RHODES UNIVERSITY
DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

BIOCHEMISTRY 302

PRACTICAL EXAMINATION (**PAPER 2**): OCT/NOV 2022

Internal Examiners: Prof H Hoppe
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MARKS: 60
DURATION: 2 hours

GENERAL INSTRUCTIONS TO CANDIDATES

1. There are **6** questions. Answer **ALL** questions.
2. Time management is very important. The value of the mark for each question should be used as a rough guide to the amount of time allocated to answer the question (60 marks in 120 minutes).
3. It is in your best interest to write legibly.
4. At the end of the examination, place all answer books and graph paper inside answer book 1.
5. Graph paper and a calculator are required.
6. The Oxford concise English dictionary may be used during this examination.

PLEASE DO NOT TURN OVER THIS PAGE UNTIL TOLD TO DO SO

Question 1

a) To perform a glutathione-S-transferase (GST) assay, the enzyme is added to the substrates 1-chloro-2,4-dinitrobenzene (CDNB) and reduced L-glutathione (GSH). You are given stock solutions of CDNB (0.2 M), GSH (0.4 M) and GST (0.2 mg/mL), and mix them with buffer in a cuvette as follows: 970 µL buffer, 10 µL CDNB, 10 µL GSH and 10 µL GST.

What is the final concentration of CDNB in the reaction? (Give your answer in **mM**). (2)

b) What product is formed in the GST reaction (*you can use abbreviations*), and what wavelength is used to detect it in a spectrophotometer? (2)

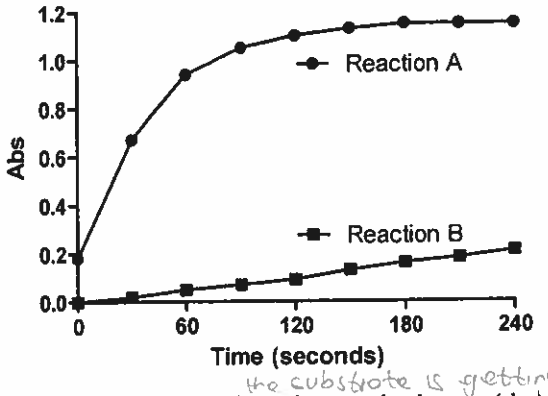
c) After starting the GST enzyme reaction described in question (a) above, you measure the absorbance of the product over a 2-minute period and obtain the following results:

Time (seconds)	Absorbance
0	0.05
30	0.11
60	0.17
90	0.23
120	0.29

i) What is the enzyme activity of GST in the reaction? (*Note: the molar extinction coefficient, ε, of the product is 9600 $M^{-1}cm^{-1}$).* (3)

ii) What is the specific activity of GST in the reaction? (2)

d) Using two different samples of GST, you perform the GST assay as described above and obtain the following results for the two reactions (reactions A and B).



i) In reaction A, why does the graph plateau (the absorbance change decreases) over time? (1)

ii) With reaction A, it would be difficult to calculate an accurate GST enzyme activity. What could you do to improve the assay? (1)

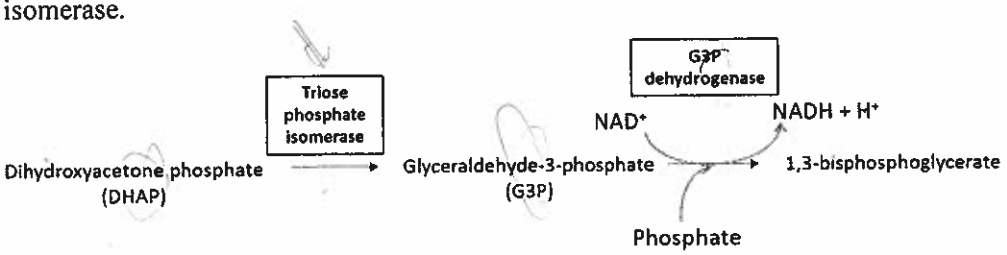
iii) Reaction B is also not ideal for obtaining an accurate GST enzyme activity. What could you do to improve the assay? (1)

e) In the Beer-Lambert equation used to calculate the concentration of a molecule based on absorbance:

i) What is "l"? (1)

ii) What is the standard (or usual) value of l? (1)

f) Coupled reactions using enzymes that produce or consume NADH (which absorbs light at 340 nm) are very useful for determining the activity of enzymes that don't have substrates or products that absorb light in the UV-Visible range. An example is the enzyme triose phosphate isomerase.

