

Introduction

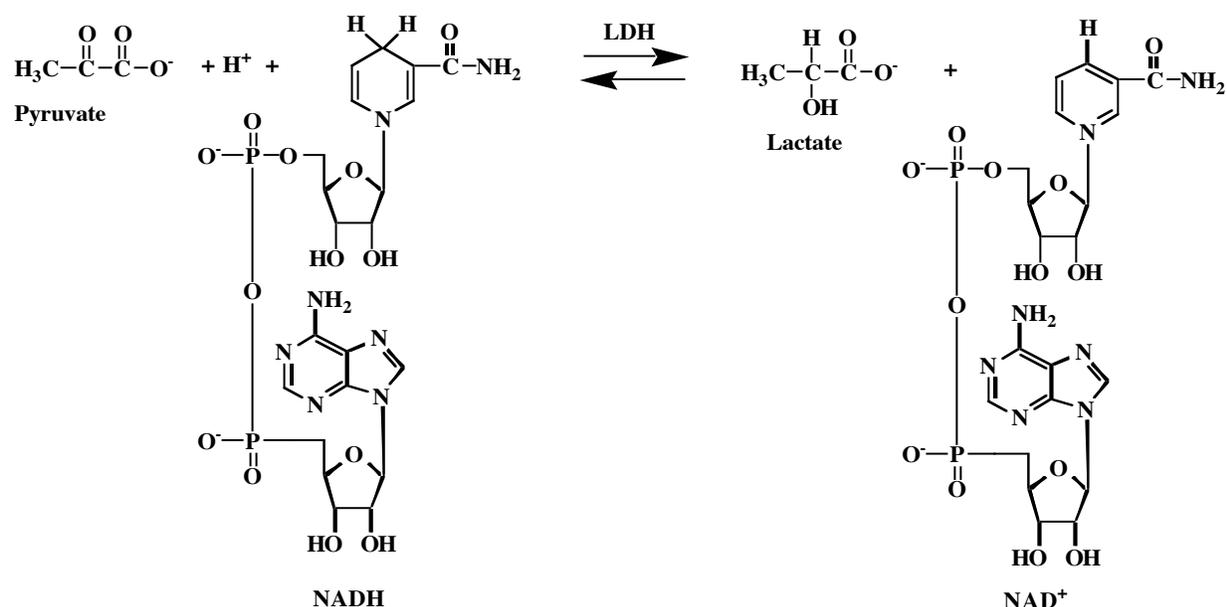
Lactate Dehydrogenase Enzymes: A Model System
for the Biochemistry Laboratory

A. Purpose

The biochemistry laboratory course is designed to familiarize students with some fundamental biochemical laboratory techniques and methodologies. Since the laboratory is intended to provide a hands-on experience of the experimental approaches discussed in the biochemistry lecture course, it will focus on the techniques for the characterization of proteins.

B. Dehydrogenase Enzymes

The dehydrogenases are a large family of enzymes which perform oxidation-reduction reactions in which hydride ions are transferred from one substrate to another. Lactate dehydrogenase (Lactate:NAD⁺ oxidoreductase; E.C. 1.1.1.27; "LDH") is an enzyme that catalyzes the reduction of the three carbon keto-acid pyruvate to the alcohol product lactate. In the LDH reaction, the hydride is transferred from a molecule of the reduced cofactor nicotinamide adenine dinucleotide (NADH) to pyruvate. As a result, NADH is oxidized to NAD⁺, and pyruvate is reduced to lactate:



This is normally the reaction catalyzed in skeletal muscle during anaerobic glycolysis. However, liver LDH catalyzes the reverse reaction in order to produce pyruvate for gluconeogenesis. The catalytic properties of LDH in various tissues may differ, since the structure of LDH can be slightly different from tissue to tissue. These different LDH molecules, encoded by different genes, are called **isoenzymes**. LDH is a tetramer of 35-36 kilodalton (kDa) protein subunits, and each subunit can be of the A type, the major subunit expressed in skeletal muscle (also referred to as or the M subunit, for skeletal muscle); the B type, the major subunit expressed in heart (also referred to as the H subunit, for heart); or the C type, the subunit expressed solely in the testis and spermatozoa. Functional isoenzymes are created by association of different subunits to form homotetramers or heterotetramers. (see Berg et al, *Biochemistry 6E*, pp. 67, 283, 468-469 for more details on the LDH enzyme)

The existence of tissue-specific LDH isozymes renders these enzymes an interesting subject for in-depth study. Each lab team will concentrate on the LDH isozymes from a single rodent tissue. In this manner the class as a whole can compare and contrast properties of the isozymes. LDH enzymes have other properties which make them amenable to study, including: high levels of the enzyme expressed in rodent brain, heart, kidney, liver, skeletal muscle; cytosolic subcellular localization; a simple spectrophotometric assay for measuring activity; and well-established purification procedures and kinetic parameters.

