D-Glucose-1-phosphate

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The quantitative determination of glucose-1-phosphate (G-1-P) by acid hydrolysis and estimation of the inorganic phosphate or glucose liberated is unspecific. On the other hand, the enzymatic determination is specific. This is particularly important for the analysis of G-1-P in the presence of glucose-6-phosphate (G-6-P) (see also p. 134) and other sugar phosphates.

Principle

According to *Cori*, *Colowick* and *Cori*[1] glucose-1-phosphate is converted to glucose-6-phosphate by phosphoglucomutase[2]:

(1) Glucose-1-phosphate $\rightleftharpoons$ glucose-6-phosphate

G-6-P is oxidized by triphosphopyridine nucleotide (TPN) and glucose-6-phosphate dehydrogenase (G6P-DH) to 6-phosphogluconate with the formation of reduced triphosphopyridine nucleotide (TPNH):

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

The TPNH formed is measured by the increase of optical density at 366 or 340 mμ. The amount of TPNH is directly proportional to the amount of G-1-P, since reaction (2) proceeds virtually quantitatively from left to right.

Reagents

1. Triethanolamine hydrochloride
2. Magnesium chloride, A. R., $MgCl_2 \cdot 6\ H_2O$
3. Ethylene-diamine-tetra-acetic acid, EDTA
   disodium salt, EDTA-$Na_2H_2 \cdot 2\ H_2O$.
4. Sodium hydroxide, A. R., 1 N
5. Triphosphopyridine nucleotide, TPN
   sodium salt, TPN-NaH_2; commercial preparation, see p. 1029.
6. Glucose-6-phosphate dehydrogenase, G6P-DH
   from yeast; suspension in 3.2 M ammonium sulphate solution; specific activity at least 70 units*)/mg.; commercial preparation, see p. 974.
7. Phosphoglucomutase, PGluM
   from rabbit skeletal muscle; suspension in 2.5 M ammonium sulphate solution; specific activity at least 6.5 units*)/mg.; commercial preparation, see p. 992.

Purity of the enzyme preparations

The glucose-6-phosphate dehydrogenase preparation must not contain more than 0.2% hexokinase, 0.1% 6-phosphogluconic dehydrogenase, 0.05% phosphohexoisomerase and 0.5% glutathione reductase (relative to the G6P-DH activity). The phosphoglucomutase preparation must not contain more than 0.1% glutathione reductase and 0.01% phosphohexoisomerase, hexokinase and 6-phosphogluconic dehydrogenase (relative to the PGluM activity).

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*) A unit is the amount of enzyme which converts 1 μmole of substrate in 1 min. at 25°C.

1) *G. T. Cori*, *S. P. Colowick* and *C. F. Cori*, J. biol. Chemistry *123*, 375 [1938].

2) See also *M. W. Slein*, chapter on "Glucose", p. 117.

Preparation of Solutions

To prevent the growth of micro-organisms sterilize the containers.

I. Triethanolamine buffer (0.05 M; pH 7.6):
Dissolve 9.3 g. triethanolamine hydrochloride in 22 ml. N NaOH and dilute to 1000 ml. with doubly distilled water. Check the pH (glass electrode).

II. Magnesium chloride (0.1 M):
Dissolve 2.03 g. $MgCl_2 \cdot 6\ H_2O$ in doubly distilled water and make up to 100 ml.

III. Ethylene-diamine-tetra-acetate, EDTA (*ca.* 0.02 M):
Dissolve 50 mg. EDTA-$Na_2H_2 \cdot 2\ H_2O$ in doubly distilled water and make up to 10 ml.

IV. Triphosphopyridine nucleotide, (*ca.* 0.012 β-TPN):
Dissolve 10 mg. TPN-NaH_2 in 1 ml. doubly distilled water.

V. Glucose-6-phosphate dehydrogenase, G6P-DH (1 mg. protein/ml.):
Dilute the stock suspension with 3.2 M ammonium sulphate solution.

