Department of Biochemical Engineering & Biotechnology

BBL433: Enzyme Science & Engineering

60 minutes

Minor –I (20 Marks)

1. A crude extract of glucose isomerase (EC 5.3.1.5) has been obtained after cultivation of Streptomyces flavogriseus and cell disruption in a homogenizer. The enzyme catalyzes the reversible isomerization of glucose into fructose. A 1mL sample of this extract, diluted as indicated in the following table, was put in contact with 4mL of 0.1M glucose ($C_6H_{12}O_6$, Mol. Wt: 180) solution. Glucose concentration was monitored during the reaction and the following results were obtained:

Reaction Time (minutes)	Glucose Concentration ($g \cdot L^{-1}$)	
	1 : 50 dilution	1 : 100 dilution
0	14.40	14.40
2	13.91	14.15
4	13.58	13.91
6	13.27	13.64
8	13.04	13.46
10	13.03	13.34

Calculate the activity of glucose isomerase in the crude extract, expressing it in IU/mL, and discuss the result. (4)

2. A certain enzyme has no preformed active site and the binding of a modulator molecule is required to build it up so that the substrate can be bound to it and acted upon by it. Develop a rate equation for such a mechanism and describe the experimental procedure used to determine the corresponding kinetic parameters. (3)

3. Phenylalanine ammonia lyase (EC 4.3.1.5) from Rhodotorula glutinis catalyzes the conversion of phenylalanine (X) into trans-cinnamic acid (Y) and ammonia (Z), where Y is a competitive inhibitor and Z a partial uncompetitive inhibitor. Develop a parametric kinetic expression. (3)

4. Write short notes on (any four) (4x2)

a. Transition state analog
b. Enzyme use in pharma industry
c. Enzyme use in biofuels
d. Near attack conformations
e. What is meta-genomics? How it is useful for Enzyme engineering?

5. If the rate of an enzyme catalyzed reaction modeled by the Michaelis-Menton mechanism levels off at V_{max}= 100 M/s when the substrate concentration is very high, and if v = 50 M/s when [S] = 1×10^{-4} M, what is the K_m (the Michaelis constant)?

a. 10^{-3} M
b. 10^{-4} M
c. 10^{-5} M
d. 10^{-6} M

6. For an enzyme that follows Michaelis-Menten kinetics, by what factor does the substrate concentration have to increase to change the rate of the reaction from 20% to 80% V_{max}?

a. A factor of 2
b. A factor of 4
c. A factor of 8
d. A factor of 16
e. Cannot be calculated

7. Based upon the definition for a competitive inhibitor, which observed kinetic parameter would you anticipate is most affected by the presence of inhibitor?

a. Vmax
b. Km and Vmax
c. Km
d. Kcat

8. The following pH dependence was found for the activity of a certain enzyme catalyzed reaction. If it is known that the only two ionizable residues in the active site are both glutamates, which conclusion can be drawn?

a. The glutamates have different microenvironments which cause their pKa's to differ.
b. One of the glutamates must be amidated.
c. Both glutamates have a pKa equal to 5.0.
d. Both glutamates are deprotonated during the reaction