L-Glyceraldehyde-3-phosphate

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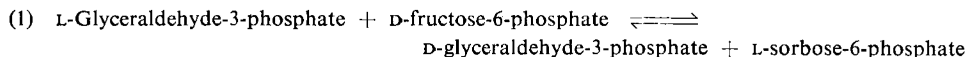
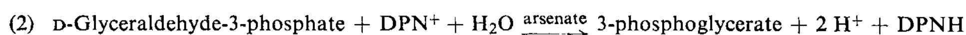
Principle

The determination of L-glyceraldehyde-3-phosphate (L-GAP) is based on the following reactions:



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



(2) D-Glyceraldehyde-3-phosphate + DPN^+ + H_2O $\xrightarrow{\text{arsenate}}$ 3-phosphoglycerate + 2 H^+ + DPNH

Reaction (1) is catalysed by transaldolase and (2) by D-glyceraldehyde-3-phosphate dehydrogenase. In the presence of excess fructose-6-phosphate both reactions proceed until all the L-glyceraldehyde-3-phosphate is completely consumed. The increase of optical density at 340 mμ due to the formation of reduced diphosphopyridine nucleotide (DPNH) is a measure of the reaction. 1 μmole of DPNH is formed for each μmole of L-glyceraldehyde-3-phosphate.

Reagents

1. Glycylglycine
2. Fructose-6-phosphate, F-6-P
   barium salt; commercial preparation, see p. 1016.
3. Trichloroacetic acid
4. Sodium hydrogen carbonate, $NaHCO_3$
5. Sodium arsenate, $Na_3AsO_4 \cdot 12\,H_2O$
6. Diphosphopyridine nucleotide, DPN
   free acid; commercial preparation, see p. 1010.
7. D-Glyceraldehyde-3-phosphate dehydrogenase, GAPDH
   from rabbit skeletal muscle; commercial preparation, see p. 979.
8. Transaldolase
   from baker's yeast[1]. Isolation, see p. 110.

Purity of the enzyme preparations

Glyceraldehyde-3-phosphate dehydrogenase: see "Xylulose-5-phosphate", p. 201. Transaldolase: see "Sedoheptulose-7-phosphate", p. 110.

Preparation of Solutions

I. Glycylglycine buffer (0.25 M; pH 7.4):
Dissolve 3.303 g. glycylglycine in 50 ml. distilled water, adjust to pH 7.4 with 0.2 N NaOH and dilute to 100 ml. with distilled water.

II. Fructose-6-phosphate (0.011 M F-6-P):
According to the F-6-P content of the preparation (assay, see p. 134) weigh out, for example, 58.0 mg. fructose-6-phosphate (Ba salt) of a preparation which contains 75% F-6-P-Ba, and dissolve in 5 ml. distilled water. Remove the Ba^{2+} with Dowex 50 (Na^+ form) and dilute the Ba^{2+}-free solution to 10 ml. with distilled water.

III. Trichloroacetic acid (5% w/v):
Dissolve 5 g. trichloroacetic acid in distilled water and make up to 100 ml.

IV. Sodium hydrogen carbonate (1 M):
Dissolve 8.4 g. $NaHCO_3$ in distilled water and make up to 100 ml.

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

V. Sodium arsenate (0.1 M):
Dissolve 4.24 g. $Na_3AsO_4 \cdot 12H_2O$ in distilled water and make up to 100 ml.

VI. Diphosphopyridine nucleotide (0.013 M β-DPN):
Dissolve 10 mg. DPN in distilled water and make up to 1 ml.

VII. D-Glyceraldehyde-3-phosphate dehydrogenase, GAPDH (20 units*)/ml.):
Dilute the commercial preparation with $2 \cdot 10^{-3}$ M EDTA solution (pH 7.4).

VIII. Transaldolase (16 units*)/ml.):
Dilute the preparation obtained according to[1] with glycylglycine buffer (solution I).

Stability of the solutions

Store all solutions, except VII and VIII, at −20°C. The DPN solution keeps for several months. Store the GAPDH solution (VII) at 2°C. It can be used for at least a year even if the specific activity decreases to half. Crystalline suspensions of transaldolase in ammonium sulphate solution[2] can be stored for months at 0°C. Partially purified preparations should be stored at −20°C.

Procedure

Deproteinization

Deproteinize the samples with 5% trichloroacetic acid (solution III), centrifuge and neutralize the supernatant with $NaHCO_3$ solution (IV) (indicator paper). Analyse a portion of the neutralized supernatant.

Spectrophotometric measurements

Preliminary remarks: Standard solutions of L-glyceraldehyde-3-phosphate are obtained from DL-glyceraldehyde-3-phosphate**) by removing the D-glyceraldehyde-3-phosphate enzymatically[2].

Method: Wavelength: 340 mμ; light path: 1 cm.; final volume: 1 ml. Read against the control cuvette.

Pipette into the cuvettes:

Experimental cuvette	*Control cuvette*
deproteinized sample (containing 0.01 to 0.08 μmoles L-glyceraldehyd-3-phosphate)	deproteinized sample (as for experimental cuvette)
0.10 ml. buffer (soln. I)	0.10 ml. buffer (soln. I)
0.05 ml. F-6-P soln. (II)	0.05 ml. F-6-P soln. (II)
0.05 ml. DPN soln. (VI)	
0.05 ml. arsenate soln. (V)	0.05 ml. arsenate soln. (V)
distilled water to 0.96 ml.	distilled water to 0.96 ml.

Read the optical density E_1. Mix into both cuvettes

0.02 ml. GAPDH solution (VII)

and on completion of the reaction read the optical density E_2. Mix into both cuvettes

0.02 ml. transaldolase solution (VIII),

wait for the end of the reaction and then read the optical density E_3.

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*) A unit is the amount of enzyme which converts 1 μmole substrate in 1 min. (refer to p. 32).
**) Obtainable *e.g.* from Schwarz Bioresearch. Inc., Orangeburg, N. Y., USA.
[2] *R. Venkataraman* and *E. Racker*, J. biol. Chemistry *236*, 1876 [1961].

Calculations

$\Delta E_{D\text{-}GAP} = E_2 - E_1$ is a measure of the D-glyceraldehyde-3-phosphate content of the assay mixture and $\Delta E_{L\text{-}GAP} = E_3 - E_2$ gives the L-glyceraldehyde-3-phosphate content. E_1 and E_2 must be corrected for the dilution of the assay mixture on addition of the enzyme solutions:

$$\frac{E_2 - 0.96 \times E_1}{6.22} = \mu\text{moles D-glyceraldehyde-3-phosphate/assay mixture}$$

$$\frac{E_3 - 0.98 \times E_2}{6.22} = \mu\text{moles L-glyceraldehyde-3-phosphate/assay mixture}$$

where

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

Sources of Error

See "Xylulose-5-phosphate", p. 204.