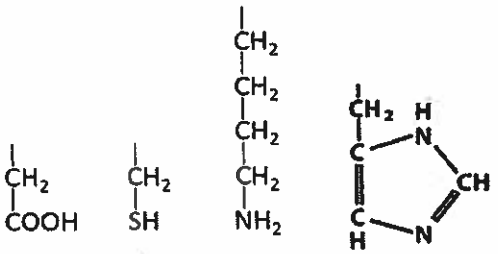


If you wanted to determine the activity of triose phosphate isomerase using the above coupled reaction, what would you add to the cuvette (besides buffer)?
(*You don't need to provide volumes or concentrations, just the names of the reagents*). (3)

[17]

Question 2

a) The table below shows the deionised (uncharged) structures of the side-chains of four amino acids found in proteins, as well as the approximate pKa values of the ionisable groups in the side-chains.

Amino acid	Asp	Cys	Lys	His
Side-chain				
pKa	3.9	8.2	10.5	6.0

At pH 9.0, what would the charge of the side-chain be (positive, negative, uncharged) in the case of:

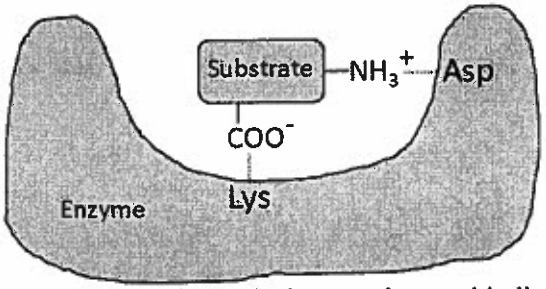
i) Aspartic acid (Asp)

ii) Cysteine (Cys)

iii) Lysine (Lys)

iv) Histidine (His) (2)

b)

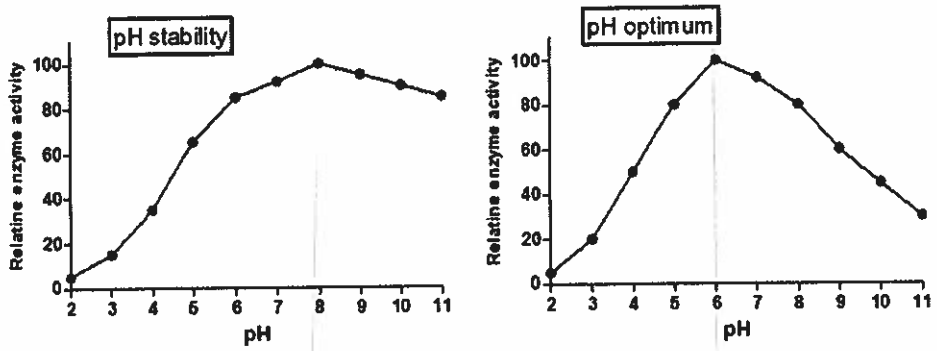


The diagram above depicts a substrate binding to the active site of an enzyme using ionic interactions. Based on this figure, a low pH (below 4) should significantly inhibit the activity of the enzyme. Explain why.

