L-Xylulose

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Touster et al.[1,2] have shown that two pyridine nucleotide dehydrogenases concerned with the metabolism of L-xylulose have xylitol as a common substrate. On the basis of these observations *Hickman* and *Ashwell*[3] developed a simple method for the enzymatic determination of microgram quantities of D- and L-xylulose. The stereospecificity of the methods depends on a satisfactory separation of the two enzymes, which is readily accomplished with acetone powder of guinea pig liver as starting material.

Principle

TPN-xylitol (L-xylulose) dehydrogenase catalyses the reaction:

(1) $$\text{L-Xylulose} + \text{TPNH} + \text{H}^+ \rightleftharpoons \text{xylitol} + \text{TPN}^+$$

The equilibrium is greatly in favour of L-xylulose reduction. In the presence of a slight excess of TPNH and enzyme, the reaction proceeds rapidly to completion with the quantitative formation of xylitol. The decrease in optical density at 340 mμ is directly proportional to the L-xylulose content of the sample being assayed.

Reagents

1. Tris-hydroxymethyl-aminomethane, tris
2. Reduced triphosphopyridine nucleotide, TPNH
   sodium salt, TPNH-Na_4; commercial preparation, see p. 1030.
3. Cysteine hydrochloride
4. Magnesium chloride, $MgCl_2 \cdot 6H_2O$
5. Perchloric acid, sp. gr. 1.54; (*ca.* 60% w/w)
6. Potassium hydroxide
7. Hydrochloric acid, 1 N
8. TPN-xylitol (L-xylulose) dehydrogenase
   from guinea pig liver[3], see p. 195.

Purity of the enzyme preparation

For the determination of L-xylulose in the presence of D-xylulose it is recommended to use an enzyme preparation of at least 60 units *)/mg. protein. However, such a highly purified preparation is unstable and loses the greater part of its activity upon storage overnight at 0°C. For many purposes a less pure fraction (1st ammonium sulphate precipitation) is adequate, since it is stable for about one week at 0°C.

Preparation of Solutions

All solutions are made up in doubly distilled water.

I. Tris buffer (0.10 M; pH 7.0):
   Dissolve 1.21 g. of tris-hydroxymethyl-aminomethane in 80 ml. distilled water, adjust to pH 7.0 with *ca.* 10 ml. 1 N HCl and dilute to 100 ml. Check pH with glass electrode.

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*) One unit is defined as the amount of enzyme which decreases the optical density of TPNH by 1.0 in 1 min. at 340 mμ, in 1 ml. assay mixture[3] with a light path of 1 cm.

1) *O. Touster, V. H. Reynolds* and *R. N. Hutcheson*, J. biol. Chemistry *221*, 697 [1956].
2) *S. Hollmann* and *O. Touster*, J. biol. Chemistry *225*, 87 [1957].
3) *J. Hickman* and *G. Ashwell*, J. biol. Chemistry *234*, 758 [1959].

II. Reduced triphosphopyridine nucleotide (*ca.* 0.01 M β-TPNH):
Dissolve 10 mg. TPNH-Na_4 in 1 ml. distilled water.

III. Cysteine hydrochloride (0.05 M):
Dissolve 79 mg. cysteine hydrochloride in 5 ml. distilled water, adjust with 1 N KOH to pH 7.0, dilute to 10 ml. with distilled water.

IV. Magnesium chloride (0.05 M):
Dissolve 1.02 g. $MgCl_2 \cdot 6H_2O$ and make up to 100 ml.

V. Potassium hydroxide (1.0 N):
Dissolve 5.6 g. KOH in distilled water and make up to 100 ml.

VI. TPN-xylitol (L-xylulose) dehydrogenase (*ca.* 60 units/ml.):
If necessary, dilute the aqueous enzyme solution prepared according to[3] with distilled water.

Stability of the solutions

The tris buffer and $MgCl_2$ solution are stable indefinitely when stored at 0° C. The cysteine solution is neutralized with KOH just before use and kept at 0° C. It must be freshly prepared each day. If necessary, the pH of the TPNH solution should be adjusted to 7.0—8.0 and it should be stored at —10° C. Under these conditions it is stable for several weeks.

Procedure

Deproteinization

Mix

20 parts of a 10—20% tissue homogenate with
1 part $HClO_4$, 60% (w/w),

cool to 0° C, centrifuge, carefully neutralize the supernatant with

1 N KOH (solution V).

Allow to stand for 30 min. at 0° C and then centrifuge. Trichloroacetic acid can be used instead of perchloric acid. Excess trichloroacetic acid is removed by 3—4 extractions of the supernatant with an equal volume of diethyl ether, followed by neutralization.

Spectrophotometric measurements

Wavelength: 340 mμ; 1.5 ml. silica cells, light path: 1.0 cm.; room temperature.
For each series of measurements prepare a control cuvette containing water instead of sample. Read against a blank cuvette containing water.

Pipette into the control cuvette and experimental cuvette:

0.40 ml. tris buffer (solution I)
0.01 ml. TPNH solution (II)
0.02 ml. cysteine solution (III)
0.10 ml. $MgCl_2$ solution (IV)
0.01—0.02 ml. enzyme solution (VI) (corresponding to 1 unit of the enzyme)
sample (containing 0.01—0.06 μmoles L-xylulose);
corresponding amount of water in control cuvette
distilled water to 1 ml.

Follow the decrease in optical density at 340 mμ in both cuvettes at 3 min intervals over a period of 15—20 min. Read the final value.

Calculations

The L-xylulose content of the assay mixture is calculated from the following formula:

$$\frac{E_C - E_E}{6.22} = \mu\text{moles L-xylulose/ml. assay mixture}$$

where
E_E = final optical density of the experimental cuvette
E_C = final optical density of the control cuvette
6.22 = extinction coefficient for TPNH [cm.²/μmole]

Example

0.01 ml. of an unknown sample containing *ca.* 4.0 μmoles of L-xylulose/ml. was taken for analysis. At the end of the reaction the following optical densities were measured at 340 mμ:

Control cuvette: 0.622
Experimental cuvette: 0.393

It follows that

$$\frac{0.622-0.393}{6.22} = \frac{0.229}{6.22} = 0.037\ \mu\text{moles L-xylulose/ml. assay mixture}$$

or

$$\frac{0.037}{0.01} = 3.7\ \mu\text{moles L-xylulose/ml. original sample.}$$

Specificity

The purified TPN-xylitol dehydrogenase appears to be highly specific for L-xylulose and TPNH, DPNH and D-xylulose do not react. The following compounds are also inactive: D-fructose, D-ribulose. D-ribose, D-xylose, L-sorbose, D-altroheptulose, L-erythrulose and D-galacturonic acid. At 20—50 times higher concentration L-ribulose reacts at about 6% of the rate of L-xylulose.

Other Methods of Determination

Xylulose can be determined colorimetrically with the aid of the cysteine-carbazole reaction of *Dische*[4]. This test does not distinguish between the stereoisomers of the ketopentose and is useless in the presence of fructose, sorbose, ribulose or sedoheptulose. It is also seriously affected by equal concentrations of DPNH and TPNH.

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[4] *G. Ashwell* and *J. Hickman*, J. biol. Chemistry *226*, 65 [1957].