2019

(October)

BIOCHEMISTRY

(Honours)

(BCHEM-301)

(Proteins and Enzymes)

Marks : 56

Time : 3 hours

The figures in the margin indicate full marks for the questions

Answer *any* **four** questions

1. *(a)* Proteins are purified using chromatographic techniques, which separate according to difference in specific properties. Mention the protein properties used during purification and discuss two techniques employed in the purification process. 10

(b) Define enzyme activity and specific enzyme activity. 4

2. *(a)* Discuss protein sequencing using Sanger's reagent and dansyl chloride. What are the disadvantages of these methods? 6+2=8

(Turn Over)

(b) A biochemist purifies a new enzyme, generating the purification table below :

Procedure	*Total protein* (mg)	*Activity* (units)
1. Crude extract	20000	4000000
2. Precipitation (Salt)	5000	3000000
3. Precipitation (pH)	4000	1000000
4. Ion-exchange chromatography	200	800000
5. Affinity chromatography	50	750000
6. Size-exclusion chromatography	45	675000

From the information given in the table, calculate the specific activity of the enzyme after each purification procedure. 6

3. (a) Name the classses of enzymes that catalyse the following reactions : 1×4=4

(i) $A + B + ATP \rightarrow A—B + ADP + P_i$

(ii) $A—B + C \rightarrow A + B—C$

(iii) $A—B + H_2O \rightarrow A—H + B—OH$

(iv) $A_{red} + B_{ox} \rightarrow A_{ox} + B_{red}$

(Continued)

(b) Discuss the statement, "enzyme enhances reaction rate by lowering the activation energy". 4

(c) Explain the importance of the binding energy, ΔG_B in catalysis. 3

(d) When the active site occupies a small portion of the enzyme, why is enzyme large protein? 3

4. (a) Explain the pH and temperature dependence of enzyme. 4+4=8

(b) What is the catalytic triad of chymotrypsin? 3

(c) What are the roles of those amino acid residues in the active site which do not have binding or catalytic function? 3

5. (a) Discuss Michaelis constant, K_M as an index of the affinity of an enzyme for its substrate. 5

(b) Under physiological conditions, enzymes usually do not operate at saturating substrate concentration. For these situations, explain the best way to compare the catalytic efficiency. 5

(Turn Over)

(c) Estimate the V_{max} and K_M by inspecting the data given below : 4

[S]M	U_0 (μM/min)
$2{\cdot}5\times10^{-6}$	28
$4{\cdot}0\times10^{-6}$	40
1×10^{-5}	70
2×10^{-5}	95
4×10^{-5}	112
1×10^{-5}	128
2×10^{-4}	139
1×10^{-2}	140

6. (a) What is enzyme inhibition? Derive the rate equation for an enzyme subjected to competitive inhibition. 2+9=11

(b) Explain why non-competitive inhibition is the most important type of inhibition for the regulation of the cell metabolism. 3

7. Explain any *one* of the following with respect to enzyme regulation : 7

(a) Proteolytic cleavage

(b) Covalent modification

8. What does protein turnover mean? How can a cell distinguish proteins that are meant for degradation? 2+5=7

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