BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI
First Semester, 2023-2024
BIO F421 ENZYMOLOGY

Comprehensive Examination [CB+OB] Date: 12.12.2023 Max. Marks: 40 Max. Time: 180min

OPEN Book Max. Marks: 20 Time: 10.00 am – 12.00 noon [120 min]

Note: *Answer all Questions. Ensure you suitably justify all your responses. Underline key terms. 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

1. A researcher purified an enzyme involved in aroma biosynthesis in a locally available plant's fruit. Study the purification table obtained by her and answer the questions that follow.

S. No.	Volume (ml)	Step	Specific activity (μkat/mg)
1	1100	Crude extract	0.41
2	150	DEAE sepharose	1.84
3	48	Phenyl sepharose	2.41
4	16	Sephacryl S-300 HR	2.96

(i) What could have been the weight (in grams) of the starting material (i.e. the plant tissue) from which the enzyme was isolated to prepare the crude extract? Justify briefly. **[1M]**

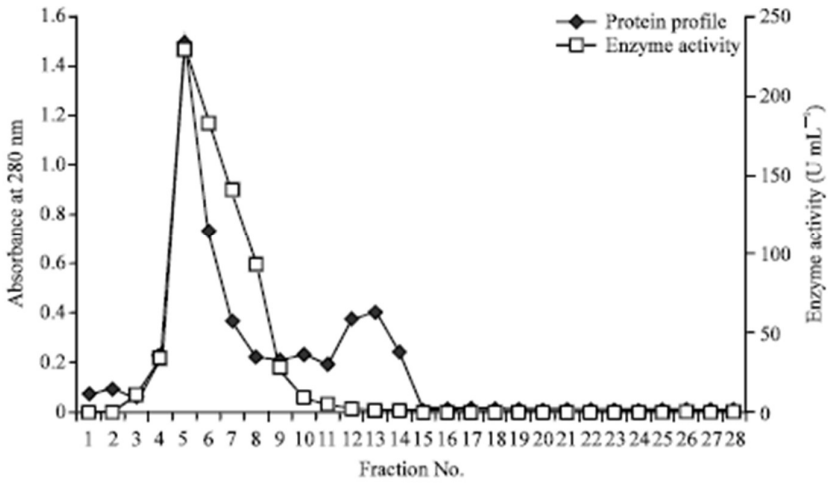
(ii) Calculate the fold-purification factor for each step. **[1M]**

(iii) *The researcher expected the final specific activity to be above 10 μkat/mg, however could not achieve the same!* Give possible reason(s) why the specific activity did not increase to the anticipated extent with the purification steps. **[1M]**

(iv) Mention a possible *in planta* substrate, the corresponding enzyme and the catalytic product that could contribute to the aroma in the fruit. **[1M]**

(v) Enlist any two specific and unique instruments / techniques you would need to carry out the experiments performed by the researcher. Give the rationale for your choices. **[2M]**

2. Study the following figure (representing elution profile for an enzyme purified via Sephadex G-75 chromatography) and answer the questions that follow. (Source for the figure: *Vijayaraghavan et al., 2011. Purification and Properties of Novel Malate Dehydrogenase Isolated from Pseudomonas aeruginosa. Asian Journal of Biotechnology, 3: 478-485.*)



(i) *For a while, step into the researchers' shoes*! You know that as a scientist, you need to appropriately discuss your results. *So, take up the challenge!* Write a discussion for the above result in approximately 150-200 words (approx. half page). **[2M]**

(ii) In this experiment, Sephadex G-75 was used as the stationary phase. *How would your results have varied if you had chosen a lower grade (SG-25, for instance) and a higher grade (SG-200, for instance) matrix for your experiment?* In each case, provide a brief justification for your insights. **[1M]**

(iii) How can you utilize gel filtration chromatography to estimate the molecular weight of a native enzyme? Mention briefly. **[1M]**

PART – B

1. Read the following research summary, and answer the related questions.

Cystathioninuria is a genetic disease in which γ-cystathionase is either deficient or inactive Cystathionase catalyzes the reaction:

Cystathionine $\rightleftharpoons$ cysteine + α-ketobutyrate

Deficiency of the enzyme leads to accumulation of cystathionine in the plasma. Since cystathionase is a pyridoxal phosphate-dependent enzyme, vitamin B_6 was administered to patients whose fibroblasts contained material that cross-reacted with antibody against cystathionase. Many responded to B_6 therapy with a fall in plasma levels of cystathionine. These patients produce the apoenzyme that reacted with the antibody. In one patient the enzyme activity was undetectable in fibroblast homogenates but increased to 31% of normal with the addition of 1 mM of pyridoxal phosphate to the assay mixture. It is thought that the K_m for pyridoxal phosphate binding to the enzyme was increased because of a mutation in the binding site. Activity is partially restored by increasing the concentration of coenzyme. Apparently these patients require a higher steady-state concentration of coenzyme to maintain γ-cystathionase activity.

