Stellenbosch University – Biochemistry Honours 2026 – Biochemistry 714

Mini-course 2 (Systems Biology)
Instructions for Practical, Data Analysis and Assessment

This assessment has been subject to internal moderation.
Examiners: Prof JM Rohwer, Dr DD van Niekerk

In the last part of the mini course, experimental data for the kinetic characterisation of lactate dehydrogenase will be measured in the practical component, and then analysed. The enzyme will be analysed in the forward direction, with pyruvate (Pyr) and NADH as substrates, and in the reverse direction with lactate (Lac) and NAD^+ as substrates. When measured in the reverse direction hydrazine is added to the assay; this binds to pyruvate that is formed, and thereby prevents product inhibition by Pyr, which would interfere with the assay. The class will be split into eight groups, and each group will analyse initial rate kinetics for LDH in terms of a substrate, for the forward, or for the reverse reaction: pyruvate (Pyr), NADH, lactate (Lac) and NAD^+. Each group will receive a specific kinetic mechanism for LDH for which they will construct a rate equation. They will fit their rate equation to the data and write a report in which the theory of the lectures, data analysis and computational aspects are combined.

Schedule

Wed 4 Mar: Introduction

Thu 5 Mar – Tue 10 Mar: Experiments

Wed 11 Mar – Fri 13 Mar: Data analysis

Deadline for handing in: 16 March (see below for details).

Tasks

Experiments

NADH absorbance changes will be recorded spectrophotometrically over an approximately 3-minute period. The experimental protocol will be discussed in more detail on the first day of the practical. Also refer to the additional protocol document on the course website.

Analysis of raw data

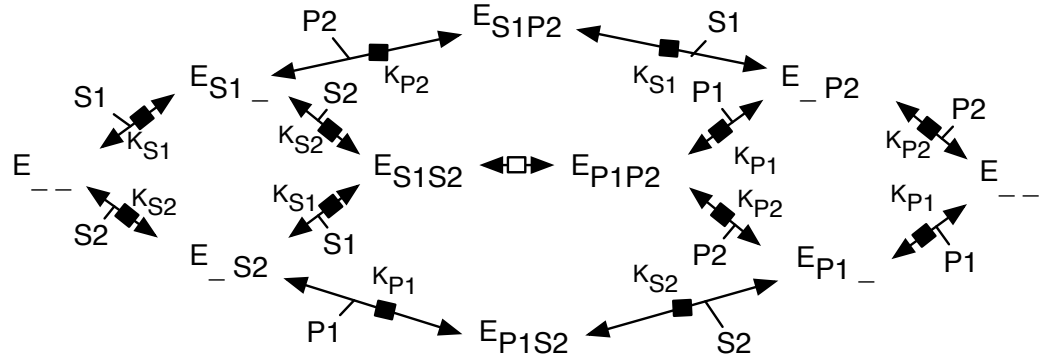
Absorbance changes over the incubation period will be used to calculate enzyme activity for each of the incubations. After deciding on a time period over which the kinetics were linear (as judged from a scatter plot), each group will analyse their data, i.e. determine a slope over the selected time period, for each of the experiments they performed. From these slopes, the activity of the LDH for the different substrate concentrations that were tested in your group will be calculated. The path length for the spectrophotometer, and the extinction coefficient for NADH will be provided. Each experiment will be performed in triplicate. Note that the triplicates should be treated independently to determine three initial rates per substrate concentration.

Derivation of rate equation

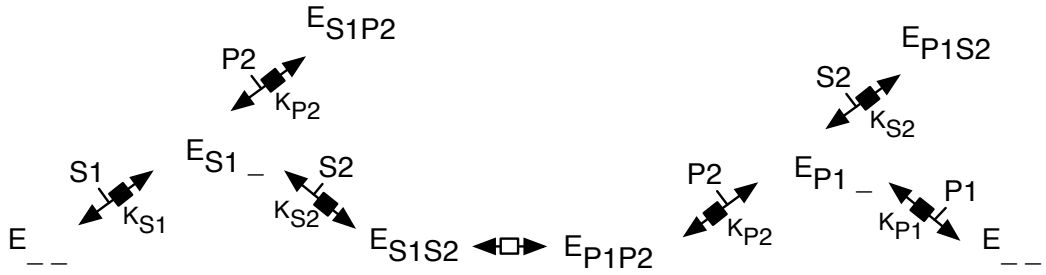
Each group will derive a unique rate equation for a specific reaction scheme. We distinguish two basic mechanisms, a random order and an ordered mechanism (with two variants), as shown in Fig. 1A–C

respectively. This enzyme displays substrate inhibition at high concentrations of Pyr. To take the substrate inhibition into account we will test three different possible mechanisms: 1) Pyr binds to all enzyme complexes, 2) Pyr binds to all enzyme complexes that already have Pyr bound, 3) Pyr binds to all enzyme complexes that have both catalytic sites occupied. For these substrate inhibition kinetics we assume rapid (equilibrium) binding of Pyr to an allosteric site with a dissociation constant $K_{i_{s1}}$. In addition, lactate inhibition is present at high concentrations. For this inhibition effect we will also assume rapid (equilibrium) binding of Lac to an allosteric site on all enzyme complexes with a dissociation constant $K_{i_{p1}}$.

