1-Phosphofructoaldolase

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So far 1-phosphofructoaldolase (PFA) has been found mainly in the liver[1, 2], and in smaller amounts in the kidney and intestinal mucosa of human beings and several other mammals[2, 3]. The enzyme is certainly related to muscle aldolase, because after treatment of muscle aldolase with carboxypeptidase its activity with fructose-1,6-diphosphate decreases considerably, while that with fructose-1-phosphate is unaltered, although only one or two amino acids have been split off from the muscle aldolase[4, 4a]. Highly purified muscle aldolase cleaves fructose-1-phosphate only slowly at high substrate concentrations. In human serum PFA occurs only after damage to the liver parenchyma[5–7]; rat serum always has a certain amount of activity with fructose-1-phosphate[2]. An enzyme with the properties of PFA has also been found in *Canavalia ensiformis*[7a].

Determination of PFA activity: Colorimetrically, by measurement of the complex formed in strongly alkaline solutions by the 2,4-dinitrophenylhydrazine derivatives of dihydroxyacetone phosphate and D-glyceraldehyde[8]. Or spectrophotometrically, by making use of the reduction of dihydroxyacetone phosphate with DPNH*) and α-glycerophosphate dehydrogenase (GDH) as indicator reaction.

Principle

1-Phosphofructoaldolase catalyses the reaction:

(1) Fructose-1-phosphate $\rightleftharpoons$ dihydroxyacetone phosphate + D-glyceraldehyde

The equilibrium lies to the left. Nevertheless, the further conversion of dihydroxyacetone phosphate by means of the indicator reaction (2) results in the quantitative conversion of fructose-1-phosphate

(2) Dihydroxyacetone phosphate + DPNH + H^+ $\rightleftharpoons$ α-glycerophosphate + DPN^+

The rate of oxidation of DPNH is proportional to the amount of dihydroxyacetone phosphate formed by reaction (1) and therefore to the activity of the enzyme. The method described here was developed by *Wolf, Forster* and *Leuthardt*[5] for measurements in serum and modified by *Holzer* and *Stewing*[9].

Optimum Conditions for Measurements

The optimum pH for the enzyme lies beween 6.7 and 7.8[1]. With a fructose-1-phosphate concentration of 1.1×10^{-2} M the reaction is linear with time[6]. For routine determinations it is recommended to work at 25°C; by increasing the temperature to 36°C the rate of the reaction is almost doubled. Normally buffer is not required, but strongly icteric serum should be diluted with 0.05 M triethanolamine buffer (pH 7.6).

The DPNH concentration should be about 3×10^{-4} M.

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*) DPN = diphosphopyridine nucleotide; DPNH = reduced diphosphopyridine nucleotide.

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7a) *C. E. Cardini*, Enzymologia *15*, 503 [1952].
8) *J. A. Sibley* and *A. Lehninger*, J. biol. Chemistry *177*, 859 [1949].
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Reagents

1. Triethanolamine hydrochloride
2. Sodium hydroxide, 1 N
3. Fructose-1-phosphate
   barium salt or crystalline dicyclohexylammonium salt; commercial preparation, see p. 1015.
4. Sodium sulphate, sat. solution
5. Reduced diphosphopyridine nucleotide, DPNH
   sodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
6. Sodium hydrogen carbonate, $NaHCO_3$, 1% solution
7. α-Glycerophosphate dehydrogenase, GDH
   crystalline suspension in ammonium sulphate solution. Commercial preparation, see p. 981.

Purity of the indicator enzyme

The GDH preparation should not contain more than the following amounts of contaminating enzymes relative to its own activity: muscle aldolase <0.01%; lactic and glyceraldehyde-3-phosphate dehydrogenase <0.05%; pyruvic kinase <0.03%.

Preparation of Solutions

All solutions should be prepared with glass distilled water.

I. Triethanolamine buffer (0.05 M; pH 7.6):
Dissolve 9.3 g. triethanolamine hydrochloride in 22 ml. 1 N NaOH and dilute to 1000 ml. with distilled water.

II. Fructose-1-phosphate (*ca.* 0.2 M F-1-P):
Dissolve 0.9 g. barium fructose-1-phosphate in 8 ml. distilled water, add the calculated amount of saturated Na_2SO_4 solution, centrifuge off the $BaSO_4$ precipitate and dilute the clear solution of the sodium salt to 10 ml. with distilled water. Or dissolve 0.9 g. dicyclohexylammonium salt in distilled water and make up to 10 ml.

