

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI
First Semester, 2023-2024
BIO F421 ENZYMOLOGY Mid-Semester Test (Closed Book)

Date: 11.10.2023

Max. Marks: 25

Time: 2 – 3.30 PM [90 min]

Note: Answer all Questions. Ensure you suitably justify all your responses.
Answer questions in the same order as they appear in the Question paper – Do not jumble the order.
Leave sufficient left margin for examiner's remarks.

- Part A –

Questions 1-4 carry 2 marks each. Q5 carries 3 marks.

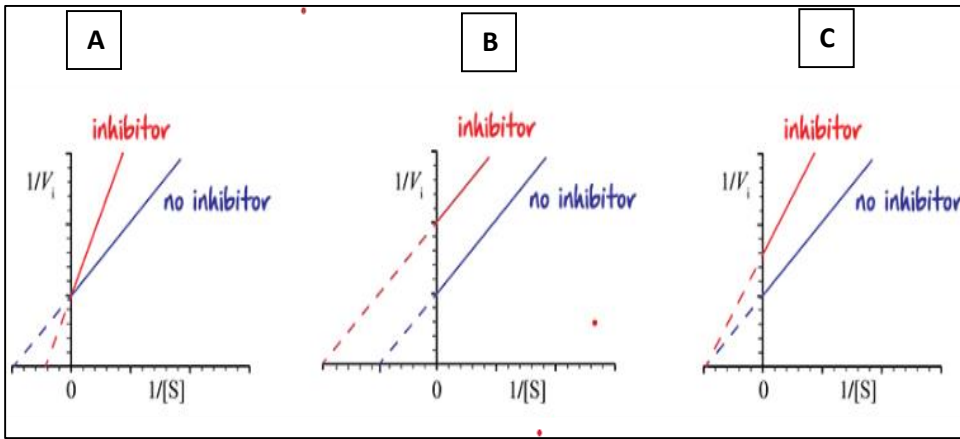
1. Briefly elaborate on Kornberg's statement: "Don't waste clean thinking on dirty enzymes!" Bring in the perspectives from both academic/scientific and commercial/industrial fields.
2. Which type(s) of column chromatography can be performed (i.e. included in the enzyme purification step) immediately after ammonium sulfate precipitation? Justify briefly – for both included and excluded chromatography types.
3. Mention briefly the role of different inclusions in the (a) enzyme extraction buffer and (b) enzyme assay buffer.
4. Discuss briefly the physiological functions and commercial applications of plant β -glucosidases.
5. Think of a hypothetical enzyme involved in plant secondary metabolism (other than β -glucosidases!). Now, design and present your purification protocol in flowchart form. Include all possible details, on the lines of our discussion in class.

- Part B –

Marks are indicated within brackets.

6. Glucose 1-phosphate is converted to G-6-P, by phosphoglucomutase. So EC number will start with? Indicate the Main class name and number, along with a brief justification. [1M]
7. What type of substrate-specificity of enzymes is responsible for the fact that mammals are able to use mostly starch or glycogen as sources of energy, and not cellulose? Give reason. [2M]
8. Name two important Vitamin B-complex molecules that act as coenzymes for redox reaction catalysing enzymes. You should also indicate the name of the corresponding coenzymes. [2M]
9. The 'hand and the woollen glove' analogy is used for which type of enzymatic mechanism? Explain the mechanism, and clearly indicate what the 'hand' and the 'glove' represent with respect to enzyme catalysis. [2M]

10. Read the graphical plots below, and answer the questions that follow:



(i) Identify the type of inhibitor depicted under A, B and C. Substantiate your answer with suitable enzyme kinetics correlations. [3M]

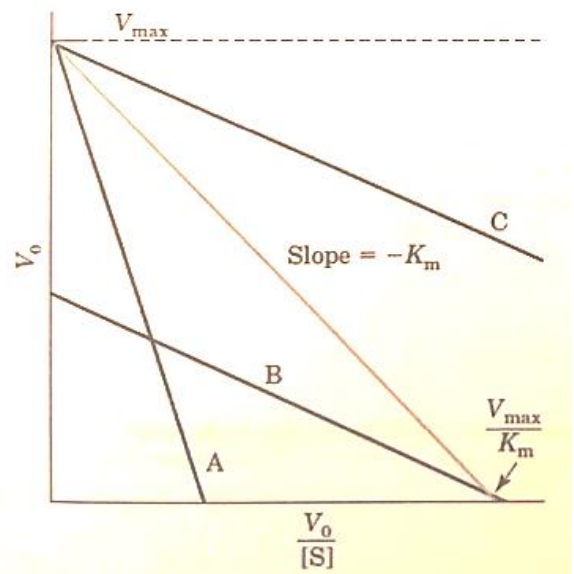
(ii) In which of the above cases (A/B/C), can increasing the substrate concentration reverse the inhibition? How does it work? [2M]

11. There are several ways to transform the Michaelis-Menten equation so as to plot data and derive kinetic parameters, each with different advantages depending on the data set being analyzed. One transformation of the Michaelis-Menten equation is the Lineweaver-Burk, or double-reciprocal, equation. Multiplying both sides of the Lineweaver-Burk equation by V_{max} and rearranging gives the Eadie-Hofstee equation:

$$v_0 = -K_m \frac{v_0}{[S_0]} + V_{max}$$

A plot of V_0 versus $V_0/[S]$ for an enzyme-catalyzed reaction is shown here. The curve in the middle (*without any tag*) was obtained in the absence of inhibitor.

So, now the Question is which of the other curves (A, B, or C) shows the enzyme activity when a competitive inhibitor was added to the reaction mixture? Justify your answer. [2M]



***** All the Best ! *****