Glutamic Dehydrogenase

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Glutamic dehydrogenase (GlDH) has been detected in micro-organisms[1-9], higher plants[10,11] and warm-blooded tissues[12-16]. In mammals the richest source is the liver. The liver of a healthy person contains 3000–4000 units*)/g. fresh weight[17], followed by kidney cortex (600–800 units/g.), cerebral cortex, gastric mucosa, lymph nodes, lung, cerebral medulla and cerebellum (100–160 units/g.)[17]. The concentration in muscle is very low[16,17]. The enzyme is not detectable with the usual methods in non-nucleated erythrocytes[17] or in normal serum[18,19]. A higher activity can occur in serum, mainly after liver cell damage[18,19].

Liver GlDH reacts with diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN)[14,15,20-24]. Of the four possible methods (two coenzymes, two reaction directions) of measuring the activity of GlDH from warm-blooded animals, the measurement of the rate of DPNH oxidation is the most convenient (limitation: very narrow optimum concentration range for DPNH[23,25], see Fig. 1.). With human liver extract and under optimum conditions, the following activities were measured: compared with DPNH, 50% activity was obtained with TPNH, 20% with DPN and only 3% with TPN.

Principle

Glutamic dehydrogenase (GlDH) catalyses the reaction:

(1) α-Oxoglutarate + NH_4^+ + DPNH $\rightleftharpoons$ L-(+)glutamate + DPN^+ + H_2O

The equilibrium lies in favour of amino acid formation. Oxidation of DPNH is directly proportional to the reduction of the substrate and can be followed by the decrease in optical density (ΔE) at 340 or 366 mμ.

*) Definition according to *Th. Bücher* et al. (p. 33).

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Optimum Conditions for Measurements

Figure 1 gives the relationship between enzyme activity in serum from patients with liver disease and substrate concentration, and pH.

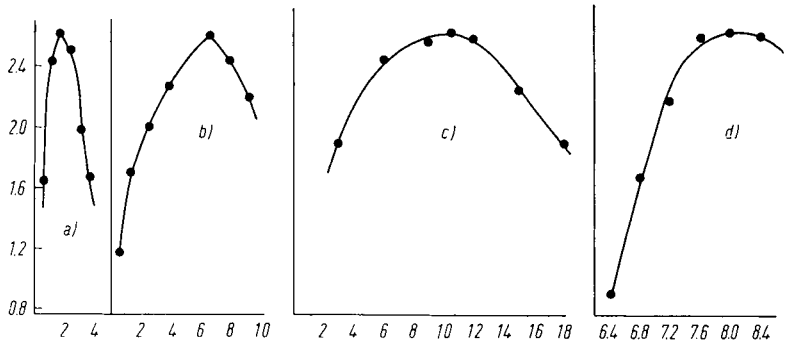

Figure 1. Dependence of GlDH activity in hepatitis serum on pH, and coenzyme and substrate concentrations. Triethanolamine buffer, 25°C.

a) Effect of varying DPNH with constant: α-oxoglutarate 6.5×10^{-3} M, NH_4^+ 10.5×10^{-2} M and pH 8.0
b) Effect of varying α-oxoglutarate with constant: DPNH 1.5×10^{-4} M, NH_4^+ 10.5×10^{-2} M and pH 8.0
c) Effect of varying NH_4^+ with constant: DPNH 1.5×10^{-4} M, α-oxoglutarate 6.5×10^{-3} M and pH 8.0
d) Effect of varying pH with constant: DPNH 1.5×10^{-4} M, α-oxoglutarate 6.5×10^{-3} M, NH_4^+ 10.5×10^{-2}

Ordinate: Units/ml. serum
Abscissa: a) DPNH ($\times10^{-4}$ M) — b) α-oxoglutarate ($\times10^{-3}$ M) — c) NH_4^+ ($\times10^{-2}$ M) — d) pH

The pH optimum depends on the buffer used and lies between 7.6 and 8.6[10, 11, 14, 21, 23, 24, 26]. The activity of GlDH is primarily dependent on the DPNH eoncentration, and secondly on the concentration of α-oxoglutarate and NH_4^+.

