L-Sorbose-6-phosphate

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Principle

The determination of L-sorbose-6-phosphate is based on the following reactions:

(1) L-Sorbose-6-phosphate + D-glyceraldehyde-3-phosphate $\rightleftharpoons$ D-fructose-6-phosphate + L-glyceraldehyde-3-phosphate

(2) D-Fructose-6-phosphate $\rightleftharpoons$ D-glucose-6-phosphate

(3) D-Glucose-6-phosphate + TPN^+ $\rightleftharpoons$ 6-phosphogluconate + TPNH + H^+

Reaction (1) is catalysed by transaldolase, (2) by phosphoglucose isomerase (PGI), and (3) by glucose-6-phosphate dehydrogenase (G6P-DH). The measure of the over-all reaction is the increase of optical density at 340 mμ due to the formation of reduced triphosphopyridine nucleotide (TPNH). In the presence of excess D-glyceraldehyde-3-phosphate the three reactions proceed until all the L-sorbose-6-phosphate is consumed. Providing that the enzyme preparation is free from 6-phosphogluconic dehydrogenase, 1 μmole of TPNH is formed for each μmole of L-sorbose-6-phosphate present.

Reagents

Reagents for deproteinization see "Sedoheptulose-7-phosphate", p. 107.

1. Glycylglycine
2. Triphosphopyridine nucleotide, TPN
   sodium salt, $TPN\text{-}NaH_2$; commercial preparation, see p. 1029.
3. Glyceraldehyde-3-phosphate, GAP
   DL-Glyceraldehyde-3-phosphate diethylacetal, barium salt; commercial preparation, see p. 1019.
4. Glucose-6-phosphate dehydrogenase, G6P-DH
   from yeast; commercial preparation, see p. 975.
5. Phosphoglucose isomerase, PGI
   from yeast; commercial preparation, see p. 993.
6. Transaldolase
   from baker's yeast[1]; isolation, see p. 110.

Purity of the enzyme preparations

See "Sedoheptulose-7-phosphate", p. 107.

Preparation of Solutions

I. Glycylglycine buffer (0.25 M; pH 7.4):
Dissolve 3.30 g. glycylglycine in 70 ml. distilled water, adjust to pH 7.4 with 0.2 N NaOH and dilute to 100 ml. with distilled water.

II. Triphosphopyridine nucleotide (5×10^{-3} M β-TPN):
Dissolve 22 mg. $TPN\text{-}NaH_2$ in distilled water and make up to 5 ml.

III. Glyceraldehyde-3-phosphate (0.02 M D-GAP):
Add 50 mg. DL-glyceraldehyde-3-phosphate diethylacetal (Ba salt) to a suspension of about 500 mg. Dowex 50 (H^+ form) in 3 ml. distilled water. Place in a boiling water bath

[1] *D. Couri* and *E. Racker*, Arch. Biochem. Biophysics *83*, 195 [1959].

and shake for 3 min. Decant the solution from the ion exchange resin. Determine the D-GAP concentration enzymatically (p. 246). If necessary, dilute the solution to 0.02 M with distilled water.

IV. Glucose-6-phosphate dehydrogenase, G6P-DH (10 units*)/ml.):
Dilute the commercial preparation with distilled water.

V. Phosphoglucose isomerase, PGI (10 units*)/ml.):
Dilute the commercial preparation with distilled water.

VI. Transaldolase (6.8 units*)/ml.):
Dilute the preparation obtained according to[1] with glycylglycine buffer (solution I).

Stability of the solutions

Store all the solutions, except IV and V, at −20° C. Ammonium sulphate suspensions of G6P-DH and PGI are stable for several months or years respectively at 0° C. Crystalline suspensions of transaldolase in ammonium sulphate solution[2] can be stored for several months at 0° C. Partially purified preparations should be stored at −20° C.

Procedure

Deproteinization

See "Sedoheptulose-7-phosphate", p. 109.

Spectrophotometric measurements

Wavelength: 340 mμ; light path: 1 cm.; final volume: 1 ml. Measure against a control cuvette.

Pipette into the cuvettes:

Experimental cuvette	*Control cuvette*
deproteinized sample (containing 0.01 to 0.08 μmoles L-sorbose-6-phosphate)	deproteinized sample (as experimental cuvette)
0.1 ml. buffer (solution I)	0.1 ml. buffer (solution I)
0.1 ml. TPN solution (II)	—
0.05 ml. glyceraldehyde-3-phosphate solution (III)	0.05 ml. glyceraldehyde-3-phosphate solution (III)
distilled water to 0.94 ml.	distilled water to 0.94 ml.

Read the optical density E_1. Pipette into both cuvettes
0.02 ml. G6P-DH solution (IV).

If the deproteinized sample contains TPN, then substitute distilled water for the enzyme solution in the control cuvette. On completion of the reaction read the optical density E_2. Mix into both cuvettes

0.02 ml. PGI solution (V)

and on completion of the reaction read the optical density E_3.

Mix into both cuvettes

0.02 ml. transaldolase solution (VI)

and when the reaction stops read the optical density E_4.

*) A unit is the amount of enzyme which converts 1 μmole of substrate in 1 min. (refer to p. 32, 33).
2) *R. Venkataraman* and *E. Racker*, J. biol. Chemistry *236*, 1876 [1961].

Calculations

$\Delta E_{G\text{-}6\text{-}P} = E_2 - E_1$ corresponds to the glucose-6-phosphate content of the assay mixture, $\Delta E_{F\text{-}6\text{-}P} = E_3 - E_2$ gives the fructose-6-phosphate content, and $\Delta E_{S\text{-}6\text{-}P} = E_4 - E_3$ gives the L-sorbose-6-phosphate content. It is necessary to correct the optical densities E_1 to E_3 for the dilution occurring on addition of the enzyme solutions.

It follows that:

$$\frac{0.96\,E_2 - 0.94\,E_1}{6.22} = \mu\text{moles D-glucose-6-phosphate/assay mixture}$$

$$\frac{0.98\,E_3 - 0.96\,E_2}{6.22} = \mu\text{moles D-fructose-6-phosphate/assay mixture}$$

$$\frac{E_4 - 0.98\,E_3}{6.22} = \mu\text{moles L-sorbose-6-phosphate/assay mixture}$$

6.22 = extinction coefficient for TPNH at 340 mμ [cm.^{2}/μmole]