Stellenbosch University – Biochemistry Honours 2017

Mini-course 4: Practical Programme and Assessment

In this practical, lactate dehydrogenase will be kinetically characterised. The class will be split into six groups and each group will analyse LDH in terms of a substrate or a product (one or two groups per metabolite). The enzyme will be characterised with initial rate kinetics. Each group will receive a specific kinetic mechanism for the 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

Tuesday 18 April – Friday 21 April: Experiments
Monday 24 April – Wednesday 26 April: Data analysis and report

Tasks

Experiments

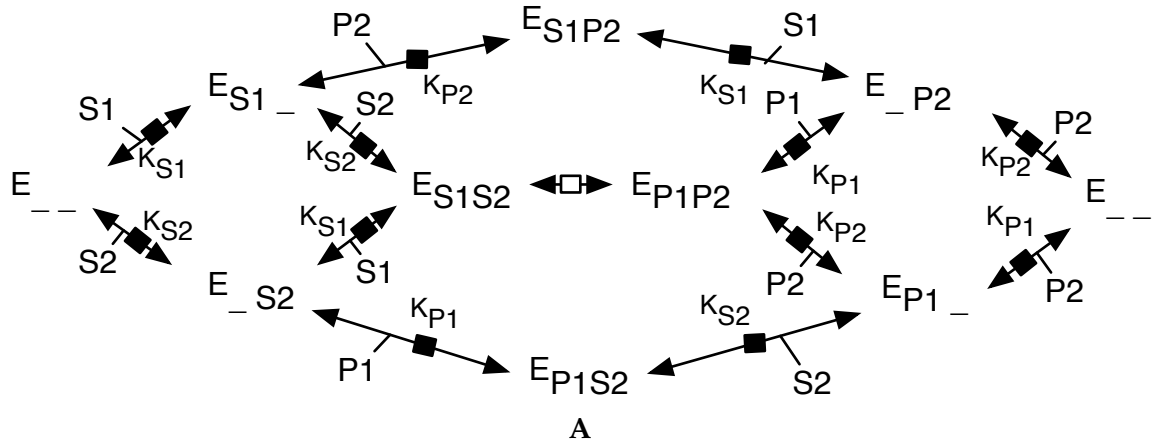
NADH absorbance changes will be followed spectrophotometrically over a three-minute period. The experimental protocol will be discussed in more detail on the first day of the practical.

Analysis of raw data

Absorbance changes over the three-minute 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, each group will analyse their data, i.e. determine a slope over the agreed time period, for each of the experiments they performed. This will result in a set of dA/dt values. Group these data in a table with five columns: [Pyr], [Lac], [NADH], [NAD], dA/dt. For example, if you have varied the pyruvate concentration for the forward reaction, you will have a first column with the respective pyruvate concentrations that you have used, and an NADH concentration (third column) that is the same for all incubations. The Lac and NAD columns will have zeros except for the incubations where you added Lac to measure product inhibition. These tables will later be combined for all groups.

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, as shown in Fig. 1A and B 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 dissociation constant of K_i. Each group will get one unique enzyme kinetic mechanism from the following: ordered with NADH binding first, ordered with Pyr binding first, or random order, with three mechanisms of pyruvate inhibition each.



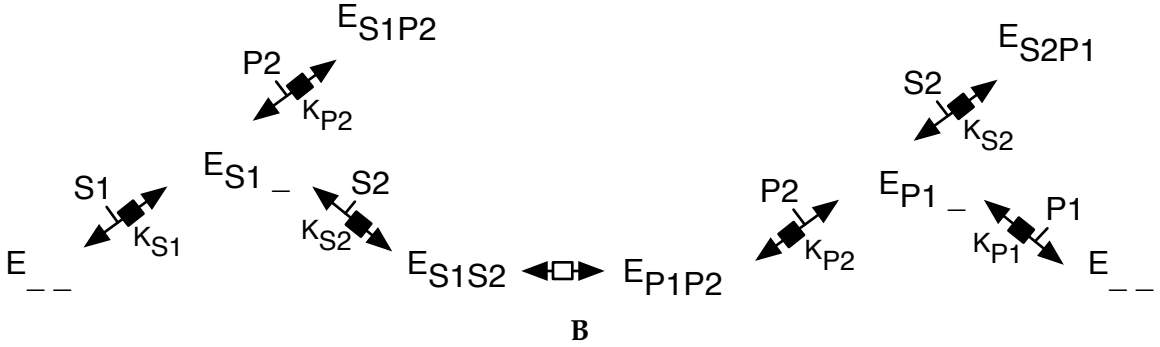


Figure 1: LDH kinetic mechanism. Binding of substrates and products to the enzyme are indicated for a random order mechanism **(A)** and an ordered mechanism **(B)**.

Fitting of simplified rate equation

Each group will first analyse their own data set to get familiar with the Mathematica fitting function. 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 product inhibition data corresponding to their specific substrate-product pair.

Additional tasks

- Calculate the equilibrium constant of the reaction using the Haldane relation and the parameter values that you have obtained, and compare the value to literature data.

- Validate the model by comparing the model prediction for a progress curve of the reverse reaction of LDH to experimental progress curves.
- Calculate equilibrium constant from experimental equilibrium concentrations.

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 [20]
6. Validation of model with product inhibition [5]
7. Use of Haldane relation to calculate K_{eq} and comparison to literature (cite) [5]
8. Use of ODE model to obtain timecourse of NADH (reverse reaction without hydrazine) and comparison to data [5]
9. Calculation of K_{eq} from experimental equilibrium concentrations [5]

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

Mark allocation

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

Each group will receive a *maximum of 110 marks times the number of group members*. For example, if a three-member group achieves a final mark of 70/110, the group will be allocated $3 \times 70 = 210$ marks, which they have to divide amongst group members according to their contribution to the overall effort. If the group cannot reach consensus on the division of marks on their own, one of the lecturers will mediate and their decision is final.

The bottom line: participate actively, and keep a note of your contributions!