Reagents

1. Triethanolamine hydrochloride
2. Sodium hydroxide, 2 N, A. R.
3. α-Oxoglutarate
   commercial preparation, see p. 1024
4. Ammonium acetate, A. R.
5. Reduced diphosphopyridine nucleotide, DPNH
   sodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
6. Ethylene-diamine-tetra-acetic acid, EDTA
   disodium salt, EDTA-$Na_2H_2\cdot2H_2O$, *e.g.* Titriplex III*)

Preparation of Solutions

I. Triethanolamine buffer (0.05 M; pH 8.0):
Dissolve *ca.* 930 mg. triethanolamine hydrochloride in *ca.* 80 ml. doubly distilled water, adjust pH to 8.0 with 1.58 ml. 2 N NaOH (glass electrode) and dilute to 100 ml.

*) e.g. from E. Merck, Darmstadt (Germany)

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II. Sodium α-oxoglutarate (*ca.* 0.4 M):
Dissolve 146 mg. α-oxoglutaric acid in *ca.* 1 ml. doubly distilled water, bring to pH 6.8 with several drops 2 N NaOH, and dilute to 2.5 ml.

III. Ammonium acetate (*ca.* 3 M):
Dissolve 2.35 g. ammonium acetate in 10 ml. doubly distilled water.

IV. Reduced diphosphopyridine nucleotide (*ca.* 0.01 M β-DPNH):
Dissolve 15 ml. DPNH-Na_2 in 1.5 ml. doubly distilled water.

V. Ethylene-diamine-tetra-acetate (*ca.* 0.26 M):
Dissolve 100 mg. EDTA-$Na_2H_2 \cdot 2H_2O$ in 1 ml. doubly distilled water.

Stability of the solutions

Store the DPNH and α-oxoglutarate solution, and the buffer at 0° to 4° C. The DPNH solution is stable for at least a week, the α-oxoglutarate solution for about four weeks if its pH is below 7.0. The other solutions are stable indefinitely.

Procedure

Before commencing the measurements bring the daily requirements of buffer to 25°C in a thermostatically controlled water bath (pour, do not pipette!). Keep the DPNH and α-oxoglutarate solutions in an ice bath.

Preferably use fresh serum. Haemolysis does not interfere with the assay because erythrocytes contain no GlDH, however considerable haemolysis can increase the "preliminary reaction" (see below) resulting in the oxidation of too large a portion of the added DPNH.

Spectrophotometric measurements

Wavelength: 366 mμ; light path: 1 cm.; final volume: 2.0 ml.; temperature: 25°C. A blank is unnecessary. Read against air or water.

Pipette into the cuvette:

1.34 ml. buffer (solution I)
0.03 ml. DPNH solution (IV)
0.02 ml. EDTA solution (V)
0.07 ml. ammonium acetate solution (III)
0.50 ml. serum.

Mix with a glass rod flattened at one end. Wait until the decrease in optical density stops (2—10 min.). This "preliminary reaction" is variable and is due to interaction of substrates and DPNH-linked dehydrogenases in the serum. If the optical density change exceeds 0.100 (corresponding to the oxidation of more than 0.06 μmoles DPNH), then another 0.01 ml. DPNH solution (IV) must be added.

Start the GlDH reaction by mixing in

0.04 ml. α-oxoglutarate solution (II).

Note the time (in sec.) taken for a decrease in optical density of 0.020 and continue readings until the total decrease in optical density is >0.120. Average the time (in sec.) taken for each optical density change of 0.02 and use this average for the calculations.

The reactions is linear with time at least until the optical density has decreased by 0.100. Premature retardation of the rate is usually due to lack of DPNH, and gradual acceleration

to excess DPNH (refer to Fig. 1). The decrease in optical density remains linear with time even if other than optimum concentrations of α-oxoglutarate or NH_4^+ are used. If the optical density decreases by less than 0.005 in 5 minutes, then measurement is discontinued; >6000 sec. ($\Delta E = 0.100$) means <0.06 units GlDH/ml. or "normal". Dilution of the serum is not necessary even with high activities.

