Lactic dehydrogenase

CAS #: 9001-60-9

Synonym: L-Lactate : NAD+ oxidoreductase lactate dehydrogenase

E.C. #: 1.1.1.27

Source: Bovine heart (151531), Porcine heart

Description:
Lactic dehydrogenase (LDH) catalyzes the following reaction:

Pyruvate + NADH ⇐⇒ Lactate + NAD

Mammalian lactate dehydrogenase (LDH) exists as five tetrameric isozymes composed of combinations of two different subunits. The isozymes differ in catalytic, physical and immunological properties. Cahn, et al. refer to the polypeptide subunits as "H" and "M", which combine to form two pure types of isozymes, H4 and M4, and three hybrids, H3M, H2M2, and HM3. Type H4 is the most negatively charged at pH 7 and in zone electrophoresis appears nearest the anode. Subunit "H" predominates in heart muscle LDH which is geared for aerobic oxidation of pyruvate. The "M" subunit predominates in skeletal muscle and liver and is concerned more with anaerobic metabolism and pyruvate reduction.

Silverstein and Boyer compared kinetics of beef heart and rabbit muscle LDH.

LDH is of interest clinically in that the serum level of certain isozymes reflects pathological condition in particular tissues. Studies have been done on structure, binding sites and kinetics.

Characteristics of LDH from Beef Heart:

Molecular Weight: 35,000/subunit. 136,700 ± 2,100/tetramer.

Composition: Valee and Williams have reported on its subunit dissociation at low pH.

Extinction coefficient: E 1%280 = 14.9.

Specificity: The enzyme is specific for L (+) lactate. Meister reports it reduces several a-keto and a,g-diketo acids but at about one-tenth the rate of reduction of pyruvate.

Inhibitors: LDH is quite stable. It is inactivated by iodide. Inhibition by p-mercuribenzoate is slow.

Activators: A number of organic compounds which stabilize the enzyme, such as dimethyl sulfoxide, ethanol, and methanol, are reported. Diethylstilbestrol and several of its derivatives also stabilize the enzyme.

Characteristics of LDH from Rabbit Muscle:

Molecular Weight: 140,000.

Composition: Lovell and Winzor report that the tetramer dissociates completely into two dimers (molecular weight 70,00) in acetate-chloride buffer pH 5 (conditions without effect on beef heart LDH). Phosphate and pyridine nucleotides stabilize the quaternary structure of the tetramer. Phosphate has an activation effect.

Activity: Reaction kinetics have been reported.
Method: The reaction velocity is determined by a decrease in absorbance at 340 nm resulting from the oxidation of NADH. One unit causes the oxidation of one micromole of NADH per minute at 25°C and pH 7.4 under the specified conditions.

Reagents

0.2 M Tris-HCl, pH 7.3

6.6 mM NADH in above 0.2 M Tris-HCl buffer, pH 7.4

30 mM Sodium pyruvate in above 0.2 Tris-HCl buffer, pH 7.4

Enzyme

Dissolve at 1 mg/ml in 0.2M Tris-HCl buffer. Dilute enzyme prior to use to obtain a rate of 0.02-0.04 DA/min. in Tris buffer and keep cold.

Determination of Protein Concentration:

Beef Heart LDH: mg/ml = A_{280} \times 0.67

Rabbit Muscle Dehydrogenase: mg/ml = A_{280} \times 1.13

Procedure

Set spectrophotometer at 340 nm and 25°C.

Pipette into cuvette as follows:

Tris-HCl, 0.2 M pH 7.4 2.8 ml

6.6 mM NADH 0.1 ml

30 mM Sodium pyruvate 0.1 ml

Incubate in the spectrophotometer 4-5 minutes to achieve temperature equilibration and establish a blank rate, if any.

Add 0.1 ml of appropriately diluted enzyme and record DA_{340}/min. from initial linear portion.

Calculation

Units/mg = DA_{340}/min.

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6.22 \times mg enzyme/ml reaction mixture

Solubility: Soluble in distilled water or dilute buffer

Availability:

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<th>Description</th>
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Lactic Dehydrogenase from human heart. Activity approximately 50-100 units/mg protein. Highly purified crystalline suspension in saturated ammonium sulfate.

Lactic Dehydrogenase from chicken heart. Activity approximately 300 units/mg solid. Lyophilized powder.

References:

– Jaenicke, R., "Reassociation and activation of lactic dehydrogenase from the unfolded subunits." Biochem., v.11, 1609 (1972).