III. Reduced diphosphopyridine nucleotide (*ca.* 1.5×10^{-2} M β-DPNH):
Dissolve 100 mg. DPNH-Na_2 in 10 ml. distilled water and adjust to pH 7.6 with 1% $NaHCO_3$ solution.

IV. α-Glycerophosphate dehydrogenase, GDH (2 mg. protein/ml.):
If necessary, dilute the commercially available, crystalline suspension with 2 M ammonium sulphate solution.

Stability of the solutions

All the solutions should be stored, stoppered, in a refrigerator at 0 to 4°C. The GDH suspension should not be frozen. The DPNH solution should be prepared freshly each week. The fructose-1-phosphate solution is stable for a longer period if bacterial contamination is avoided by sterilization of the container and aseptic sampling of the solution.

Procedure

Spectrophotometric measurements

Wavelength: 366 mμ; light path: 1 cm.; final volume: 2.07 ml.; temperature: constant 25°C. Measure against air or distilled water.

Pipette successively into the cuvette:

1.8 ml. serum
0.05 ml. DPNH solution (III).

Allow to stand for 15 min. After this interval the substrates contained in the serum (especially pyruvate) have reacted. Start the reaction with

0.02 ml. GDH suspension (IV) and
0.20 ml. fructose-1-phosphate solution (II).

Mix well. About 3–4 min. after beginning the reaction note the time required for an easily readable change in optical density ΔE. If the needle of a direct reading photometer has previously been adjusted to a favourable part of the scale, optical density changes of 0.020 to 0.050 are sufficient.

Calculations

According to[5b] a unit is the amount of enzyme in an assay mixture of 2.07 ml., which decreases the optical density of DPNH at 366 mμ by 0.100 in 100 min. (25° C). ΔE is therefore multiplied by 1000. The units/ml. are obtained by division by the ml. serum used for the assay.
The PFA reaction is linear with time up to $\Delta E \approx 0.200$, but it is not necessary to measure the time taken for $\Delta E = 0.100$. The units are calculated from the more rapidly measured, smaller ΔE values according to the formula

$$\frac{\Delta E_{366} \times 1000}{\text{ml. serum} \times \text{time [min.]}} = \text{PFA units/ml. serum}$$

If the serum is strongly icteric, then in order to decrease the optical density, it should be diluted with triethanolamine buffer (solution I) until $\log I_0/I < 1.0$ (including the DPNH absorption). This dilution should be taken into account in the calculations.

Example

Amount of serum: 1.8 ml. Optical density change in 10 min. $\Delta E = 0.040$

$$\frac{0.040 \times 1000}{1.8 \times 10} = 2.2 \text{ PFA units/ml. serum}$$

or: optical density change in 20 min. $\Delta E = 0.080$

$$\frac{0.080 \times 1000}{1.8 \times 20} = 2.2 \text{ PFA units/ml. serum}$$

Values up to 1.1 units/ml. serum are normal; higher values indicate damage to the liver parenchyma.

Conversion to other units

Conversion to other units, *e.g.* according to *Bücher*[10], is not advisable, because this unit is defined as an optical density change of 0.100/100 sec. (366 mμ) and therefore with the slow PFA reaction values of a low order would be obtained.

Stability of the Enzyme in the Serum Sample

The serum sample can be stored for several days between 0 and 4° C without noticeable change in the PFA activity.

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

Sources of Error

The alcohol dehydrogenase present in liver homogenates, which reduces the D-glyceraldehyde formed according to equation (1) with DPNH to give glycerol, and so simulates a raised PFA activity, may be ignored, because under the conditions chosen only insignificant amounts of D-glyceraldehyde are reduced.

Determination in Tissues

PFA can be determined directly in tissue homogenates[11], extracts of acetone-dried powders[1], or protein fractions which have been obtained by ammonium sulphate fractionation[12]. However, in the last instance it is necessary to dialyse the protein against glass distilled water, because PFA is inhibited by high concentrations of ammonium sulphate[13].

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[11] *E. Schmidt, F. W. Schmidt* and *E. Wildhirt*, Klin. Wschr. *37*, 1221 [1959].
[12] *U. Kaletta-Gmünder, H. P. Wolf* and *F. Leuthardt*, Helv. chim. Acta *40*, 1027 [1957].
[13] *H. P. Wolf* and *F. Leuthardt*, Helv. chim. Acta *40*, 237 [1957].