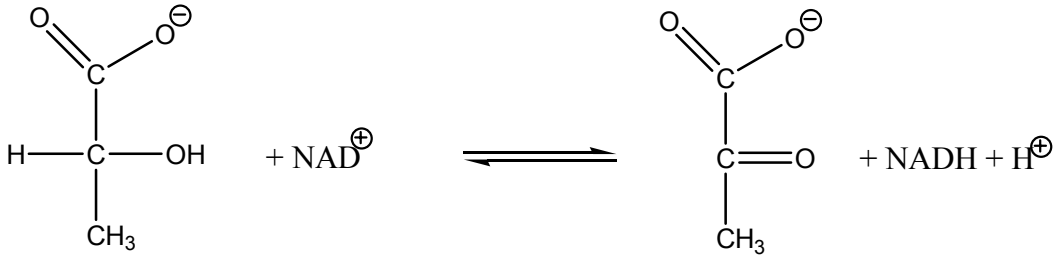
Enzyme Kinetics and Inhibition – Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is a key enzyme in energy metabolism in many cells. This enzyme catalyzed the reversible oxidation of pyruvate to lactate (Figure 1.) In muscles (when O_2 levels are low) pyruvate from glycolysis is converted by LDH to lactate. The lactate is secreted into the blood and transported to the liver where LDH converts the lactate back to pyruvate where it is mainly converted back to glucose via gluconeogenesis. This cycle allows the muscle to keep producing energy (via glycolysis) under high load; i.e. when O_2, and therefore respiration, is limiting.



Lactate pyruvate

Figure 1.

LDH can be assayed by taking advantage of the difference in molar absorbtivity between NAD^+ and NADH. NAD^+ has a lower molar absorbtivity than does NADH.. Thus if we assay LDH using pyruvate and NADH as the substrates we would expect to see a decrease in absorbance at 340 nm (λ_{max} for NADH).

In this experiment you will determine the K_m for either pyruvate or NADH (assigned by the instructor) and V_{max}. You will also determine k_{cat} and K_i for at least one inhibitor (assigned by the instructor).

You will be provided with reagents and a general assay procedure (see below). You are responsible for the detailed design of the experiment as well as collection and analysis of the data.

Experimental Procedures

Solutions Provided

Buffer (100 mM Tris HCl, pH 7.5)

50 mM sodium pyruvate in buffer

100 mM α-ketoglutarate in buffer

15 mM oxamate in buffer

50 mM $ZnSO_4$ in buffer

50 mM $CuSO_4$ in buffer

Solutions to be prepared fresh for each experiment

(Conserve expensive reagents! Prepare only enough of each of these solutions for the day's experiments plus about 10% extra.)

1 mM β-NADH in buffer

0.5 U LDH / mL in buffer (need 0.1 mL / assay)

General Assay Procedure

1) Place 2.0 mL of working solution (see below) in cuvette
2) Add appropriate volumes of substrate, inhibitor and buffer; keeping total volume of all additions equal to 0.75 mL.
3) Add 0.1 mL of LDH (to start reaction)
4) Quickly mix contents of cuvette by gentle inversion
5) Determine A_{340} at 30 sec. intervals for about 5 min. (You may need to adjust the interval and total time as conditions warrant. Ask for advice!)

Important Details

for experiments in which you will vary [pyruvate] –

Working solution is 180 μM β-NADH in buffer
Vary [pyruvate] from 0 to 4 mM

for experiments in which you will vary [NADH] –

Working solution is 2.5 mM pyruvate in buffer
Vary [β-NADH] from 0 to 200 μM

for determining experiments in which you will vary [LDH] –

Working solution is 180 μM β-NADH and 2.5 mM pyruvate in buffer
Vary [LDH] from 0 to 0.025 U/mL

for experiments in which you will determine K_i use final concentrations of the inhibitors as follows –

α-ketoglutarate: 0 – 10 mM

Oxamate: 0 – 1.5 mM

$ZnSO_4$: 0 – 1.0 mM

$CuSO_4$: 0 – 5.0 mM