L-Erythrulose

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The discovery by *Hollmann* and *Touster*[1] that polyol dehydrogenase catalyses the reduction of L-erythrulose by reduced diphosphopyridine nucleotide (DPNH) can be used for the quantitative determination of L-erythrulose (according to the principle of spectrophotometric assay developed by *O. Warburg*[2]).

Principle

Polyol dehydrogenase (PDH) catalyses the reduction of L-erythrulose with DPNH according to the equation:

$$\text{L-Erythrulose} + \text{DPNH} + \text{H}^+ \rightleftharpoons \text{L-threitol} + \text{DPN}^+$$

L-Threitol was identified as the reduction product by *Hollmann* and *Touster*[1,3]. The equilibrium of the reaction lies to the right. The Michaelis constant K_M with L-erythrulose is 2.5×10^{-2} M[4]. There fore as the affinity of the enzyme for L-erythrulose is very low, highly purified enzyme must be added in high concentration to obtain quantitative conversion of small amounts of L-erythrulose in a convenient time.

Reagents

1. Triethanolamine
2. Reduced diphosphopyridine nucleotide, DPNH
   disodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
3. Hydrochloric acid, A. R., 2 N
4. Polyol dehydrogenase, PDH
   The enzyme is purified according to *Holzer* and *Goedde*[4] from acetone-dried sheep liver by extraction, acid precipitation, ethanol precipitation, acetone precipitation, adsorption on alumina-C_γ-gel and DEAE-cellulose, and finally ammonium sulphate precipitation.

   Purity of the enzyme preparation

   The PDH preparation used for the quantitative determination of erythrulose should have a specific activity of 100×10^2 to 300×10^2 units*/mg. If L-erythrulose is to be determined in the presence of α-ketoacids and aldehydes (refer to[4]), the enzyme preparation must not contain lactic dehydrogenase or liver alcohol dehydrogenase. Preparations obtained according to the method described in[4] fulfil these conditions and keep for several months at −18°C without loss of activity.

Preparation of Solutions

I. Triethanolamine buffer (0.20 M; pH 7.4):
   Dissolve 7.46 g. triethanolamine in about 100 ml. doubly distilled water, adjust pH to 7.4 with *ca.* 17 ml. 2 N HCl. After cooling, dilute to 250 ml. and check pH with glass electrode.

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*) One unit is defined as the amount of enzyme which decreases the optical density of DPNH by 0.001/min. at 366 mμ in a total volume of 3.0 ml. and with a light path of 1 cm.[4].

1) *S. Hollmann* and *O. Touster*, J. biol. Chemistry *225*, 87 [1957].
2) *O. Warburg:* Wasserstoffübertragende Fermente. Verlag Dr. Werner Saenger GmbH, Berlin 1948.
3) *S. Hollmann*, Hoppe-Seylers Z. physiol. Chem. *317*, 193 [1959].
4) *H. Holzer* and *H. W. Goedde*, Biochim. biophysica Acta *40*, 297 [1960].

II. Reduced diphosphopyridine nucleotide (*ca.* 2×10^{-2} M β-DPNH):
Dissolve 10 mg. DPNH-Na_2 in doubly distilled water and make up to 1.0 ml.

III. Polyol dehydrogenase, PDH (*ca.* 10 mg. protein/ml.):
If necessary, dilute the preparation obtained according to[4] with 0.01 M tris-hydroxymethyl-aminomethane buffer (pH 7.4).

Procedure

For preparation and extraction of experimental material (blood, tissue, *etc.*), see determination of pyruvate (p. 254).

Spectrophotometric measurements

Wavelength: 340 or 366 mμ; light path: 0.5 cm.; final volume: 0.4 ml.; room temperature. Read experimental and control cuvettes against a water blank.

Bring buffer and sample to room temperature; pipette successively into the cuvettes:

Experimental cuvette	*Control cuvette*
0.21 ml. buffer (solution I)	0.21 ml. buffer (solution I)
0.10 ml. sample	0.10 ml. distilled water
0.03 ml. DPNH solution (II)	0.03 ml. DPNH solution (II)

Read optical densities of both cuvettes. If the change in optical density in both cuvettes does not exceed 0.001 to 0.002 per 30 seconds, mix

0.06 ml. PDH solution (III)

into both cuvettes. The reaction is considered complete (usually after about 60 min.) when the same changes in optical density with time are obtained for the experimental and control cuvettes. A control containing all the components of the assay mixture, but with water instead of enzyme, usually shows no significant change in optical density with time. The ΔE value required for the calculations is obtained by subtracting the optical density difference between sample and control before the start of the reaction with PDH, from the optical density difference between the sample and control after completion of the reaction.

The optical density change due to the absorption of the enzyme and the dilution of the cuvette contents may be obtained by the addition of the enzyme to the control cuvette. This optical density change can be either positive or negative according to the magnitude of the initial optical density and the absorption of the enzyme solution; usually it can be neglected.

Calculations

$$\frac{\Delta E \times V}{\varepsilon \times d} = \mu\text{moles erythrulose/cuvette}$$

ΔE is the decrease in optical density after addition of PDH, corrected as stated above, ε is the extinction coefficient (in $cm.^2/\mu$mole) for DPNH, which is 3.3 at 360 mμ, 6.2 at 340 mμ and 5.9 at 334 mμ; d is the light path in cm., V is the final volume of the assay mixture in ml.

Other Determinations

Using the same sample and test mixture other substrates can be determined before the estimation of erythrulose by addition of specific enzymes, *e.g.* hydroxypyruvate with crystalline lactic dehydrogenase and glycolaldehyde with crystalline yeast alcohol dehydrogenase (refer to[4]).

Specificity

Polyol dehydrogenase from different tissues and bacteria reacts with numerous keto-sugars and alcohols, as the studies of *Blakley*[5], *Williams-Ashman* et al.[6], *McCorkindale* et al.[7], *Shaw*[8], *Arcus* et al.[9] and *Hollmann* and *Touster*[1,3] have shown. The polyol dehydrogenase from sheep liver used in the above test does not catalyse the reduction of the following compounds by DPNH: pyruvate, hydroxypyruvate, α-oxoglutarate, acetaldehyde and glycolaldehyde (in a final concentration of 0.01 M). A mixture of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate was reduced at about 3% the rate of 0.01 M erythrulose[4].

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5) *R. L. Blakley*, Biochem. J. *49*, 257 [1951].
6) *H. G. Williams-Ashman* and *J. Banks*, Arch. Biochem. Biophysics *50*, 513 [1954].
7) *J. McCorkindale* and *N. L. Edson*, Biochem. J. *57*, 518 [1954].
8) *D. R. D. Shaw*, Biochem. J. *64*, 394 [1956].
9) *A. C. Arcus* and *N. L. Edson*, Biochem. J. *64*, 385 [1956].