Chemistry 4000/5001/7001 Fall 2010 Assignment 7

Due: Friday, Nov. 4, 4:00 p.m.

Marks: 46

1. Lactate dehydrogenase catalyzes the conversion of lactate to pyruvate in mitochondria using NAD^+ as a cofactor. To my knowledge, the concentration of lactate dehydrogenase in mitochondria is unknown, but for the sake of argument, we can assume that it is around $1\,\mu$M. The volume of a mitochondrion is about $0.5\,(\mu\text{m})^3$. Estimate the number of molecules of lactate dehydrogenase in a mitochondrion. [1 mark]

2. Denoting the enzyme by E, lactate by L and pyruvate by P, the enzyme carries out the following reaction steps:

$$\mathrm{E} + \mathrm{NAD}^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{E}^{\mathrm{NAD}^+}$$

$$\mathrm{E}^{\mathrm{NAD}^+} + \mathrm{L} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E}_{\mathrm{L}}^{\mathrm{NAD}^+}$$

$$\mathrm{E}_{\mathrm{L}}^{\mathrm{NAD}^+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{E}_{\mathrm{P}}^{\mathrm{NADH}}$$

$$\mathrm{E}_{\mathrm{P}}^{\mathrm{NADH}} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \mathrm{E}^{\mathrm{NADH}} + \mathrm{P}$$

$$\mathrm{E}^{\mathrm{NADH}} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \mathrm{E} + \mathrm{NADH}$$

The concentrations of the substrate lactate (about 5 mM) and of the product pyruvate (about $100\,\mu$M) are both much higher than the concentration of the enzyme and can be taken to be constant. Similarly, the cofactor NAD^+ is present at a concentration of about 1 mM, while its reduced version NADH has a concentration of about $200\,\mu$M, both again much higher than the concentration of the enzyme. All the rate constants are known [1], and are given in table 1. Assuming that the concentrations of lactate, pyruvate, NAD^+ and NADH are constant, determine the pseudo-first-order rate constants for each of the bimolecular steps. [2 marks]

Constant	Value	Units	Constant	Value	Units
k_1	8.74×10^6	$\mathrm{M^{-1}s^{-1}}$	k_{-1}	526	$\mathrm{s^{-1}}$
k_2	6.07×10^4	$\mathrm{M^{-1}s^{-1}}$	k_{-2}	1200	$\mathrm{s^{-1}}$
k_3	1002	$\mathrm{s^{-1}}$	k_{-3}	246	$\mathrm{s^{-1}}$
k_4	190	$\mathrm{s^{-1}}$	k_{-4}	1.21×10^6	$\mathrm{M^{-1}s^{-1}}$
k_5	16	$\mathrm{s^{-1}}$	k_{-5}	3.63×10^7	$\mathrm{M^{-1}s^{-1}}$

Table 1: Rate constants for the lactate dehydrogenase reaction

3. Create an `xppaut` input file for the stochastic simulation of the simplified model consisting of the pseudo-first-order conversions between the enzyme forms. Submit this input file with your assignment. [5 marks]

4. Determine the average and standard deviation of the stationary probability density with respect to each of the enzyme forms. Explain briefly how you did the calculation. [10 marks]

5. The standard deviation is sometimes a bit difficult to interpret because it tends to be larger when the underlying quantity (in our case, concentrations of various enzyme forms) is larger. Instead, we often use the coefficient of variation (CV) as a measure of the importance of fluctuations. The CV is defined as σ/μ, where σ is the standard deviation and μ is the average of a quantity. Calculate the CV for each enzyme concentration. Which has the largest relative fluctuations? [4 marks]

6. Vary the enzyme concentration in several steps from 10^{-7} to 10^{-5} M. Repeat the above calculations. It is not necessary to show all your calculations, but summarize key quantities in one or more tables. [10 marks]

   Note: For some of the steps that follow, it will be a good idea to distribute the concentrations logarithmically.

7. Calculate the average proportion of enzyme molecules in each of the five forms at each enzyme concentration. Are these proportions maintained as you vary the enzyme concentration? [4 marks]

8. The CVs typically obey a scaling law of the form $CV \sim N_E^{\alpha}$, where N_E is the number of enzyme molecules. Plot ln(CV) vs $\ln N_E$ for each form of the enzyme. If the scaling law given above is obeyed, these plots should be linear with slope α. Comment on the results, and give the value(s) of α if appropriate. [10 marks]

References

[1] Janos Südi. How to draw kinetic barrier diagrams for enzyme-catalyzed reactions. *Biochem. J.*, 276:265–268, 1991.