Magnesium in Plasma

R. Czok

Principle

Magnesium ions activate isocitric dehydrogenase (ICDH). This enzyme catalyses the reaction:

$$\text{(1)} \qquad \text{Isocitrate} + \text{TPN}^+ \xrightleftharpoons[Mg^{2+}]{\text{ICDH}} \text{TPNH} + H^+ + \alpha\text{-oxoglutarate} + CO_2$$

and with constant amounts of enzyme the rate is dependent on the Mg^{2+} concentration.

Reagents

1. Triethanolamine hydrochloride, A. R.
2. DL-Isocitric acid-γ-lactone
   free from the *allo* form *)
3. Triphosphopyridine nucleotide, TPN
   sodium salt, TPN-NaH_2; commercial preparation, see p. 1029
4. $MgCl_2$ standard solution **)
5. Sodium hydroxide, A. R. 1 N
6. Trichloroacetic acid, A. R.
7. Sodium oxalate, A. R.
8. Isocitric dehydrogenase, ICDH
   from pig heart [1], lyophilized dry powder; commercial preparation, see p. 985

Purity of the enzyme preparation

The ICDH preparation need not be very pure. A sixteenth of the purity given by *Siebert* et al. [2] for the highly purified enzyme is sufficient.

Preparation of Solutions

Prepare all solutions with doubly distilled water.

I. Triethanolamine buffer (0.2 M; pH 7.45):
Dissolve 9.3 g. triethanolamine hydrochloride in about 200 ml. distilled water, adjust to pH 7.45 with 16.5 ml. 1 N NaOH and dilute to 250 ml. with distilled water.

II. DL-Isocitrate (0.08 M):
Dissolve 348 mg. isocitric acid-γ-lactone in *ca.* 15 ml. distilled water, bring to pH 9–10 (indicator paper) with about 0.2 ml. 1 N NaOH. Heat solution for 10 min. in boiling water bath, keeping pH above 7 by addition of small amounts of alkali. Adjust the cooled solution to pH 7.4 and dilute with distilled water to 25 ml.

III. Triphosphopyridine nucleotide (0.015 M β-TPN):
Dissolve 13 mg. TPN-NaH_2 in 1 ml. distilled water.

IV. Magnesium chloride standard solutions (0.04 to 140 mM):
Dissolve the contents of a Fixanal **) ampoule (10 g. magnesium as magnesium

*) *e.g.* from the California Corp. for Biochemical Research, Los Angeles, Cal. (USA).
**) *e.g.* Fixanal from Riedel-de Haën, Hannover (Germany)
1) *P. Baum* and *R. Czok*, Biochem. Z. *332*, 121 [1959].
2) *G. Siebert* et al., J. biol. Chemistry *226*, 965 [1957].

chloride) in distilled water and make up to 1030 ml. (0.4 M Mg^{2+}) and prepare from this stock solution the following dilutions:

a) 0.1 ml. stock solution to 1000 ml. with distilled water (0.04 mM)
b) 0.5 ml. stock solution to 1000 ml. with distilled water (0.2 mM)
c) 1.0 ml. stock solution to 1000 ml. with distilled water (0.4 mM)
d) 3.5 ml. stock solution to 10 ml. with distilled water (140 mM)

V. Isocitric dehydrogenase, ICDH (2 mg. protein/ml.):
Dissolve 10 mg. of the enzyme preparation in 1 ml. isocitrate solution (II), 1 ml. buffer (solution I) and 3 ml. distilled water.

VI. Trichloroacetic acid (*ca.* 6% w/v):
Dissolve about 6 g. trichloroacetic acid in distilled water and make up to 100 ml.

VII. Sodium oxalate (0.1 M):
Dissolve 1.34 g. sodium oxalate in distilled water and make up to 100 ml.

Stability of the solutions

The TPN and enzyme solutions are stable for several weeks when stored at −20° C. Only the amounts required for a series of measurements should be thawed. All other solutions are stable indefinitely at 2°–4° C.

Procedure

Preliminary treatment of plasma

Mix whole blood from fasting patients with 0.1 M sodium oxalate solution (VII) in the ratio of 9 : 1 and separate the plasma within 2 hours.

For the determination of the *ionized* Mg^{2+} use 0.01 ml. of the oxalated plasma. In order to determine the *total* magnesium the oxalated plasma must be deproteinized: dilute 0.1 ml. of oxalated plasma with 0.5 ml. water, add 0.5 ml. trichloroacetic acid (VI) and after 10 min. centrifuge off the precipitate. Wash the sediment with a little trichloroacetic acid solution (VI) and centrifuge again. Heat the combined supernatants in a drying oven at 120° C for 30 min., evaporate to dryness at 80° C. Trichloroacetic acid is destroyed by this treatment and bound magnesium is liberated. Dissolve the dry residue in 1 ml. water. The solution is approximately neutral; use 0.1 ml. for analysis.

