Malic Dehydrogenase

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Malic dehydrogenase (MDH) was discovered by *Thunberg*[1] and *Batelli* and *Stern*[2] in 1910, and was first isolated in the pure state from pig heart by *Straub*[3]. The enzyme occurs in animal and plant tissue and in micro-organisms. As it is an enzyme of the citric cycle it is mainly found in mitochondria and sarcosomes[4]. *Bücher et al.*[5,6] have shown that MDH from mitochondria and from the cell sap differ with respect to pH optimum and substrate affinity. The distribution of the enzyme between the cytoplasm and the mitochondria is different with different organs; in heart muscle practically all the MDH is located in the cytoplasm. The absolute activity in the cytoplasm is greatest in liver, followed by heart, skeletal muscle and brain.

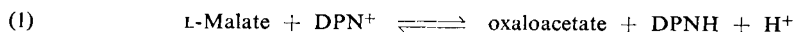
The concentration of malic dehydrogenase in the cell is several orders of magnitude higher than that in the extracellular space. The large concentration gradient leads to an increase of MDH in the serum when the cell membrane is damaged. *Hess et al.*[7,8], *Wacker et al.*[9] and *Siegel et al.*[10] were first to measure the MDH activity in serum in different diseases.

The MDH activity can be measured by the *Thunberg* technique (see p. 31), manometrically (see p. 29) or best of all spectrophotometrically.

The MDH activity of serum is due to different enzyme proteins with the same action and substrate specificity, but of different origin and with different activity. As is the case with lactic dehydrogenase (see p. 736), the existence of several isoenzymes[11,12] has been demonstrated by *Vesell et al.*[13], *Hess et al.*[14], *Schmidt et al.*[15], *Tsao*[16] and others. Generally the cytoplasmic enzyme passes into the serum, but with considerable cell damage the mitochondrial enzyme may also occur.

Principle

Malic dehydrogenase (MDH) catalyses the reaction:



$$(1) \qquad \text{L-Malate} + \text{DPN}^+ \rightleftharpoons \text{oxaloacetate} + \text{DPNH} + \text{H}^+$$

At neutral pH the equilibrium is far to the left[3]. According to[17] $K' = [\text{oxaloacetate}] \times [\text{DPNH}] / [\text{L-malate}] \times [\text{DPN}^+]$ is 2.33×10^{-5} at 22°C and pH 7.4. Therefore the measurements of activity are made with oxaloacetate as substrate and DPNH as coenzyme[18]. The decrease in optical density at 340 or 366 mμ is measured.

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1) *T. Thunberg*, Scand. Arch. Physiol. *24*, 23 [1910].
2) *F. Batelli* and *L. Stern*, Biochem. Z. *31*, 478 [1911].
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17) *J. R. Stern*, *S. Ochoa* and *F. Lynen*, J. biol. Chemistry *198*, 313 [1952].
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Oxaloacetate is unstable in aqueous solution, being partly decarboxylated to pyruvate. Therefore with old oxaloacetate solutions the measured activity is partly due to the lactic dehydrogenase activity of serum[14]. This difficulty can be overcome if just before the MDH determination, the optimum amount of oxaloacetate is produced in the cuvette from α-oxoglutarate and L-aspartate by means of glutamate-oxaloacetate transaminase[19]; for the equation for this reaction, see p. 837.

The amount of oxaloacetate converted per unit time as determined by the decrease in the optical density of DPNH is a measure of the MDH activity.

Optimum Conditions for Measurements

The most important characteristics of the MDH proteins for measuring their activities are their different substrate and pH optima. These have been specially studied by *Schmidt*[15]. The DPNH dependence of the reaction is low; at pH 7.5 the following oxaloacetate concentrations are optimum for measurements in serum: 1.7×10^{-3} M in acute hepatitis; 0.8×10^{-3} M in myocardial infarction; 1.4×10^{-3} M in haemolytic anaemia; 3.7×10^{-3} M in bronchial carcinoma. If the MDH activity is measured in serum several days after the excretion of the enzyme from a certain organ, the optimum oxaloacetate concentration is found to be different, *i.e.* with the original optimum concentration the measured values are too low[15]. Research and discussion on this point are still in progress.

In the following method, 1×10^{-3} M oxaloacetate, 2×10^{-4} M DPNH and pH 7.4 are used as these concentrations approximate to the most satisfactory conditions for measurements in serum after myocardial infarction and in hepatitis.

Reagents *)

1. Potassium dihydrogen phosphate, KH_2PO_4,
2. Dipotassium hydrogen phosphate, K_2HPO_4,
3. L-Aspartic acid or sodium-L-aspartate
4. α-Oxoglutaric acid
   free acid (commercial preparation, see p. 1024) or sodium salt.
5. Sodium hydroxide, A. R., 0.1 N
6. Sodium hydrogen carbonate, A. R., 1% (w/v)
7. Reduced diphosphopyridine nucleotide, DPNH
   disodium salt, DPNH-Na_2; commercial preparation, see p. 1011.
8. Glutamate-oxaloacetate transaminase, GOT
   from pig heart, suspension in 3 M ammonium sulphate solution (pH 6.0) containing 2.5×10^{-3} α-oxoglutarate and 2.5×10^{-2} M maleate to stabilize the preparation[20,21]. Commercial preparation, see p. 976.

Purity of the enzyme preparation

The GOT preparation should contain about 150 units **)/mg. It must be free from malic dehydrogenase. Contamination with glutamic dehydrogenase, lactic dehydrogenase and oxaloacetic decarboxylase should be $< 0.05\%$ (relative to the GOT activity).

