Triphosphopyridine Nucleotide (TPN)

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Principle

TPN is reduced by glucose-6-phosphate and glucose-6-phosphate dehydrogenase (G6P-DH, zwischenferment) to TPNH:

(1) Glucose-6-phosphate + TPN^+ $\rightleftharpoons$ 6-phosphogluconate + TPNH + H^+

The equilibrium constant

$$K_{pH} = \frac{K}{[H^+]} = \frac{[\text{6-phosphogluconate}] \times [\text{TPNH}]}{[\text{glucose-6-phosphate}] \times [\text{TPN}^+]}$$

is $K_7 = 10^5$ at pH 7 and 25°C. Therefore the equilibrium is far in favour of TPNH formation.

Reagents

1. Perchloric acid, A. R., sp. gr. 1.67; *ca.* 70% (w/w)
2. Potassium hydroxide, A. R., 3 N
3. Dipotassium hydrogen phosphate, K_2HPO_4, A. R.
4. Sodium hydroxide, A. R., 2 N
5. Magnesium sulphate, $MgSO_4 \cdot 7H_2O$, A. R.
6. Glucose-6-phosphate, G-6-P
   disodium salt; commercial preparation, see p. 1017.
7. Glucose-6-phosphate dehydrogenase, G6P-DH
   from yeast, suspension in 3.3 M ammonium sulphate solution. Commercial preparation, see p. 975.

Purity of the enzyme preparation

The specific activity of the G6P-DH preparation should be about 4000 units/mg. according to *Bücher*[1] or 70 units/mg. according to *Racker*[2]. G6P-DH preparations may contain glutathione reductase. The values for TPN in an extract containing glutathione will be too low because of the re-oxidation of the TPNH by this enzyme. Therefore the glutathione reductase content of the glucose-6-phosphate dehydrogenase preparation must be $<0.2\%$ (relative to the specific activity of the G6P-DH).

Preparation of Solutions

I. and II. Perchloric acid solutions as described on p. 528.

III. Dipotassium hydrogen phosphate (1 M) as described on p. 528.

IV. Magnesium sulphate (1 M):
Dissolve 2.4 g. $MgSO_4 \cdot 7H_2O$ in doubly distilled water and make up to 10 ml.

V. Glucose-6-phosphate (*ca.* 0.1 M G-6-P):
Dissolve 0.31 g. G-6-P-Na_2 in doubly distilled water and make up to 10 ml.

VI. Glucose-6-phosphate dehydrogenase, G6P-DH (1 mg. protein/ml.):
Dilute the stock suspension with 3.3 M ammonium sulphate solution.

Stability of the solutions

All solutions are stable for several months when stored, stoppered, in a refrigerator.

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[1] *G. Beisenherz, H. J. Boltze, Th. Bücher, R. Czok, K. H. Garbade, E. Meyer-Arendt* and *G. Pfleiderer*, Z. Naturforsch. *8b*, 555 [1953].

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

Procedure

Experimental material and deproteinization

Extract TPN from experimental material by the method described for DPN (p. 529). Use undiluted extract for the measurements.

Spectrophotometric measurements

Since the TPN concentration in biological material is very low, it is necessary to use the most sensitive method of measurement available (cuvettes with a long light path and a spectrophotometer of high sensitivity).

Wavelength: 340 mμ (not 334 or 366 mμ); light path: 4 cm.; final volume: 4.080 ml.; room temperature. Measure against air or water.

Pipette successively into the cuvette:

4.000 ml. extract
0.020 ml. magnesium sulphate solution (IV)
0.040 ml. G-6-P solution (V).

Mix, read optical density E_1, then mix in

0.020 ml. G6P-DH suspension (VI)

and after about 15 min. read the final optical density E_2

$\Delta E = E_2 - E_1$ is used for the calculations.

Calculations

The procedure for the calculations is the same as for DPN, see p. 530.

Sources of Error

The presence of glutathione reductase in the G6P-DH preparation interferes with the determination (see "Purity of the enzyme preparation"); TPNH oxidase also interferes. A slow decrease in the optical density on completion of the reaction can be corrected for by graphical extrapolation, see p. 39. G6P-DH is absolutely specific for TPN.

Other Methods for the Determination of TPN

The reaction catalysed by isocitric dehydrogenase:

(2) $$\text{Isocitrate} + \text{TPN}^+ \rightleftharpoons \alpha\text{-oxoglutarate} + \text{TPNH} + CO_2 + H^+$$

can also be used for the estimation of TPN[3]. The equilibrium of this reaction is also greatly in favour of TPNH formation. For the isolation of an isocitric dehydrogenase preparation suitable for the determination of TPN, see[4,5]. Commercial preparation, see p. 985.

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[3] *M. Klingenberg* and *W. Slenczka*, Biochem. Z. *331*, 486 [1959].
[4] *G. Siebert*, *J. Dubuc*, *R. C. Warner* and *G. W. E. Plaut*, J. biol. Chemistry *226*, 965 [1957].
[5] *P. Baum* and *R. Czok*, Biochem. Z. *332*, 121 [1959].