D-Erythrose-4-phosphate

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Principle

The estimation of erythrose-4-phosphate is based on the following reactions[1]:

(1) Erythrose-4-phosphate + fructose-6-phosphate $\rightleftharpoons$ sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate

(2) Glyceraldehyde-3-phosphate $\rightleftharpoons$ dihydroxyacetone phosphate

(3) Dihydroxyacetone phosphate + DPNH + H^+ $\rightleftharpoons$ α-glycerophosphate + DPN^+

Reaction (1) is catalysed by transaldolase, (2) by triosephosphate isomerase, and (3) by α-glycerophosphate dehydrogenase. Under the conditions described in the following method the reduction of dihydroxyacetone phosphate is virtually quantitative. In the presence of excess fructose-6-phosphate the amount of DPNH oxidized is equivalent to the erythrose-4-phosphate present.

Reagents

1. Trichloroacetic acid
2. Potassium carbonate, K_2CO_3, A. R.
3. Sodium hydroxide, 0.2 N
4. Glycylglycine
5. Reduced diphosphopyridine nucleotide, DPNH
   sodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
6. Fructose-6-phosphate, F-6-P
   barium salt, F-6-P-Ba; commercial preparation, see p. 1016.
7. Triosephosphate isomerase/α-glycerophosphate dehydrogenase, TIM/GDH
   mixed crystalline suspension; commercial preparation, see p. 999.
8. Transaldolase
   from baker's yeast[2]. Isolation, see p. 110.

Purity of reagents and enzymes

Commercial preparations of fructose-6-phosphate occasionally contain impurities which react with transaldolase giving rise to DPNH oxidation. This oxidation should be determined before use of the preparation and be taken into account when calculating the results. The transaldolase should be free from transketolase. Preparations satisfying the following conditions are suitable for use:

a) In the absence of erythrose-4-phosphate no DPNH oxidation takes place in the complete reaction mixture. If a little DPNH is oxidized, but the reaction soon stops, corrections can be applied to the experimental results.

b) The reaction with known amounts of erythrose-4-phosphate goes to completion in less than 10 minutes.

c) Addition of a mixture of xylulose-5-phosphate and ribose-5-phosphate does not give rise to oxidation of DPNH.

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[1] *P. Srere, J. R. Cooper, M. Tabachnick* and *E. Racker*, Arch. Biochem. Biophysics *74*, 295 [1958].
[2] *D. Couri* and *E. Racker*, Arch. Biochem. Biophysics *83*, 195 [1959].

Preparation of Solutions

I. Trichloroacetic acid (10% w/v):
Dissolve 10 g. trichloroacetic acid in 100 ml. distilled water.

II. Potassium carbonate (*ca.* 5 M):
Dissolve *ca.* 69 g. K_2CO_3, A. R. in distilled water and make up to 100 ml.

III. Glycylglycine buffer (0.25 M; pH 7.4):
Dissolve 3.303 g. glycylglycine in *ca.* 50 ml. distilled water, adjust pH to 7.4 with *ca.* 21 ml. 0.2 N NaOH, and dilute to 100 ml. with distilled water. Check pH on glass electrode.

IV. Reduced diphosphopyridine nucleotide (*ca.* 0.004 M β-DPNH; pH 9):
Dissolve 7 mg. DPNH-Na_2 in 2 ml. distilled water, adjust pH to *ca.* 9 with KOH.

V. Fructose-6-phosphate (0.006 M F-6-P):
Weigh out according to the F-6-P content of the preparation, *e. g.* for a preparation containing 75% F-6-P·Ba: 31.64 mg., and dissolve in *ca.* 5 ml. distilled water. Remove barium with Dowex 50 (Na^+ form), dilute Ba^{2+}-free solution with distilled water to 100 ml.

VI. Triosephosphate isomerase/α-glycerophosphate dehydrogenase, TIM/GDH (50 μg protein/ml.):
Dilute 0.1 ml. of the mixed crystalline preparation suspended in ammonium sulphate solution to 0.4 ml. with glycylglycine buffer (0.001 M; pH 7.4).

VII. Transaldolase (15 units*)/ml.):
Make a suitable dilution of the transaldolase preparation, obtained from baker's yeast [2], with glycylglycine buffer (solution III).

Stability of the solutions

All solutions are stored at −20° C except for VI. The DPNH solution keeps for several weeks. The mixed suspension of triosephosphate isomerase/α-glycerophosphate dehydrogenase is diluted freshly for each set of determinations, unused suspension is discarded.

Procedure

Deproteinization

Deproteinize sample with sufficient trichloroacetic acid to give a final concentration of 5% (w/v) and centrifuge. Neutralize a portion of the supernatant with K_2CO_3 solution (II) (glass electrode).

Spectrophotometric measurements

Wavelength: 340 mμ; light path: 1 cm.; final volume: 1 ml.

Read optical density of experimental cuvette against control cuvette.

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

To two quartz cuvettes add sufficient water to give a final volume in the assay mixture of 1 ml. Then add:

Experimental cuvette	*Control cuvette*
neutralized supernatant (containing 0.01–0.06 μmoles erythrose-4-phosphate)	distilled water
0.10 ml. buffer (soln. III)	0.10 ml. buffer (soln. III)
0.03 ml. DPNH soln. (IV)	0.03 ml. distilled water
0.05 ml. fructose-6-phosphate soln. (V)	0.05 ml. fructose-6-phosphate soln. (V)

Measure optical density E_1 at 340 mμ. Add to both cuvettes

0.02 ml. TIM/GDH suspension (VI).

Wait for end of reaction, then read optical density E_2 at 340 mμ. The decrease in optical density $E_1 - E_2$ corresponds to the triose phosphate content of the sample.

Add to both cuvettes,

0.02 ml. transaldolase solution (VII).

At the end of the reaction measure optical density E_3 at 340 mμ.

Calculations

A decrease in optical density of 6.22 corresponds to the oxidation of 1 μmole DPNH. The erythrose-4-phosphate content of the test mixture is calculated from the formula:

$$\frac{0.98\ E_2 - E_3}{6.22} = \text{μmoles erythrose-4-phosphate/ml. test mixture.}$$

The factor 0.98 corrects for the 2% dilution on addition of transaldolase solution.

Sources of Error

Impurities in the fructose-6-phosphate may interfere with the assay, but can be corrected for if relatively small. Acetaldehyde in the sample interferes, if the transaldolase preparation contains alcohol dehydrogenase. Controls without the addition of triosephosphate isomerase/α-glycerophosphate dehydrogenase give values which can be used to correct the assay results provided that only a small amount of acetaldehyde is present in the sample.