Ahmedabad University

School of Arts and Sciences

BIO210 Intermediate Biochemistry

End Term Examination 2024 (Winter Semester)

Total Marks = 50 **Time – 3 hours**

Date: 25-04-2024 (Exam date)
No. of printed pages: 4 (Pages of question paper)

- **This is an open book exam. Hence, use of physical copy of the course pack is permitted.**
- **Use of scientific calculator is permitted.**
- **Use of mobile devices to connect to the internet such as laptops or mobile phones is NOT permitted. Violation of this rule would lead to immediate cancellation of the exam for the student.**

1. Radio-labeling carbon atoms with either C13 or C14 is a powerful way of diagnosing flux through the citric acid cycle and cellular utilization of metabolites for a variety of needs through the pathway. For this question trace the the course of the radioactive label in [2-14C] pyruvate (pyruvate with a C14 radiolabel at carbon-2) through the citric acid cycle until all of the C14 from a single pyruvate molecule has been released as CO2. How many times did you have to draw out the cycle? Please show your work by drawing the molecular structure of each citric acid cycle intermediate and where the C14 labeled carbon is. Draw the entire cycle until the C14 labeled carbon has been removed as carbon dioxide. Hint: Keep in mind that succinate is a symmetric molecule and when acted upon by an enzyme can bind in two different orientations. (5 marks)
2. In the paper by Krebs, there are two experiments that make use of two poisons of the citric acid cycle (poisons = inhibitors). For instance, in Table III, pigeon breast muscle is incubated with arsenite, and addition of citrate leads to a loss of citrate. In Table V, Krebs uses malonate. What he shows is the formation of succinic acid under aerobic and anaerobic conditions, starting with OAA. Given the results in the Table, compare these results with what might be expected from glycolysis – in other words, why is this result NOT consistent with a mechanism that has a definite start and end point. (5 marks)

TABLE III.

Disappearance of citric acid in pigeon breast muscle in the presence of arsenite (3.10^{-3} mol.).

(3 ccm muscle suspension containing 750 mg wet muscle were shaken for 40 min. at 40°)

μl citrate added	μl citrate found after 40 min.	μl citrate used	$Q_{citrate}$
1120	30	1090	—10,9
2240	972	1268	—12,7
4480	2790	1690	—16,9

TABLE V.

Effect of malonate on the aerobic and anaerobic conversion of oxaloacetic into succinic acid.

(0,75 grammes wet muscle in 3 ccm phosphate buffer; 40° C; ph = 7,4)

Experimental conditions (final concentration of the substrates)	μl succinic acid formed in 40 min.
1. O_2; 0,1 M oxaloacetate;	1086
2. O_2; 0,1 M oxaloacetate; 0,06 M malonate	1410
3. N_2; 0,1 M oxaloacetate;	1270
4. N_2; 0,1 M oxaloacetate; 0,06 M malonate	834

3. A scientist undertook an experiment with all the necessary reagents to study the β-oxidation of a fatty acyl-CoA derived from myristic acid (14:0) in Tube 1. In Tube 2, coenzyme A was omitted. In Tube 3, NAD+ was omitted. In Tube 4, FAD was omitted. The catabolism of myristic acid was observed in Tube 1 but not Tubes 2, 3 or 4. Based on your knowledge of lipid metabolism from class answer the following. State the key step directly involving water in Tube 1. State the key step impaired in Tubes 2,3 and 4, respectively. The reaction in Tube 1 resulted in the complete catabolism of myristic acid. State the products, including how many of each were produced from this reaction. (5 marks)
4. A patient is suspected of having a problem with B-oxidation. You have the patient fast for 24 h intervals, administer different fatty acids with/without L-carnitine and measure the levels of plasma acetoacetate and y-hydroxybutyrate. The results are in the table below.

