2022   4/BIT C 401
(July)
BIOTECHNOLOGY & BIOINFORMATICS
(Animal Cell Science and Basic Enzymology)
Course No: BIT C 401
Full Marks: 75
Time: 3 hours

The figures in the margin indicate full marks for the questions
Answer Question No.1 and any four from the rest

1. A. Match the terms given in Column A with those of Column-B.   1x5 = 5

Column-A		Column-B
(i)	Survival Assay	(a) HAT medium
(ii)	Enzyme substrate binding	(b) Polyethylene glycol
(iii)	Ara-C	(c) Induced fit model
(iv)	iPSC	(d) Complex system
(v)	Feedback inhibition	(e) Regenerative medicine
		(f) Cryopreservation
		(g) Cell synchronization
		(h) Cytotoxicity

B.   Write True or False?   1x5 = 5
   (a)   Moses Kunitz coined the term *enzyme*.
   (b)   Oxidoreductases transfer groups within molecules to yield isomeric forms.
   (c)   Transition state analogues can bind enzyme more tightly than substrate.
   (d)   Binding energy contributes to reaction specificity and catalysis.
   (e)   miRNA can be used for iPSC development.

C. Choose the best answer: 1x5=5

(a) K_M can be considered to be the same as the dissociation constant K_S for an enzymatic reaction if:
   (i) This statement cannot be completed because K_M can never approximate K_S
   (ii) ES $\rightarrow$ E + P is fast compared to ES $\rightarrow$ E + S
   (iii) The turnover number is very large
   (iv) $k_2 \ll k_{-1}$

(b) In irreversible inhibition, increasing the concentration of substrate:
   (i) Decreases the reaction rate
   (ii) Double the reaction rate
   (iii) Has no effect on the reaction rate
   (iv) Overcomes the inhibition, increasing the reaction rate up to ½Vmax

(c) Enzymes classified under a new EC class (EC7) are
   (i) Ligase
   (ii) Translocase
   (iii) Transportase
   (iv) Isomerase

(d) Which of the following best describes the assumption made in steady state kinetic analysis?

   (i) The concentration of [ES] is constant

   (ii) The concentration of [S] is constant

   (iii) The total amount of enzyme decreases

   (iv) Both '(i)' and '(ii)' are correct

(e) Which of the following statement is incorrect for Allosteric enzymes?

   (i) Allosteric enzymes have sigmoid kinetics

   (ii) The value of the substrate concentration corresponding to half-maximal velocity is designated as $K_{0.5}$

   (iii) Allosteric enzymes follow Michaelis-Menten equation

   (iv) Effector binding can alter either the $K_{0.5}$ or the V_{max} of the enzyme

2. (a) What are the characteristic features of isoenzymes?   5

 (b) Explain the mechanism of acid-base catalysis, covalent catalysis, electrostatic catalysis and desolvation with example of enzyme.   8

 (c) Explain the difference between prosthetic groups and cosubstrates.   2

3. (a) Describe how multi-substrate reactions are classified based on the mechanisms and represent the diagramatic summary of the reaction sequence using Cleland plot. How these mechanism can be differentiated using Lineweaver-Burk plot?   4+2+3=9

 (b) A competitive inhibitor for an enzyme is studied by the Lineweaver-Burk method. The K_i for the competitive inhibitor is 10 mM. The K_m value without inhibitor is 50 mM. Calculate the apparent K_m when 40 mM inhibitor is present.   4

(c) Draw the Eadie-Hofstee plot, a graphical representation of enzyme kinetics.   2

4. (a) Discuss briefly the types of irreversible inhibitors. Do irreversible inhibitors affect the K_m and V_{max} of a reaction? if Yes/No. Explain it briefly. Give one point as how irreversible inhibitors differ from reversible inhibitor.   5+2+1=8

(b) An enzyme "E" catalyse the reaction by converting the substrate "A" to "B" where the K_m of "E" for its substrate "A" is 3.2×10^{-5} M. When the substrate concentration [A]=24mM and the rate of reaction, v_0 is 73.3 μmol/(mL.s), (i) what is the V_{max} for this enzyme? (ii) Assuming the enzyme concentration "E" in this experiment was 3nmol/mL what is k_{cat} for this enzyme? (iii) What is the catalytic efficiency for "E"? (iv) Does the value of k_{cat}/K_m reveal that "E" approaches "catalytic perfection"?   2+2+2+1=7

5. (a) What is the significance of the enzyme HGPRT and TK in cell fusion?   3

 (b) How do you metabolically label the cells and what purpose will it serve?   4

 (c) Differentiate between cell survival assay and cytotoxicity assay.   4

 (d) Why MTT assay is used and how the assay is done?   1+3= 4

6. (a) What is stem cells? How normal cells can be converted to stem cells? Both cancer cells and normal stem cells have self-renewal ability. Write your view on this   1+2+6=9

(b) What are the advantages and disadvantages of monolayer compared to suspension culture system during scaling up?   6

7. (a) Differentiate between primary culture and cell line. Why and when do we use DNAse during initiation of primary culture?   2+1=3
   (b) What are the major constituents of serum? Write down the advantages and disadvantages of using serum in cell culture.   4+4=8
   (c) What is transformation in the context of cell culture? Write down various process of cell transformation.   2+2=4

8. Write short notes on any three of the following: 5x3=15
   a. Ribozymes
   b. Multienzymes
   c. 3-dimensional culture
   d. Cell synchrony
   e. Roller culture

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