D-Sedoheptulose-7-phosphate

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Principle

The determination of sedoheptulose-7-phosphate depends on the following reactions[1]:

(1) Fructose-1,6-diphosphate $\rightleftharpoons$ glyceraldehyde-3-phosphate + dihydroxyacetone phosphate

(2) Sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate $\rightleftharpoons$ fructose-6-phosphate + erythrose-4-phosphate

(3) Fructose-6-phosphate $\rightleftharpoons$ glucose-6-phosphate

(4) Glucose-6-phosphate + TPN^+ $\longrightarrow$ 6-phosphogluconate + TPNH + H^+

Reaction (1) is catalysed by aldolase, (2) by transaldolase, (3) by phosphoglucose isomerase, and (4) by glucose-6-phosphate dehydrogenase. In the presence of excess glyceraldehyde-3-phosphate which is generated from fructose-1,6-diphosphate (equation 1), the reaction proceeds until the sedoheptulose-7-phosphate present is completely consumed. Provided that no 6-phosphogluconate is present in the experimental material, 1 μmole sedoheptulose-7-phosphate forms 1 μmole TPNH.

Reagents

1. Perchloric acid, A. R.; sp. gr. 1.67; *ca.* 70% (w/w)
2. Potassium hydroxide.
3. Glycylglycine
4. Triphosphopyridine nucleotide, TPN
   sodium salt, $TPN\text{-}NaH_2$; commercial preparation, see p. 1029.
5. Fructose-1,6-diphosphate, FDP
   sodium salt, $FDP\text{-}Na_3H$; commercial preparation, see p. 1014.
6. Aldolase
   from skeletal muscle, crystalline suspension in 2 M ammonium sulphate solution. Commercial preparation, see p. 970.
7. Phosphoglucose isomerase, PGI
   from rabbit muscle[3]. Commercial preparation, see p. 993.
8. Glucose-6-phosphate dehydrogenase, G6P-DH
   from yeast, suspension in 3.3 M ammonium sulphate solution. Commercial preparation (see p. 975) or preparation from yeast obtained according to[2].
9. Transaldolase
   from yeast[4], suspension in phosphate buffer. Isolation, see p. 110.

Purity of the enzyme preparations

Glucose-6-phosphate dehydrogenase must not contain 6-phosphogluconic dehydrogenase or TPNH oxidase. The suitability of a preparation is tested as follows:

a) The amount of enzyme used in the assay system should catalyse the complete oxidation of 0.05 μmole of glucose-6-phosphate in less than 5 minutes.

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[1] *J. Cooper, P. A. Srere, M. Tabachnick* and *E. Racker*, Arch. Biochem. Biophysics *74*, 306 [1958].
[2] *P. Srere, J. R. Cooper, M. Tabachnick* and *E. Racker*, Arch. Biochem. Biophysics *74*, 295 [1958].
[3] *M. W. Slein* in *S. P. Colowick* and *N. O. Kaplan:* Methods in Enzymology. Academic Press, New York 1955, Vol. I, p. 299.
[4] *D. Couri* and *E. Racker*, Arch. Biochem. Biophysics *83*, 195 [1959].

b) On completion of the reaction in the assay system the optical density at 340 mμ should remain constant for at least 10 minutes. A continuous decrease in optical density indicates the presence of TPNH oxidase.

If a glucose-6-phosphate dehydrogenase preparation does not fulfil these requirements then it should be purified by chromatography on a DEAE-cellulose column[2]. Glucose-6-phosphate dehydrogenase prepared according to[2] as well as commercially available preparations usually contain appreciable amounts of glutathione reductase. Transaldolase should be free from transketolase (particularly if pentose-5-phosphates are present in the sample), 6-phosphogluconic dehydrogenase and TPNH oxidase. The transaldolase must be added in sufficient amounts to the test mixture to allow completion of the reaction in less than 15 minutes.

Preparation of Solutions

I. Perchloric acid (*ca.* 10% w/w):
Dilute 10 ml. perchloric acid (sp. gr. 1.67) with distilled water to 110 ml.

II. Potassium hydroxide (1 N):
Dissolve 5.6 g KOH in distilled water and make up to 100 ml.

III. Glycylglycine buffer (0.25 M; pH 7.4):
Dissolve 3.30 g. glycylglycine in *ca.* 70 ml. distilled water, adjust pH to 7.4 with *ca.* 21 ml. 0.2 N NaOH and dilute with distilled water to 100 ml. Check pH with glass electrode.

IV. Triphosphopyridine nucleotide (0.007 M β-TPN):
Dissolve 6 mg. $TPN\text{-}NaH_2$ in distilled water and make up to 1 ml.

