D-Ribose-5-phosphate

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Principle

In the presence of ribose-5-phosphate isomerase and xylulose-5-phosphate epimerase, ribose-5-phosphate is isomerized to ribulose-5-phosphate and epimerized to xylulose-5-phosphate[1]:

(1) Ribose-5-phosphate $\overset{\text{ribose-5-phosphate isomerase}}{\rightleftharpoons}$ ribulose-5-phosphate

(2) Ribulose-5-phosphate $\overset{\text{xylulose-5-phosphate epimerase}}{\rightleftharpoons}$ xylulose-5-phosphate

According to p. 201 xylulose-5-phosphate can be determined enzymatically after conversion to glyceraldehyde-3-phosphate (for determination, see p. 246):

(3) Xylulose-5-phosphate + ribose-5-phosphate $\overset{\text{transketolase}}{\rightleftharpoons}$ glyceraldehyde-3-phosphate + sedoheptulose-7-phosphate

No extra acceptor aldehyde, ribose-5-phosphate, has to be added since it is present already in the sample. Therefore only 0.5 mole glyceraldehyde-3-phosphate is obtained from each mole of ribose-5-phosphate.

As this method measures both ribulose-5-phosphate and xylulose-5-phosphate, they must be removed from the sample by treatment with 1 N NaOH (see p. 201). Small losses ($<10\%$) of ribose-5-phosphate due to this treatment are within the error of the complete analysis.

Reagents

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

11. Ribose-5-phosphate isomerase

    from spinach leaves[2] or alfalfa leaves[3]. Isolation, see p. 176.

12. Xylulose-5-phosphate epimerase

    from skeletal muscle[2]. Isolation, see p. 177.

Purity of the enzyme preparations

Since both xylulose-5-phosphate epimerase and ribose-5-phosphate isomerase are used in the test described here, the complete separation of the two enzymes is not necessary. However, the preparations must be checked for contamination with a non-specific phosphatase. Traces of this enzyme, which are often present in ribose-5-phosphate isomerase preparations, do not usually interfere. On the other hand the reaction with ribose-5-phosphate is only rapid when large amounts of ribose-5-phosphate isomerase are added. The suitability of the enzyme preparations is tested by quantitative recovery of known amounts of ribose-5-phosphate under the assay conditions.

Preparation of Solutions

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

X. Ribose-5-phosphate isomerase (100 units*)/ml.):
   If necessary, dilute the stock suspension with distilled water.

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*) A unit is 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].
3) *B. Axelrod, R. S. Bandurski, C. M. Greiner* and *R. Jang*, J. biol. Chemistry *202*, 619 [1953].

XI. Xylulose-5-phosphate epimerase (200 units*)/ml.):
If necessary, dilute the stock suspension with distilled water.

Stability of the solutions

See determination of xylulose-5-phosphate (p. 201) and of ribulose-5-phosphate (p. 186).

Procedure

Deproteinization

Deproteinize sample as described for the determination of xylulose-5-phosphate (p. 203). Treat the deproteinized and neutralized supernatant with 1 N NaOH to remove traces of ketopentose phosphates (see p. 201). Use a portion of the neutralized solution (containing 0.02 to 0.16 μmoles ribose-5-phosphate) for the analysis.

Spectrophotometric measurements

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

Pipette into the experimental and control cuvettes the solutions as for the determination of xylulose-5-phosphate, omitting the ribose-5-phosphate solution and adding the transketolase solution before the addition of the deproteinized sample. After addition of the sample the volume of the test mixture must be adjusted to 0.98 ml. with water. If the treatment with alkali has been carried out properly the sample should not contain glyceraldehyde-3-phosphate or xylulose-5-phosphate; no optical density change takes place. Mix into both cuvettes

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

and

0.01 ml. ribose-5-phosphate isomerase solution (X).

Continue readings until end of reaction.

Calculations

For each mole of ribose-5-phosphate present in the cuvette 0.5 mole of DPN is reduced. An increase in optical density of 6.22 corresponds to the reduction of 1 μmole DPN. From the optical density increase ΔE the ribose-5-phosphate content of the test mixture is calculated according to the formula:

$$\frac{\Delta E}{3.11} = \mu\text{moles ribose-5-phosphate/ml. test mixture.}$$

Sources of Error

The interfering substances mentioned in the determination of xylulose-5-phosphate (p. 204) and ribulose-5-phosphate (p. 187) also interfere in this estimation. In addition, the ribose-5-phosphate isomerase preparation must not contain more than traces of a phosphatase which hydrolyses ribose-5-phosphate.

Appendix

Isolation of ribose-5-phosphate isomerase[2])

Carry out all the operations in a cold room at 2 to 4°C. Wash spinach leaves and press with a hydraulic press. To every 100 ml. of expressed juice add 22.6 g. $(NH_4)_2SO_4$ and filter overnight. To every 100 ml. of filtrate add 18.2 g. $(NH_4)_2SO_4$ and filter. Dissolve the filter cake in 50 ml. water and dialyse for 2 hours against 7 litres 0.01 M cysteine-HCl solution (pH 2.3) (stir). Neutralize the dialysed solution and centrifuge. Adjust the supernatant with distilled water to give 5 mg. protein/ml. and stir

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

in $^1/_{10}$ volume of calcium phosphate gel. Centrifuge, wash the gel twice with 40 ml. portions of water. Elute the enzyme from the gel with four 10 ml. portions of 0.01 M potassium phosphate buffer (pH 7.6). Dialyse the extract for 3 hours against 7 litres distilled water. Store the dilute enzyme solution (*ca.* 0.5 mg. protein/ml.) at −20°C.

Isolation of xylulose-5-phosphate epimerase [2]

Mince rabbit skeletal muscle, stir for 10 min. with the same volume (g.:ml.) of EDTA solution (1.8 mg. EDTA-$Na_2 \cdot 2\ H_2O$/ml.; 0.03 M KOH) and press through cheesecloth. Extract the residue once again with $^1/_2$ volume of the above solution and combine the extracts. To every 100 ml. of extract add 30.5 g. $(NH_4)_2SO_4$ and filter. Extract the filter cake with two 50 ml. portions of distilled water. Combine the aqueous extracts and dialyse overnight against 7 litres of EDTA solution (containing 600 µg. EDTA-$Na_2 \cdot 2\ H_2O$/ml.; pH 7.0). Centrifuge the contents of the dialysis sac, add 0.2 volumes calcium phosphate gel (20 mg./ml.) to the supernatant, centrifuge and wash the gel twice with 40 ml. portions of distilled water. Elute the enzyme from the gel with two 10 ml. portions of 0.01 M potassium phosphate puffer (pH 7.6). Lyophilize the eluates. Dissolve the freeze-dried material in the smallest amount of distilled water and dialyse for 4 hours against 7 litres distilled water. Store the contents of the dialysis sac at −20°C. The enzyme solution loses about 27% of its activity in 6 months.