Calculations

According to *Bücher* et al.[27] a unit of enzyme activity for DPN and TPN-dependent dehydrogenases is the amount of enzyme contained in 1 ml., which at 25° C and with a light path of 1.0 cm, decreases the optical density of DPNH (TPNH) by 0.100 in 100 seconds at 366 mμ. A unit corresponds to the reduction of 1.09 μmoles of substrate/ml./hour[27]. Therefore with a reaction volume of 2 ml., a light path of 1 cm, $\Delta E = 0.020$ and 0.5 ml. of serum added:

$$\frac{100}{\text{sec.}} \times \frac{2.0}{0.5} \times \frac{0.020}{0.100} = \frac{80}{\text{sec.}} = \text{units/ml. serum}$$

Stability of the Enzyme in the Serum Sample

Serum can be stored in a refrigerator (<7° C) for at least 48 hours without loss of GlDH activity.

Sources of Error

On completion of the "preliminary reaction" no interference by other enzymes occurring in serum has been observed.

Effect of Exercise or Corticosteroid Therapy

A significant rise in the GlDH activity of serum from healthy people occurs with severe muscular exercise, the origin of which is still unknown[28]. With corticosteroid therapy the GlDH activity in serum falls significantly[28] as do all the other "key pathway" enzymes*) so far investigated. The enzyme is inhibited by sulphonylurea derivatives[29].

Details for Measurements in Tissues

In liver the GlDH is located in the mitochondria[20, 26, 30-32]. On cell fractionation it sediments with cytochrome oxidase, but leaks out into the surrounding medium. It can be measured within the mitochondria if the membrane is made permeable to coenzymes and substrates[25, 33]. Therefore it follows that the extracting agent and the degree of mechanical disintegration of the mitochondria are of great importance for the quantitative measurement of the enzyme in tissues[25, 34]. After homogenizing human liver for 2 min. in 0.15 M NaCl in a Potter-Elvehjem glass homogenizer and immediately centrifuging, less than 10% of the GlDH activity is found in the supernatant. Even after standing for 3 hours only *ca.* 70% of the total GlDH activity is found in the whole homogenate after disintegration as described. In the course of 24–72 hours this percentage rises to *ca.* 80% in

*) "Key pathway" enzymes are found in all types of cell. They represent the basic elements for respiration, glycolysis and general amino acid metabolism. (cf. *Th. Bücher, E. Schmidt* and *F. W. Schmidt:* Serum Patterns of "Key Pathway" Enzymes. Lecture, 9th Middle East Medical Assembly, May 1959, American Univ., Beirut, Lebanon).

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the whole homogenate and to *ca.* 60% in the supernatant obtained immediately before measurement[25,33]. If doubly distilled water is used instead of NaCl solution then about 80% of the GlDH is detectable in the supernatant after standing for 2 hours[25,34].

Homogenization for 2 min. in an Ultra-Turrax (Janke and Kunkel & Co., see p. 51) results in 92–97% of the GlDH activity being found in the supernatant, and the total activity after standing for 24 hours[25]. A determination in duplicate on liver requires 1 mg. fresh weight. Other tissues require other conditions of disintegration.

For assay conditions for human liver (2 min. extraction in 0.15 M NaCl in an Ultra-Turrax), see Table 1. These data are not valid for isolated liver mitochondria and in the same way the optimum substrate concentrations obtained for human serum or liver must not be assumed for other tissues from other species.

Table 1. Conditions for the determination of GlDH from liver

Species	Buffer	pH	DPNH	OxoG	NH_4^+	pH	DPN	Glu	EDTA	Ref.
Cattle [a,b]	phosphate					7.6	0.1	33.0		21)
Chick [a,b]	tris	7.6	0.15	11.1	150.0	8.0	0.2	13.3		14)
Cattle [b]	phosphate					8.0	8.3	11.0		35)
Rat [a]	phosphate	7.7	0.28	10.0	50.0	7.7	1.4	13.0	+	26)
Cattle [b]	tris	8.0	0.10	50.0	100.0	8.0	3.3	50.0	+	23)
Human [a]	TEA	8.0	0.13	8.0	70.0	8.0	3.3	60.0	2.6	25)
			TPNH				TPN			
Human [a]	TEA	8.0	0.22	10.0	120.0	8.0	1.8	25.0	2.6	25)

All concentrations are given in μmoles/ml.
a) Crude or fractionated tissue extract
b) Purified or crystalline enzyme preparation

Abbreviations:
OxoG = α-oxoglutarate
Glu = glutamate
EDTA = ethylene-diamine-tetra-acetate
Tris = tris-hydroxymethyl-aminomethane buffer
TEA = triethanolamine buffer

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