V. Fructose-1,6-diphosphate (0.04 M FDP):
Dissolve about 162 mg. $FDP\text{-}Na_3H$ in distilled water and make up to 10 ml. Vary the amount taken according to the purity and water content of the preparation. It is preferable to determine the FDP content of the preparation before use (see p. 160)

VI. Aldolase (76 units *)/ml.):
Dilute crystalline suspension with distilled water.

VII. Phosphoglucose isomerase, PGI (10 units *)/ml.):
Dilute suspension with distilled water.

VIII. Glucose-6-phosphate dehydrogenase, G6P-DH (15 units *)/ml.):
Dilute suspension with distilled water.

IX. Transaldolase (15 units *)/ml.):
Dilute suspension with distilled water.

Stability of the solutions

Suspensions of glucose-6-phosphate dehydrogenase and aldolase in ammonium sulphate solution are stable for several months at 0°C, while phosphoglucose isomerase is stable for several years. Solutions IV and V should be stored frozen at −20°C. Transaldolase preparations are still suitable for use after storage for more than a year in the frozen state.

Procedure

Deproteinization

Trichloroacetic acid inhibits glucose-6-phosphate dehydrogenase, therefore perchloric acid is used for deproteinization.

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*) A unit is the amount of enzyme which converts 1 μmole of substrate in 1 min.

To the chilled sample add

an equal volume of $HClO_4$ solution (I),

centrifuge in the cold and immediately neutralize with the previously determined amount of

1 N KOH (solution II).

Allow to stand for 15 min. at 0°C, remove the $KClO_4$ precipitate by centrifuging. Take a portion of the supernatant for analysis.

Spectrophotometric measurements

Wavelength: 340 mμ; silica micro-cuvettes, light path: 1 cm.; final volume: 1 ml. Read experimental against control cuvette. Pipette successively into the cuvettes:

Experimental cuvette	*Control cuvette*
0.10 ml. buffer (solution III)	0.10 ml. buffer (solution III)
0.05 ml. TPN solution (IV)	0.05 ml. distilled water
0.05 ml. FDP solution (V)	0.05 ml. FDP solution (V)
0.05 ml. aldolase solution (VI)	0.05 ml. aldolase solution (VI)
0.03 ml. PGI solution (VII)	0.03 ml. PGI solution (VII)

Pipette into both cuvettes:

deproteinized and neutralized sample, (containing 0.01 — 0.08 μmoles sedoheptulose-7-phosphate)

distilled water to give a final volume of 0.98 ml.

Mix, read optical density E_1. Then mix into both cuvettes

0.02 ml. G6P-DH solution (VIII).

If TPN is expected to be present in the sample, then add 0.02 ml. distilled water to the control cuvette instead of the G6P-DH solution. The presence of fructose or glucose-6-phosphate in the sample causes an increase in optical density (reduction of TPN). Wait for the reaction to stop, then read optical density E_2.

Add

0.02 ml. transaldolase solution (IX)

to both cuvettes. Follow the absorption changes and on completion of the reaction read optical density E_3.

Calculations

An increase in optical density of 6.22 corresponds to the reduction of 1 μmole TPN. The sedoheptulose-7-phosphate content of the sample is calculated from the formula:

$$\frac{E_3 - 0.98 \times E_2}{6.22} = \mu\text{moles sedoheptulose-7-phosphate/ml. test mixture.}$$

0.98 is the correction factor for the dilution caused by the addition of the transaldolase solution.

Sources of Error

Trichloroacetic acid inhibits glucose-6-phosphate dehydrogenase. The samples deproteinized with perchloric acid should be analysed as soon as possible, since storage results in decreased sedoheptulose-7-phosphate values. Glutathione should not be present in the experimental material because the oxidized form reoxidizes TPNH with the aid of glutathione reductase, which is usually present in the glucose-6-phosphate dehydrogenase preparations.

Appendix

Isolation of transaldolase [4]

The isolation from baker's yeast includes the following steps: Drying the yeast at room temperature. Heating the crude extract for 2.5 hours at 40° C. Acetone fractionation at −2° C and dialysis overnight. Nucleic acid precipitation. Adsorption and elution from calcium phosphate gel. Fractionation with $(NH_4)_2SO_4$; most of the enzyme precipitates between 65−75% and 75−85% saturation. Dialysis. Chromatography on DEAE-cellulose. Concentration of the eluate to a small volume by lyophilization. Specific activity *ca.* 3.4 units *)/mg. Store the suspension at −20° C.

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*) A unit is the amount of enzyme which converts 1 μmole of substrate in 1 min.