(*Note: you can use the amino acid side-chain structures in question (a) as a guide to help answer the question*). (2)

c) Besides substrate binding, when the pH is above or below the optimum pH of an enzyme, there are two other main factors that can inhibit the activity of an enzyme. Briefly describe one of them. (*One sentence should be sufficient*) (1)

d) The graphs below show the results obtained from pH stability and pH optimum experiments carried out on an enzyme.



i) At what pH would you carry out the enzyme assay (or reaction)? (1)

ii) At what pH would you store the enzyme? (1)

iii) In the pH stability experiment, why is the enzyme activity low at pH 2-3? (1)

[8]

Question 3

a) A commonly used protein assay was used in a practical to determine the concentration of GST. What is the assay called? (1)

b) To determine the concentration of a protein using the above assay, a standard curve is prepared using dilutions of a standard protein. Which standard protein was used in the practical? (1)

c) You are provided with a stock solution of the standard protein, with a concentration of 2 mg/mL. You prepare a series of dilutions of the standard protein in buffer and use the protein assay above to obtain absorbance readings at 595 nm. The results are shown in the table below:

Volume of standard stock solution	Volume of buffer	Abs_{595}
0.5 mL	0.5 mL	1.2
0.4 mL	0.6 mL	0.95
0.3 mL	0.7 mL	0.70
0.2 mL	0.8 mL	0.50
0.1 mL	0.9 mL	0.25

i) On the graph paper provided, draw a graph of the standard curve obtained with the above values. (3)

ii) If you included a GST sample and obtained an absorbance value of 0.8, what is the GST concentration? (1)

d) In one of the practicals, an ELISA method was used to detect the presence of GST in samples. In the method, what primary antibody was used? (1)

e) In the ELISA experiment, what enzyme was the secondary antibody conjugated (attached) to? (1)

[8]

Question 4

Assume that for your GST enzyme (in your practical), you obtained the following data for your enzyme assays below with a particular substrate.

Degrees Celsius	Activity (V_{max} in µmol product formed per min) (µmol.min^{-1})
20	0.103
30	0.411
40	0.719
45	0.932
50	1.041
55	1.084
60	1.033
70	0.752
80	0.657

(a) Draw the corresponding graph, labelling all axes, scales and titles properly. (3)

(b) What is the temperature optimum of the GST? (1)

(c) What is the significance of the term "temperature optimum" in enzymology? Does it relate to the properties of the enzyme or of the enzyme assay/reaction catalyzed? (2)

(d) Why does the activity of the GST decrease above 55°C? (1)

(e) Briefly explain how you would go about calculating the activation energy, E_a, of the GST, using the data in the table above. (3)

[10]

Question 5

A single-substrate enzyme-catalysed reaction was investigated in the presence of 1.0 mmol l^{-1} inhibitor and in the absence of inhibitor, the initial enzyme concentration being constant throughout. The following results were obtained:

[Substrate] (mmol l^{-1})		[Product] (µmol l^{-1}) at time t (s)					
t =		0	60	120	180	240	300
5.0	inhibited	0	110	221	333	430	480
5.0	uninhibited	0	161	320	482	598	662
6.67	inhibited	0	142	281	420	531	598
6.67	uninhibited	0	194	388	581	745	796
10.0	inhibited	0	183	367	549	705	752
10.0	uninhibited	0	263	525	789	998	1120
20.0	inhibited	0	279	558	837	1050	1170
20.0	uninhibited	0	400	798	1200	1520	1760
50.0	inhibited	0	398	798	1190	1520	1760
50.0	uninhibited	0	576	1150	1730	2170	2460

(a) Determine the type of inhibition using a Lineweaver-Burk plot. (10)

(b) Calculate the values of V_{max}' and K_m' (these are the V_{max} and K_m values when there's an inhibitor present) in the presence of the 1.0 mmol l^{-1} inhibitor. (2)

[12]

Question 6

In your GST practical, the initial velocity (v_0) of the reaction in the direction of GS-DNB conjugate formation was followed spectrophotometrically at different initial concentrations of reduced glutathione (GSH) and CDNB. The following kinetic data were obtained:

	Reduced glutathione (GSH) (mM)				
	1.25	2	2.5	5	10
CDNB (mM)	Initial velocity (µmol/min)				
2	0.019	0.025	0.028	0.038	0.045
3	0.024	0.032	0.036	0.049	0.059
4	0.027	0.037	0.042	0.057	0.069
6	0.031	0.043	0.049	0.067	0.083

Identify the reaction mechanism of the GST enzyme.

[5]

END OF THE EXAMINATION PAPER