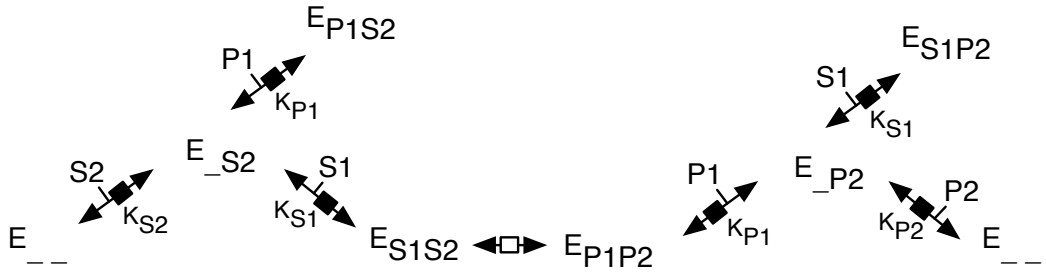
Each group will get one unique enzyme kinetic mechanism from the following: ordered with the cofactor binding first, ordered with the carbon substrate/product binding first, or random order, with three mechanisms of pyruvate inhibition each, and lactate inhibition in all cases.



A



B



C

Figure 1: LDH kinetic mechanism. Binding of substrates and products to the enzyme are indicated for a random order mechanism **(A)**, and two ordered mechanisms **(B)** and **(C)**. Let S1 = Pyr, S2 = NADH, P1 = Lac and P2 = NAD^+.

Fitting of simplified rate equation

Each group will first analyse their own data set to familiarise themselves. For this they will fit the data with a simplified rate equation. This rate equation can be derived from the complete rate equation by setting the products to zero and substituting a value of 10 for the s/K_s value of the non-varied substrate. From this fitting procedure, V_m and a K_s value for the specific (varied) substrate will be estimated.

Fitting of full rate equation

The complete data set of the Honours group will be combined and fitted to the full rate equation to obtain values for all parameters in the rate equation.

Model validation

Each group will validate their model using additional product inhibition data (which they have generated in the lab), corresponding to their specific substrate-product pair. In addition, the model prediction for a progress curve of the reverse reaction of LDH will be compared to experimental data collected in the absence of hydrazine (so that the Pyr is not removed from the reaction mixture). This will be performed by setting up an ordinary differential equation (ODE) model, integrating it, and comparing the result to the experimental progress curve.

Additional tasks

- Calculate the equilibrium constant of the reaction using the Haldane relation and the parameter values that you have obtained, and compare your result to literature values.
- Calculate the equilibrium constant from experimental equilibrium concentrations.
- Determine the specific activity of the enzyme.

Reports

Each group must submit one *combined report*. The report should be in scientific manuscript form and be close to five pages (not including graphs). Your Results section must contain the following topics, and be written with a logical story-line (i.e. not just listing of results):

1. Experimental results (description and plots) [5]
2. Derivation of detailed kinetic rate equation [10]
3. Reduced rate equation for group-specific experiment [5]
4. Results of fitting of reduced equation on your data; show table of fitted values; include plot with data and model [10]
5. Fitting of full equation on complete dataset; show table of fitted values; include 4 plots with data and model for each of the varied substrates/products [15]
6. Validation of model with product inhibition [5]
7. Use of ODE model to obtain timecourse of NADH (reverse reaction without hydrazine) and comparison to data [10]
8. Use of Haldane relation to calculate K_{eq} and comparison to literature (cite) [5]

9. Calculation of K_{eq} from experimental equilibrium concentrations [2]
10. Calculation of specific activity of the enzyme [3]

You will also be marked on the completeness of your Introduction, Methods, Discussion and Conclusion sections as well as your writing style.

Handing in

- Submit your group's report in **PDF format** via the **STEMLearn** submission system. On the first page, be sure to include **your group number** (e.g. 2B) and the **names and student numbers** of your group members.
- Only **one** report to be submitted per group! Any one of the group members can submit the report on STEMLearn.
- Reports will be checked for plagiarism via Turnitin.
- Deadline: **Mon 16 March 2026 at 23h59**

Mark allocation

Total Content (see above):	70
Introduction:	5
Methods:	5
Discussion and Conclusion:	20
Style and Presentation:	10
TOTAL:	**110**