Pascal, T. A., Gaull, G. E., Beratis, N. G., Gillam, B. M., Tallan, H. H., and Hirschhorn, K. Vitamin B_6-responsive and unresponsive cystathionuria: two variant molecular forms.

a. There is another term for such genetic diseases. Can you mention the term? **[0.5M]**

b. Why do the Cystathioninuria patients need a higher dosage administration of the coenzyme? Pinpoint the specific enzyme-kinetics property here. **[1M]**

c. Do you think in all patients with Cystathioninuria, such coenzyme treatment would work? Why or why not? Explain any pre-screening technique that will help us decide this choice of treatment with coenzyme. **[1M]**

2. You know that the substrate interacts *only* with the active- site of an enzyme, which is a tiny cleft in a huge enzyme molecule. Then, why is there a need for an enzyme to be so big? Provide at least two major justification points. **[1M]**

3. The *activation barriers* in molecular reactions are crucial for the existence of life itself!

a. Why do you think activation barriers are needed? What would be the consequence(s) of their absence? **[1M]**

b. Nonetheless, In living cells, for energy and structural needs, these barriers must be overcome. So how is this achieved in the biological system? Further, list the three key distinguishing features of this mechanism. **[1M]**

4. Here is the abstract of a recent research article. Respond to the questions that follow.

Tournier, et al. An engineered PET depolymerase to break down and recycle plastic bottles. *Nature* 580, 216–219 (2020).

> *Present estimates suggest that of the 359 million tons of plastics produced annually worldwide, 150–200 million tons accumulate in landfill or in the natural environment. Poly(ethylene terephthalate) (PET) is the most abundant polyester plastic, with almost 70 million tons manufactured annually worldwide for use in textiles and packaging. The main recycling process for PET, via thermomechanical means, results in a loss of mechanical properties. Consequently, de novo synthesis is preferred and PET waste continues to accumulate. With a high ratio of aromatic terephthalate units—which reduce chain mobility—PET is a polyester that is extremely difficult to hydrolyse. Several PET hydrolase enzymes have been reported, but show limited productivity. Here we describe an improved PET hydrolase that ultimately achieves, over 10 hours, a minimum of 90 per cent PET depolymerization into monomers, with a productivity of 16.7 grams of terephthalate per litre per hour (200 grams per kilogram of PET suspension, with an enzyme concentration of 3 milligrams per gram of PET). This highly efficient, optimized enzyme outperforms all PET hydrolases reported so far, including an enzyme from the bacterium Ideonella sakaiensis strain 201-F6 (even assisted by a secondary enzyme) and related improved variants that have attracted recent interest. We also show that biologically recycled PET exhibiting the same properties as petrochemical PET can be produced from enzymatically depolymerized PET waste, before being processed into bottles, thereby contributing towards the concept of a circular PET economy.*

a. What enzyme class does the PET depolymerase belong to? Identify its substrate and product. **[1.5M]**

b. Why is this enzyme assisted PET recycling process considered a *sustainable* process? Provide at least <u>two</u> key points. **[1M]**

c. In this work, the scientists have improved the PET depolymerase enzyme by protein engineering. From the abstract, <u>identify</u> the specific structural difficulty faced in this enzyme catalysis.

In your semester-long Enzymology course, you have come across several enzymes with different kinetics, specificities, and mechanism of action. if you were to attempt engineering the improved variant of the enzyme, which <u>other enzyme</u> you would study as an *inspiration* or *guide* to overcome the structural bottleneck / hurdle in this PET depolymerase enzyme? <u>Specify</u> the enzyme and <u>justify</u> your choice. **[0.5 + 1= 1.5M]**

d. In general, what is the pre-requisite information for conducting rational design of enzyme engineering? **[0.5M]**

**********All the best**********