VI. Phosphoglucomutase, PGluM (2 mg. protein/ml.):
Dilute the stock suspension with 2.5 M ammonium sulphate solution.

Stability of the solutions

Store all solutions and suspensions, stoppered, in a refrigerator at 0 to 4° C. In this state they are stable for several weeks.

Procedure

Experimental material

The method has so far only been used for the determination of the purity of aqueous solutions of G-1-P and G-6-P preparations. It has not been tested with biological material (see under "Sources of Error").

Spectrophotometric measurements

Wavelength: 366 or 340 mμ; light path: 1 cm.; final volume: 3.0 ml.; room temperature. Measure against the blank.

Pipette successively into the cuvettes:

Blank: 2.88 ml. buffer (solution I)
0.02 ml. sample

Experimental: 2.69 ml. buffer (solution I)
0.10 ml. $MgCl_2$ solution (II)
0.10 ml. EDTA solution (III)
0.05 ml. TPN solution (IV)
0.02 ml. sample.

Mix thoroughly with a glass or plastic rod flattened at one end and read the optical density E_1. Mix in

0.02 ml. G6P-DH suspension (V),

wait for the end of the reaction (increase in optical density caused by the G-6-P contained in the sample reacting according to equation (1)) and then read the optical density E_2. Mix in

0.02 ml. PGluM suspension (VI).

Follow the increase in optical density at 2 min. intervals until the reaction stops. Read the optical density E_3.

$E_2 - E_1 = \Delta E_{G\text{-}6\text{-}P}$

$E_3 - E_2 = \Delta E_{G\text{-}1\text{-}P}$

These values are used for the calculations.

Calculations

For a final volume in the cuvette of 3.0 ml. (refer to p. 37).

$$\text{at } 340\,m\mu: \frac{\Delta E_{G\text{-}1\text{-}P} \times 3.0}{6.22} = \mu\text{moles G-1-P/assay mixture}$$

$$\text{at } 366\,m\mu: \frac{\Delta E_{G\text{-}1\text{-}P} \times 3.0}{3.3} = \mu\text{moles G-1-P/assay mixture}$$

$$\mu\text{moles G-1-P} \times 260 = \mu\text{g. G-1-P}$$

To obtain the amount of G-1-P per ml. of sample, the results must be multiplied by 50 if 0.02 ml. of sample is taken for the assay.

To calculate the amount of G-6-P in the sample use the same formula, but multiply the $\Delta E_{G\text{-}6\text{-}P}$ by 2.98/3.00 because of the smaller assay volume.

Sources of Error

Enzymes which are not sufficiently pure can lead to false results if the sample contains, for example, 6-phosphogluconate, glucose, fructose, ATP or fructose-6-phosphate. If large amounts of fructose-6-phosphate are present, the reaction before and after the addition of PGluM may not stop completely. In this case, extrapolate to the time of addition of the PGluM and so obtain $\Delta E_{G\text{-}1\text{-}P}$ (refer to p. 39).

In the application of the method to biological material, for example, tissue homogenates, the "quick-freeze" method (refer to p. 47) should be used, otherwise G-1-P will be rapidly converted to glucose or G-6-P because of the high phosphatase or phosphoglucomutase activity of the tissue.

Specificity

The reaction shown in equation (1) is specific for glucose-1-phosphate. According to *Najjar*[3] the conversion of G-1-P to G-6-P proceeds by way of glucose-1,6-diphosphate. If the sample contains no glucose-1,6-diphosphate, the phosphoglucomutase reaction requires an induction period of *ca.* 2 min. in which the necessary catalytic amounts of glucose-1,6-diphosphate are formed.

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[3] *V. A. Najjar* in *W. D. McElroy* and *B. Glass:* The Mechanism of Enzyme Action. Johns-Hopkins Press, Baltimore 1954.