2015

BIOCHEMISTRY

Paper – BCT – 101

(Enzymology)

Full Marks – 25

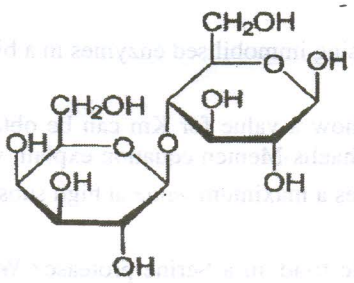
The figures in the margin indicate full marks

Candidates are required to give their answers in their own words as far as practicable

Question no. 1 is compulsory and answers ***any two*** from ***Question nos. 2*** to ***5*** and ***any one*** from ***Question nos. 6*** and ***7***

1. Lactose is a disaccharide found in milk. Although milk is a very good source of nutrients, many adults throughout the world get sick from drinking milk because they cannot digest lactose. When someone who is lactose intolerant ingests milk, the lactose accumulates in the lumen of the small intestine because there is no mechanism for uptake of the disaccharide. This causes abdominal distension, cramping and watery diarrhea.

(a) Based on the structure of Lactose given below, explain why lactose can't diffuse across the membranes of the intestinal epithelial cells in the absence of a carrier-mediated uptake system?



(b)

[Lactose] µmoles/liter	Velocity (moles/min)	
	lactose only	Lactose +Maltose (5umoles/liter)
2	7	5
5	17	10
10	29	20
20	44	35
50	67	60
100	80	78
200	89	85
500	91	91
1000	91	91

Adults who can drink milk can do so because of the enzyme lactase which is located on the outer surface of epithelial cells lining the small intestine. Lactase hydrolyzes lactose into its two component monosaccharides, glucose and galactose. Both glucose and galactose can cross the epithelial cells, and therefore do not cause illness. You decide to study lactase further, and find that maltose is NOT cleaved by lactase, and furthermore, maltose appears to have some kind of inhibitory effect on lactase's ability to cleave lactose. You suspect that maltose is a likely candidate for competitive inhibition of lactase. Explain. In order to confirm your hypothesis in part, you quantitatively study the kinetics of lactase with lactose alone, and in the presence of both lactose and maltose. You measure the initial velocity of the reaction (rate at which lactose is cleaved) at varying concentrations of substrate. The data is given in the adjoining table. Make a Lineweaver-Burke plot for lactase both with and without maltose based on the above data.

(c) Does your graph confirm or contradict your hypothesis? Why? 1+3+1

2. An enzyme has a Km for substrate (S) of 10 mM and V_{max} of 5 $\mu mol.L^{-1}.sec^{-1}$ at a total enzyme concentration of 1 nM. Find Kcat at [S] = 10 mM. Predict whether the V_{max} will increase or decrease if enzyme concentration is made 1.5nM. $2+\frac{1}{2}$

3. You are supplied with two unlabelled vials suspected to contain either Acid phosphatase or Alkaline Phosphatase. Propose an experimental strategy to verify the identity of the enzymes in each of the vials. $2\frac{1}{2}$

4. State the advantages of using immobilised enzymes in a bioreactor. $2\frac{1}{2}$

5. Explain mathematically how a value for Km can be obtained from the V_0 vs [S] graph. How does the Michaelis-Menten equation explain why the rate of an enzyme-catalyzed reaction reaches a maximum value at high substrate? $1\frac{1}{2}+1$

6. (a) What is a "catalytic triad' in a Serine protease? Why are they so called? Just the triad is not at all enzymatically efficient. Justify briefly by listing your reasons (no detailed description).

(b) Give an experimental evidence to show that the efficiency of reaction between two functional groups increases with increased restriction of their movements. How do you correlate this phenomenon with the efficiency of an enzyme? Cite two probable functions of polypeptide regions other than the active site of a protein enzyme. (2+3+3)+(3+2+2)

7. (a) What is meant by covalent modification of enzymes? Give two examples. Illustrate with appropriate example the regulation of enzyme activity by substrate analogue in vivo.

(b) What is an 'oxyanion hole'? How is it significant in a serine protease?

(c) Define transition state analogue. Explain briefly the significance with an appropriate example. Cite two important uses of such analogue(s). (2+1+2)+ (2+3)+(2+2+1)