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*) Complete reagent kits are available commercially (see p. 1037).
**) A unit is the amount of enzyme which converts 1 μmole of substrate per min.

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[20] *W. T. Jenkins* and *J. W. Sizer*, J. Amer. chem. Soc. *79*, 2655 [1957].
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Preparation of Solutions (for *ca.* 25 determinations)

I. Phosphate-aspartate solution (0.1 M phosphate buffer, pH 7.4; 4.2×10^{-2} M aspartate): Dissolve 0.2 g. KH_2PO_4, 1.5 g. K_2HPO_4, 0.66 g. Na-L-aspartate or 0.56 g. L-aspartic acid in 50 ml. doubly distilled water, adjust to pH 7.4 with 0.1 N NaOH and dilute to 100 ml. with doubly distilled water.

II. Sodium α-oxoglutarate (6×10^{-2} M):
Dissolve 17 mg. Na-α-oxoglutarate in 1.5 ml. doubly distilled water or 13 mg. α-oxoglutaric acid in *ca.* 1 ml. doubly distilled water, neutralize with *ca.* 0.2 ml. 0.1 N NaOH and dilute to 1.5 ml. with doubly distilled water.

III. Reduced diphosphopyridine nucleotide (1.2×10^{-2} M β-DPNH):
Dissolve 15 mg. DPNH-Na_2 in 1.5 ml. 1% $NaHCO_3$ solution.

IV. Glutamate-oxaloacetate transaminase, GOT (1 mg. protein/ml.):
If necessary, dilute the stock solution with 3 M ammonium sulphate solution.

Stability of the solutions

The phosphate-aspartate solution is stable as long as no bacterial contamination occurs. Prepare the α-oxoglutarate and DPNH solutions freshly each week. The GOT suspension keeps for several months. Store all solutions at 0–4°C.

Procedure

Use only fresh serum free from haemolysis.

Spectrophotometric measurements

Wavelength: 340 or 366 mμ: light path: 1 cm.; final volume: 3.00 ml.; temperature 25°C (constant temperature cuvette holder). A control cuvette is not necessary. Measure against air or a cuvette containing water.

Pipette successively into the cuvette*):

2.75 ml. phosphate-aspartate solution (I)
0.05 ml. α-oxoglutarate solution (II)
0.05 ml. DPNH solution (III)
0.05 ml. GOT suspension (IV).

Mix with a small glass or plastic rod flattened at one end. Wait 5 min. until the amount of aspartate equivalent to the α-oxoglutarate added is completely converted to oxaloacetate. Mix in

0.10 ml. sample (serum or other biological fluid)

and start stopwatch. Read the optical density every minute for 5 to 10 min.

The values for ΔE/min. at 366 mμ should not be greater than 0.030. Otherwise dilute the serum five to ten-fold with solution I or measure at shorter time intervals.

Calculations

According to the American literature the usual definition (*e.g.*[9]) of a unit is the amount of enzyme in 1 ml. serum, which changes the optical density of DPNH at 340 mμ by 0.001 in 1 min. at 25°C, with an assay volume of 3 ml.

It follows that taking 0.1 ml. serum for assay

$$(\Delta E_{340}/\text{min.}) \times 1000 \times 10 = (\Delta E_{340}/\text{min.}) \times 10000 = \text{MDH units/ml. serum.}$$

*) Bring the solutions to room temperature before the start of the assay.

For measurements at 366 mμ it is necessary to multiply by 1.89 because of the ratio of the extinction coefficients for DPNH at 340 and 366 mμ:

$$(\Delta E_{340}/\text{min.}) \times 10000 = (E_{366}/\text{min.}) \times 18900 = \text{MDH units/ml. serum.}$$

For conversion to other units, see p. 33; Normal values, see p. 706.

Example

0.1 ml. serum was analysed. The following optical densities were measured at 366 mμ:

0 min.	0.445	
		ΔE = 0.006
1 min.	0.439	
		ΔE = 0.005
2 min.	0.434	
		ΔE = 0.004
3 min.	0.430	
		ΔE = 0.005
4 min.	0.425	
		ΔE = 0.005
5 min.	0.420	
		ΔE = 0.004
6 min.	0.416	
	Mean:	ΔE_{366}/min. = 0.005

$$0.005 \times 18900 = 94 \text{ MDH units/ml. serum.}$$

Stability of the Enzyme in the Serum Sample

According to [22] the enzyme in serum loses *ca.* 17% of its activity in 24 hours at room temperature, *ca.* 11% at 4°C and *ca.* 2% in the frozen state. This does not take into account the change in the optimum substrate concentration due to aging of the enzyme[15].

Sources of Error

Interference due to the decarboxylation of the oxaloacetate is reduced to a minimum under the experimental conditions described here. Since the added α-oxoglutarate is completely converted before the start of the MDH reaction, the presence of glutamic dehydrogenase in the sample does not interfere.

Details for Measurements in Tissues

The total malic dehydrogenase content of the cell is only quantitatively extracted after complete homogenization of the tissue[5,6,23], because a portion of the activity (another malic dehydrogenase, see p. 657) is located in the mitochondria. By variation of the homogenization technique it is possible to distinguish between the mitochondrial and the cytoplasmic MDH. Since the optimum pH and substrate concentration vary considerably for malic dehydrogenases from different tissues, these should be determined in preliminary experiments.

Potter[23] has published values for the MDH activity of several organs (aqueous homogenates) from laboratory animals.

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