ABE 580

Spring 2018

Homework 5

Due March 1, 2019

1. In order to develop a new lactase, a series of preliminary experiments were made. Using the results of these experiments, calculate the constants that describe this reaction. Assume that gluconolactone is a classic competitive inhibitor.

Initial Lactose	Initial Gluconolactone	
Concentration (mM)	Concentration (mM)	V (mM hr ⁻¹)
0.25	0.86	0.76
0.5	0.81	1.36
1	0.63	1.98
2	0.75	2.64
4	2	3.14
0.25	0	1.06
0.5	0	1.62
1	0	2.24
2	0	2.89
4	0	3.55

- a. Determine the constants of V_{max} , K_m and K_I .
- b. Include all the graphs and equations used.
- 2. This data does not let you rule out non-competitive inhibition as the mechanism of gluconolactone on lactase. Design an experiment to investigate the actual mechanism of inhibition in this case. Describe the concentrations for enzyme, lactose and gluconolactone that should be used in all experiments. Use reciprocal plots (e.g. Lineweaver-Burke) to show which constants can be estimated from it. Compare the expected results for which of the classical inhibition mechanisms.

3. Derive the ODEs that describe the concentrations of all components in the following enzymatic reaction.

$$E + S \xrightarrow{K_{1}} ES \xrightarrow{K_{5}} P + E$$

$$+ \qquad \qquad \downarrow$$

$$K_{2} \downarrow \downarrow K_{2} \qquad K_{4} \downarrow \downarrow K_{4}$$

$$EI + S \xrightarrow{K_{3}} ESI \xrightarrow{K_{6}} P + EI$$