Stellenbosch University – Biochemistry Honours 2024 – Biochemistry 714

Mini-course 3 (Systems Biology)
Data Analysis Programme and Assessment

This assessment has been subject to internal moderation.
Examiners:Prof JM Rohwer, Prof JL Snoep and 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^+. All incubations will be made in the presence of the allosteric activator fructose 1,6-bisphosphate (F16BP), at a high and at a low concentration. This gives 16 data sets, two for each group. In addition to the initial rate experiments, two longer incubations (for two different enzyme concentrations), both with high F16BP, were previously followed until equilibrium was reached. This data will be provided to all groups. 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 data, perform model validation, and write a report in which the theory of the lectures, data analysis and computational aspects are combined.

Schedule

Mon 8 – Thu 11 April: Practical kinetic experiments

Wed 10 – Mon 15 April: Data analysis

Tue 16 April: Finish final report.

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

Tasks

Experiments performed in practical component

NADH absorbance changes were followed spectrophotometrically over an approximately 10-minute period. Details for the experimental procedure will be provided, so that specific activities can be calculated from the raw experimental data.

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 own data set, i.e. determine a slope over the selected time period. Note that the triplicates should be treated independently to determine initial rates, so you will have three data sets and three linear regressions per substrate concentration, giving a triplicate dataset of initial rates. From the gradients of the fitted lines, calculate the specific activity of the LDH in (U/mg prot) for the different substrate concentrations that were tested in your group. Note that the protein concentration of the cell extract, and the dilution factor for the extract in the assays are

listed in the Excel data files (the dilution factor might vary for different substrate concentrations). The path length for the spectrophotometer, and the extinction coefficient for NADH will be provided.

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. A unique mechanism is assigned to each group:

1. ordered with NADH binding first (groups 1A, 1B, and 1D),
2. ordered with Lac binding first (groups 2B and 2D),
3. random order (groups 1C, 2A and 2C).

Additionally, it is known that the activator F16BP can affect K_m and V_m values but for the purpose of this study we will not be modelling the binding of the activator explicitly. This means that you are, in actual fact, working with apparent constants in your derivation and fitting procedure.

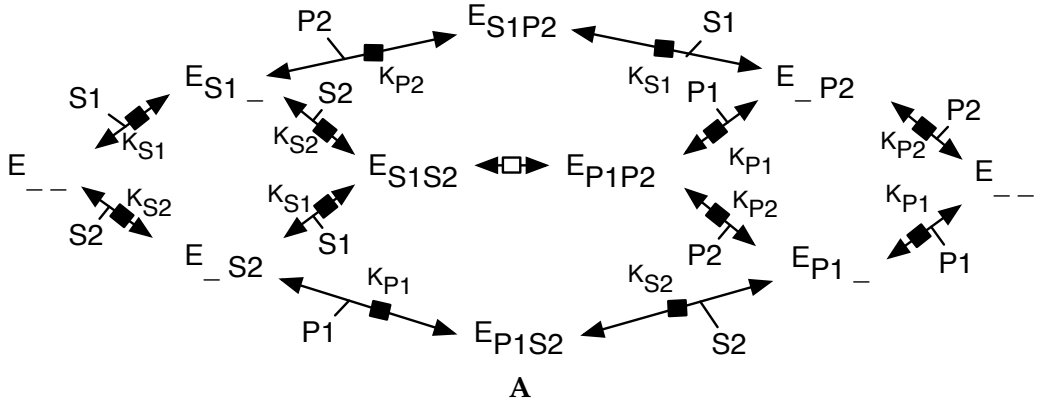

Figure 1: LDH kinetic mechanism. Binding of substrates and products to the enzyme are indicated for a random order mechanism **(A)** and an ordered mechanism **(B)**. Binding steps are indicted by filled squares; catalytic steps by open squares.

Fitting of simplified rate equation

Each group will first analyse their own dataset with a single variable to familiarise themselves with the Python fitting code. For this they will fit the data for high F16BP 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^{app} value of the non-varied substrate. From this fitting procedure, V_m^{app} and a K_s^{app} value for the specific (varied) substrate will be estimated.

Fitting of full rate equation

The data from all the groups will be combined and fitted to the full rate equation to obtain values for all parameters in the rate equation. This will be done for each F16BP concentration resulting in two sets of fitted parameters.

Model validation

Validate the full rate equation model by comparing the model prediction for progress curves of the reaction to two sets of experimental data for NADH production/consumption (which will be provided). This data reflects two enzyme concentrations (detail will be provided) and the high F16BP concentration (as above) in both cases. Set up an ordinary differential equation (ODE) model, integrate this and compare it to an experimental progress curves.

Additional tasks

- For the low and high F16BP concentration scenarios independently, calculate the equilibrium constant of the reaction using the Haldane relation and the parameter values that you have obtained (i.e. two K_{eq} values), and compare to each other and to a literature value.

- Calculate the equilibrium constants from the two sets of provided experimental equilibrium data.

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 of conditions, data and plots) [5]
2. Derivation of detailed kinetic rate equation [10]
3. Reduced rate equation for group-specific data [5]
4. Results of fitting of reduced equation on group-specific data for high F16BP [8]
    - show table of fitted parameter values;
    - include plot with data and model fit.
5. Fitting of full equation on complete dataset for low and high F16BP separately [22]
    - show the two tables of fitted values;
    - include 4 plots with data and the model fit for each of the varied substrates/products, at low/high F16BP (i.e. two curves per plot);
    - explain the effect of the activator on the enzyme in terms of its effect on kinetic parameters.
6. Use of ODE model to obtain timecourses of NADH (reverse reaction without hydrazine and with high F16BP) and comparison to data [10]

7. Use of Haldane relation to calculate K_{eq} for both low and high F16BP scenarios. Comparison to each other and to literature (cite your source) [5]

8. 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.

Handing in

- Submit your group's report in **PDF format** via the **SUNLearn** 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 SUNLearn.
- Reports will be checked for plagiarism via Turnitin.
- Deadline: **Tue 16 April 2024 at 23h59**

Mark allocation

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