D-Xylulose

Determination with DPN-xylitol Dehydrogenase

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It has long been known that certain individuals with a mild metabolic disorder excrete L-xylulose in the urine[1]. In contrast the role of D-xylulose and its phosphates in mammalian metabolism has only recently been recognized[2–4]. The method of determination described here is similar to that for L-xylulose[5] (p. 191).

Principle

DPN-xylitol (D-xylulose) dehydrogenase catalyses the reaction:

(1) D-Xylulose + DPNH + H^+ $\rightleftharpoons$ xylitol + DPN^+

The equilibrium lies in favour of xylitol. In the presence of slight excess of reduced diphosphopyridine nucleotide (DPNH) and enzyme the reaction proceeds quickly to completion with quantitative formation of xylitol. The decrease in optical density at 340 mμ is proportional to the D-xylulose content of the test mixture.

Reagents

See determination of L-xylulose (p. 191), but substitute for TPNH and TPN-xylitol (L-xylulose) dehydrogenase:

2. Reduced diphosphopyridine nucleotide, DPNH
disodium salt, DPNH-Na_2; commercial preparation, see p. 1011.

7. DPN-xylitol (D-xylulose) dehydrogenase
from guinea pig liver[5]; see p. 195.

Purity of the enzyme preparation

An enzyme preparation containing at least 23 units *)/mg. protein is preferable for the determination of D-xylulose in the presence of L-xylulose. This highly purified preparation is unstable. For many purposes the less pure, but considerably more stable ammonium sulphate precipitate is suitable.

Preparation of Solutions

See determination of L-xylulose (p. 191). Additional:

II. Reduced diphosphopyridine nucleotide (*ca.* 0.01 M β-DPNH):
Dissolve 10 mg. DPNH-Na_2 in 1 ml. distilled water.

VI. DPN-xylitol (D-xylulose) dehydrogenase (50–100 units/ml.):
If necessary, dilute the aqueous enzyme solution prepared according to[5] with water.

*) One unit is defined as the amount of enzyme which decreases the optical density of DPNH at 340 mμ by 1.0 in 1 min. and in a 1 ml. test mixture[5] with a 1 cm. light path.

1) *P. A. Levene* and *F. B. LaForge*, J. biol. Chemistry *18*, 319 [1914].
2) *G. Ashwell* and *J. Hickman*, J. Amer. chem. Soc. *76*, 5889 [1954].
3) *P. A. Srere*, *J. R. Cooper*, *V. Klybas* and *E. Racker*, Arch. Biochem. Biophysics *59*, 535 [1955].
4) *J. Hickman* and *G. Ashwell*, J. biol. Chemistry *232*, 737 [1958].
5) *J. Hickman* and *G. Ashwell*, J. biol. Chemistry *234*, 758 [1959].

Stability of the solutions

See determination of L-xylulose (p. 192). Instead of TPNH solution read DPNH solution.

Procedure

Deproteinization

See determination of L-xylulose (p. 192).

Spectrophotometric measurements

See determination of L-xylulose (p. 192). Instead of TPNH solution read DPNH solution, instead of L-xylulose read D-xylulose.

Calculations

See determination of L-xylulose (p. 193). Instead of L-xylulose and TPNH read D-xylulose and DPNH.

Specificity

DPN-xylitol (D-xylulose) dehydrogenase has not yet been purified to the extent of its TPN analogue. Also it is not so specific. Preparations obtained according to [5] are inactive with L-xylulose and TPNH, but they react with L-erythrulose and DPNH. This lack of enzyme specificity can be corrected for, since L-erythrulose can be determined colorimetrically according to *Dische*[6]. The fact that L-erythrulose is reduced could prove of value in studies on tetrose metabolism.

Other Methods of Determination

See determination of L-xylulose (p. 193).

Appendix

Isolation of TPN-xylitol (L-xylulose) dehydrogenase and DPN-xylitol (D-xylulose) dehydrogenase[5].

The starting material is an acetone-dried powder of guinea pig liver. The isolation and separation of the dehydrogenases from an aqueous extract of the acetone powder includes the following steps: Heating to 50° C, calcium phosphate gel adsorption to remove inactive protein, half saturation with ammonium sulphate (separation of the dehydrogenases: supernatant contains the DPN-specific, the precipitate the TPN-specific enzyme). Low temperature acetone fractionation yields the TPN dehydrogenase which is completely free from the DPN dehydrogenase. The DPN enzyme is further purified by acetone fractionation.

TPN-specific dehydrogenase: *ca.* 100-fold purification. Final product as a solution in water.

DPN-specific dehydrogenase: *ca.* 40-fold purification. Final product as a solution in water.

For pH optimum, inhibitors and activators, substrate specificity, *etc.* see[5].

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[6] *Z. Dische* and *M. R. Dische*, Biochim. biophysica Acta *27*, 184 [1958].