D-Ribulose-5-phosphate

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Principle

The determination of ribulose-5-phosphate is based on the formation of xylulose-5-phosphate in the presence of xylulose-5-phosphate epimerase[1]. The xylulose-5-phosphate is then determined with transketolase (see p. 201). The estimation of ribulose-5-phosphate can be carried out in the same assay mixture that was used to estimate glyceraldehyde-3-phosphate and xylulose-5-phosphate. If the volume of the xylulose-5-phosphate epimerase added is small (0.01 ml.), no correction for the dilution of the cuvette contents is required.

Reagents

See determination of xylulose-5-phosphate (p. 201). Additional:

12. Xylulose-5-phosphate epimerase from rabbit muscle[2] Isolation, see p. 177.

Purity of reagents and enzyme preparations

See determination of xylulose-5-phosphate (p. 201). Additional:

Xylulose-5-phosphate epimerase should be virtually free from ribose-5-phosphate isomerase. A contamination of less than 1% of the latter enzyme does not interfere, since under the conditions of the method it only reacts very slowly with ribose-5-phosphate. Suitable xylulose-5-phosphate epimerase preparations do not react with ribose-5-phosphate in the complete test mixture but on addition of a limited amount of ribulose-5-phosphate cause a rapid epimerisation.

Preparation of Solutions

See determination of xylulose-5-phosphate (p. 202). Additional:

X. Xylulose-5-phosphate epimerase (200 units*)/ml.):

The lyophilized enzyme preparation, prepared from rabbit muscle according to[2], is dissolved in distilled water and diluted to 200 units/ml.

Stability of the solutions

See determination of xylulose-5-phosphate (p. 202). Additional:

The lyophilized xylulose-5-phosphate epimerase, prepared according to[2], keeps for several months when stored at 0°C in a desiccator over silica gel. Concentrated solutions of the enzyme in distilled water can be stored at −20°C for 2 to 3 weeks, but slowly lose activity.

Procedure

Spectrophotometric measurements

See determination of xylulose-5-phosphate (p. 203). After addition of the transketolase suspension wait for the reaction to stop (3 to 5 min.), measure optical density at 340 mμ. Then add

0.01 ml. xylulose-5-phosphate epimerase solution (X)

to the experimental and control cuvettes. The reaction is complete in about 15 min. Measure optical density at 340 mμ. The sum of the xylulose-5-phosphate and ribulose-

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

1) *J. Cooper, P. A. Srere, M. Tabachnick* and *E. Racker*, Arch. Biochem. Biophysics *74*, 306 [1958].
2) *M. Tabachnick, P. A. Srere, J. Cooper* and *E. Racker*, Arch. Biochem. Biophysics *74*, 315 [1958].

5-phosphate in the test mixture must not exceed 0.1 μmole, since the amount of acceptor aldehyde (ribose-5-phosphate) is not present in excess.
If the ribose-5-phosphate preparation used is very pure the concentration in the test mixture can be increased provided this does not cause a slow reduction of DPN.

Calculations
The ribulose-5-phosphate content of the test mixture is calculated from the optical density change ΔE on addition of xylulose-5-phosphate epimerase according to the formula:

$$\frac{\Delta E}{6.22} = \mu\text{moles ribulose-5-phosphate/test mixture.}$$

Sources of Error

The xylulose-5-phosphate epimerase should contain virtually no ribose-5-phosphate isomerase or α-glycerophosphate dehydrogenase. If the sample and the epimerase preparation contain pyruvate and lactic dehydrogenase respectively, then the pyruvate must be reduced to lactate before analysis of the pentose phosphate. This is done by adding a small excess of DPNH and extra lactic dehydrogenase. This procedure is unnecessary if the epimerase is free from lactic dehydrogenase. As the same amount of sample is added to the control cuvette, it is not necessary to correct for the excess DPNH. In the presence of large amounts of glyceraldehyde-3-phosphate dehydrogenase, ribose-5-phosphate slowly reduces DPN. The glyceraldehyde-3-phosphate dehydrogenase content of the assay system is not sufficient to cause this effect.