Assay of activity of ICDH preparation

The enzyme has to be saturated with magnesium in order to obtain optimal activity. Therefore add 0.1 ml. standard solution (IVd) to the test mixture (= 7 mM Mg^{2+} final concentration). Immediately after addition of the enzyme measure the time taken for an increase in optical density of 0.100. It should take 15–20 seconds. If it is less, then use correspondingly less enzyme for the Mg^{2+} determination.

Standard curve

In three separate determinations add 0.1 ml. of the standard magnesium solutions (IVa, b or c) to the test mixture, corresponding to a final concentration of 2, 10, 20 μM respectively. Plot the values on semi-logarithmic millimeter paper: time in seconds (linear), magnesium concentration of the test mixture in μM (logarithmic). The measured values should lie on a straight line.

Spectrophotometric measurements

Wavelength: 340 mμ; light path: 1 cm.; temperature: 25°±0.5°C; final volume: 2.0 ml.

Pipette successively into the cuvettes:

0.06 ml. isocitrate solution (II)
0.50 ml. buffer (solution I)
0.03 ml. TPN solution (III)
0.10 ml. magnesium standard solution (IVa, b, c or d)
or 0.01 ml. oxalated plasma
or 0.10 ml. deproteinized oxalated plasma
water to 2.0 ml.

Incubate for 5 min. in a constant temperature cuvette chamber at 25°±0.5°C. Start the reaction by mixing in

0.04 ml. ICDH solution (V).

About 2 minutes after the enzyme addition the optical density change is constant per unit time. Measure the time taken for an increase of 0.100. It must lie between 35 and 120 seconds, otherwise repeat with more or less enzyme solution. (For measurement of reaction rates, refer to p. 8).

Calculations

Read from the standard curve the Mg^{2+} concentration in the test mixture corresponding to the measured times. Multiplying by 222 gives μM Mg^{2+} in plasma *).

The calculations are also possible without the use of a standard curve. The rate of reaction is $v = \Delta E/\Delta t$; as ΔE is always a constant value (0.100), the relative rates ($v = 1/\Delta t$ [1/sec.]) can be used for the calculation.

When v_x = rate of reaction with an unknown Mg^{2+} concentration
v_1 and v_2 = rates of reactions with two known Mg^{2+} concentrations
c_x, c_1 and c_2 = unknown and known Mg^{2+} concentrations respectively
then it follows:

$$\log c_x = \log c_1 + \frac{\log \frac{c_2}{c_1} \times (v_x - v_1)}{v_2 - v_1}$$

$$= \log c_1 + \frac{\log \frac{c_2}{c_1} \times \left[\left(\frac{1}{\text{sec}}\right)_x - \left(\frac{1}{\text{sec}}\right)_1\right]}{\left(\frac{1}{\text{sec}}\right)_2 - \left(\frac{1}{\text{sec}}\right)_1}$$

The correct functioning of the assay system can be examined with the aid of this equation: by use of standard magnesium solutions IVa (2 μM Mg^{2+}), IVb (10 μM) and IVc (20 μM), it follows that when solution IVb is treated as an unknown and the time taken for $\Delta E = 0.100$ is substituted for $(1/\text{sec.})_x$ in the formula, the result should be 10 μM Mg^{2+}.

The value for log c_x gives the magnesium concentration in the test mixture. The molarity of the magnesium in the plasma is obtained by multiplying by 222.

*) The factor 222 is obtained from the dilution of the plasma by addition of the oxalate and from the 1 : 200 dilution of the oxalated plasma in the test mixture.

If the Mg^{2+} concentrations of the two reference standards used in this method differ by an order of magnitude then $\log \frac{c_2}{c_1} = \log 10 = 1$, and the equation simplifies to

$$\log c_x = \log c_1 + \frac{v_x - v_1}{v_2 - v_1}$$

Notes

Only chemicals which have been found to be free of magnesium are suitable, because of the high sensitivity of the determination. Contamination of commercial TPN with magnesium results in the relatively high initial activity of ICDH. This must not exceed 1% of the activity with magnesium saturation (see "Assay of activity of ICDH preparation") or 10% of the activity with the standard solution IVc.

With this kinetic determination care must be taken to keep the temperature constant.

Since in spite of storing in the frozen state the activity of the enzyme solution changes slightly from day to day, standard values must be measured for each series of estimations.

Calcium ions interfere with the determination but are removed by the preliminary treatment with oxalate. Manganese ions produce a greater activation than magnesium ions[2]. However, their concentration in biological material is considerably lower.