C. Your mission

Extensive biochemical characterization of LDH will involve a variety of experiments that everyone will perform in lab teams, including: tissue homogenization and differential centrifugation; determination of specific LDH activity; purification via affinity chromatography; and gel electrophoresis. Then, each group will be assigned a mini-project for further characterization of the purified LDH protein. The mini-projects may include: activity staining for isozyme detection, kinetic studies for isozyme characterization and pathology of LDH inhibition, Western blot detection of enzyme, a comparative study with enzyme from a different species, or a computational/bioinformatics analysis of LDH sequence, structure, and drug targeting. Each pair will also give an oral presentation on their mini-project. Finally, two written lab reports will be submitted by each pair. The first will be a rough draft describing the isolation and purification (Sessions 3-6). The second will be a revised report with the results of your mini-project included.

For each lab session you **must** bring the following: safety glasses, your laboratory notebook, a calculator, a lab marker and pen, a well-organized strategy for performing the day's task outlined in your lab notebook, and your lab partner. Please note that attendance is mandatory at all laboratory sessions on the scheduled lab day. Lab makeups are not feasible. The practical and theoretical aspects of each experiment will be presented during the discussion session; you will not be allowed into the lab without prior attendance at the discussion session. The nature of biochemical experimentation is such that it doesn't always fit well into short, defined time slots. I recommend that if possible you refrain from scheduling a 4:00 activity on the day of your lab, since a few of the experiments may extend into the late afternoon. I understand, however, that Biochemistry Lab is not your sole focus of time and effort this semester, so in order to compensate you for extra lab time on certain days, some lab days will be short!

D. Grading

Your performance in the laboratory course will be evaluated as follows:

- Organization and lab technique and preparedness **15%**
- Lab data sets I and II **20%**
- Lab Report I (1st draft, sessions 3-6) **15%**
- Mini-project oral presentation **15%**
- Lab Report II (revision of Rept 1 + mini-project) **35%**

BBMB 335 LAB SYLLABUS SECTION A (Wed) & B (Thurs)

DATE	TOPIC	DUE AT 1:00 pm
21-22 Jan	Disc: Lab intro; Literature/Database searching	
	Lab: Tour; Safety; Pipettors	
28-29 Jan	Disc: Biochemical reagents	
	Lab: Session 1 Reagent prep	
4-5 Feb	Disc: Enzyme assay; Tissue fractionation	
	Lab: Session 2 LDH assay with Sigma LDH	
11-12 Feb	Disc: NONE	
	Lab: Session 3 Tissue frac & LDH assay of cyto	
18-19 Feb	Disc: Protein assay; Enzyme purification	
	Lab: Session 4 Protein assay of cytosol	
25-26 Feb	Disc: NONE	Data set I : Sessions 3/4
	Lab: Session 5 Purification of LDH by Aff chrom	
4-5 Mar	Disc: Gel sample prep & SDS-PAGE	
	Lab: Session 5 cont Protein assay/prep gel samples	
11-12 Mar	Disc: Lab reports; Mini Projects	Data set II: Session 5
	Lab: Session 6 SDS-PAGE	
16-27 Mar	SPRING BREAK	
1-2 Apr	Lab: Data analysis for Lab report 1	
6 Apr M		Lab report I (draft)
8-9 Apr	Lab: Mini projects I	
15-16 Apr	Lab: Mini projects II	
22-23 Apr	Lab: Mini project III; Clean up	
29-30 Apr	Disc: Presentations of Mini-projects	
4 May M		Lab report II(final)
	* Disc: meet in S-142; Lab: meet in S-317	

Biochemistry Lab Safety Regulations

General Regulations The following rules will be strictly enforced; failure to comply with these rules may result in lowering of lab grades or dismissal from lab.

- 1) Eye protection must be worn at **all times** in the laboratory. Regular glasses may be used in lieu of safety glasses.
- 2) No one will work alone in the lab. There must be an instructor or assistant present for supervision. *Exceptions:* specific shut-down operations.
- 3) No unauthorized experiments shall be performed at any time.
- 4) No smoking, eating or drinking will be allowed in the laboratory.
- 5) Shoes must be worn at all times. Sandals or open-toed shoes are not sufficient.
- 6) Long hair should be secured behind your head.
- 7) Legs must be covered.

Safety Precautions

- 1) Know the location and use of the first aid kit, fire extinguishers, and eye wash.
- 2) Report all accidents immediately to the instructor.
- 3) Pay close attention to any potential hazards noted in protocols or labels, especially in the use of **acrylamide, mercaptoethanol, and azide**.
- 4) Check labels carefully to insure that you are using what you intended to use.
- 5) Never pipet by mouth
- 6) Never pour water into concentrated acid. Always add the acid slowly with stirring into a larger volume of aqueous solution.
- 7) Avoid prolonged exposure to skin or breathing of organic compounds. Common organic chemicals such as methanol, phenol, mercaptoethanol, and others. are toxic. Use the hood when possible. Also avoid inhalation of fine particle size materials such as acrylamide and SDS.
- 8) Clean up your mess before leaving the laboratory.
- 9) Wash your hands well before leaving the laboratory.

I have read the above regulations and safety precautions and have discussed any questions or concerns with my instructor:

Signed _____ Date _____