location to the authentic samples of sedoheptulose, D-fructose, and D-xylulose. No other spots were detectable. Under these conditions D-xylulose, D-ribulose, and D-ribose are well separated and the last can be distinguished by use of the anilinephthalate spray.

DISCUSSION

The formation of heptulose phosphate and pentose phosphate from D-fructose 6-phosphate has now been established to be the result of the action of transketolase and transaldolase according to the following equations.

D-Fructose-6-P + transaldolase ⇌ D-glyceraldehyde-3-P + transaldolase dihydroxyacetone complex

D-Fructose-6-P + transketolase ⇌ D-erythrose-4-P + transketolase glycolaldehyde complex

D-Erythrose-4-P + transaldolase dihydroxyacetone complex ⇌ sedoheptulose-7-P + transaldolase

D-Glyceraldehyde-3-P + transketolase glycolaldehyde complex ⇌ D-xylulose-5-P + transketolase

The stoichiometry of the reaction and the identification of the ketopentose phosphate as D-xylulose 5-phosphate, together with reverse synthesis of D-fructose 6-phosphate,

$$\text{D-Xylulose-5-P} + \text{sedoheptulose-7-P} \xrightleftharpoons[\text{+ transaldolase}]{\text{transketolase}} \text{D-fructose-6-P}$$

represent convincing evidence for the mechanism reported.

The results reported in the present paper exclude the requirement for priming quantities of D-glucose 6-phosphate which might serve as acceptor, since our enzyme preparations are completely free of hexose phosphate isomerase. The addition of this enzyme resulted in a decrease in heptulose phosphate formation from D-fructose 6-phosphate.

The formation of sedoheptulose 7-phosphate from D-fructose 6-phosphate and the reverse process, the synthesis of D-fructose 6-phosphate from sedoheptulose 7-phosphate and D-xylulose 5-phosphate, involve the two intermediate complexes "active glycolaldehyde" and "active dihydroxyacetone" with the enzymes transketolase and transaldolase, respectively. Evidence for the first has been obtained by Datta and Racker (29). The dihydroxyacetone transaldolase intermediate has been studied in several laboratories (30, 31). Recent work by Cheng and Horecker[3] suggests that the limiting step in the coupled mechanism is the reaction of the transaldolase dihydroxyacetone complex with D-erythrose 4-phosphate. This would explain our previous observation that in the coupled reaction it is the enzyme transketolase, and not transaldolase, which is present in limiting quantities (8).

In agreement with the experimental data, when only transketolase and transaldolase are present, the stoichiometry of heptulose phosphate synthesis follows the equation

$$2\ \text{Fructose-6-P} \xrightleftharpoons[\text{+ transaldolase}]{\text{transketolase}} \text{sedoheptulose-7-P} + \text{xylulose-5-P}$$

When D-xylulose 5-phosphate 3-epimerase and D-ribose 5-phosphate isomerase are also present the ketopentose phosphate formed can be utilized as an acceptor and therefore other reactions will follow the one indicated.

$$1)\ 2\ \text{Fructose-6-P} \xrightleftharpoons[\text{+ transaldolase}]{\text{transketolase}} \text{sedoheptulose-7-P} + \text{xylulose-5-P}$$

$$2)\ \text{Xylulose-5-P} \xrightleftharpoons{\text{PPE}} \text{ribulose-5-P}$$

$$3)\ \text{Ribulose-5-P} \xrightleftharpoons{\text{PPI}} \text{ribose-5-P}$$

$$4)\ \text{Fructose-6-P} + \text{ribose-5-P} \xrightleftharpoons{\text{transketolase}} \text{sedoheptulose-7-P} + \text{erythrose-4-P}$$

$$5)\ \text{Fructose-6-P} + \text{erythrose-4-P} \xrightleftharpoons{\text{transaldolase}} \text{sedoheptulose-7-P} + \text{triose-P}$$

$$6)\ \text{Xylulose-5-P} + \text{ribose-5-P} \xrightleftharpoons{\text{transketolase}} \text{sedoheptulose-7-P} + \text{triose-P}$$

Either sequence 1 to 5 or 1 to 3 + 6 will account for the stoichiometry observed (0.75 mole sedoheptulose of 7-phosphate and approximately 0.25 mole of triose phosphate formed for each mole of fructose 6-phosphate utilized).

[3] T. Cheng, and B. L. Horecker, personal communication.

Whichever path may account for the synthesis of additional heptulose phosphate, the final equilibrium constant of the entire system (equal to the product of the single reaction constants) cannot be far from the value of Reaction 1 since the constants for Reactions 2 to 6 are not greatly different from 1.0. The value for Reaction 1 was calculated by us to be 0.15 according to the following equation

$$K = \frac{(\text{sedoheptulose-7-P})(\text{xylulose-5-P})}{(\text{fructose-6-P})^2}$$

A value of 0.16 was obtained if the ratio of the compounds, present in the final equilibrium mixture, was calculated according to the following equation

$$R = \frac{(\text{sedoheptulose-7-P})^3\,(\text{triose-P})}{(\text{fructose-6-P})^4}$$

derived from the sequence 1 + 2 + 3 + 4 + 5 and which is in good agreement with the expected theoretical value.

In the presence of hexose phosphate isomerase, the over-all stoichiometry based on total hexose monophosphate remains unchanged but with a different equilibrium ratio. The new equilibrium approaches closely that expected from the effect of the hexose phosphate isomerase equilibrium.

SUMMARY

1. The synthesis of sedoheptulose 7-phosphate from D-fructose 6-phosphate has been shown to proceed reversibly in the presence of highly purified transketolase and transaldolase preparations, free of hexose phosphate isomerase, according to the following equation

$$2\ \text{D-Fructose-6-P} \rightleftharpoons \text{sedoheptulose-7-P} + \text{D-xylulose-5-P}$$

2. The mechanism of the over-all reaction which involves the formation of the two intermediates transaldolase dihydroxyacetone and transketolase glycolaldehyde complexes is briefly discussed.

3. Evidence is also presented according to which when D-xylulose 5-phosphate 3-epimerase and D-ribose 5-phosphate isomerase are added a new stoichiometry is obtained as the result of the operation of known transketolase and transaldolase reactions.

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