	Acetoacetate levels	**γ-hydroxybutyrate levels**
Short chain fatty acids	Normal	Normal
Medium chain fatty acids	Normal	Normal
Long chain fatty acids	Not detected	Not detected
Short chain fatty acids + L-carnitine	Normal	Normal
Medium chain fatty acids + L-carnitine	Normal	Normal
Long chain fatty acids + L-carnitine	Not detected	Not detected

What do these results tell you? Why do the experiment where L-carnitine was added? What would you recommend the patient do to their diet? (5 marks)

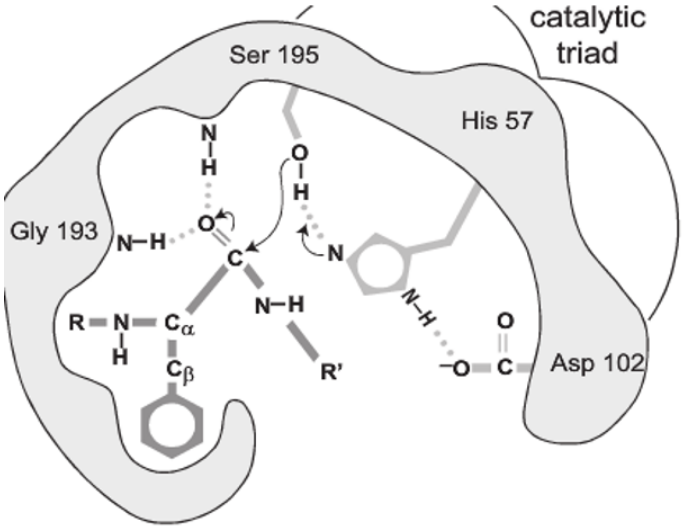
5. The urea cycle is a series of reactions in your body that allow you to excrete excess nitrogen (in the form of urea) from the breakdown of amino acids from the food you eat. During an early step in this process, an enzyme called ornithine transcarbamoylase (OTC) transfers the (CONH) group of carbamoyl phosphate to ornithine, yielding citrulline by the reaction below. The reaction has a 1:1 mole ratio of ornithine to carbamoyl phosphate. Ornithine Transcarbamoylase Deficiency (OTC) is an inherited disorder that affects the metabolism of nitrogen and as a summer research project you are interested in studying this disease.

   In order to study this process, you conduct a series of experiments in the laboratory. In the first. you combine 11.50 ml of 3.52×10^{-3}M ornithine with 29.38 ml of 2.11×10^{-3}M carbamoyl phosphate, and enough enzyme to carry out the reaction
   a) How many moles of citrulline can be formed in this reaction ? (2.5 marks)
   b) What is the concentration of citrulline that results when the reaction is complete ? (2.5 marks)
6. There is a rare medical condition in which a person is deficient in the pyruvate dehydrogenase enzyme that converts pyruvate to acetyl CoA-a precursor for the Krebs Cycle, Signs and symptoms vary, but there are generally two main manifestations. First, patients can have an elevated blood lactate (lactic acid) level. Second, patients may have neurological defects, including microcephaly (a small head circumference) and/or mental retardation. Treatment is currently limited and not very effective. Moreover, damage to the brain is often irreversible.
   a) Would you expect a diet rich in fat and low in carbohydrates to worsen or improve a patient's symptoms in someone who has pyruvate dehydrogenase deficiency? (2.5 marks)
   b) One treatment for pyruvate dehydrogenase deficiency is to eat a diet high in the amino acids lysine and leucine to decrease blood lactic acid levels. Why is this a plausible therapy? (2.5 marks)
7. You cut an apple into slices and laid out in a beautiful pattern on a platter. After 20 minutes, the apple turns brown because of an oxidation reaction catalyzed by an enzyme. No one eats your brown apples. Your friend suggests that next time you toss the apples in lemon juice to stop the browning." You're skeptical, but you try it anyway. Lo, and behold! The apples do not turn brown!
   a) What feature of the lemon juice made the difference? How did that feature stop the oxidation reaction from occurring? (2.5 marks)
   b) Suppose you tossed apple slices in lemon juice, left them alone for 10 minutes and then rinsed them in water to remove the lemon juice. Would they then start to brown again? Would that oxidation reaction still take place? Justify your answer. (2.5 marks)
8. Lactase, also known as β-galatosidase, catalyzes the hydrolysis of lactose to produce glucose and galactose from milk and whey. Experiments are carried out to determine the kinetic parameters for the enzyme. The initial rate data are as follows:

Lactose Concentration (mM)	Initial Reaction Velocity (mM/min)
25.0	1.94
22.7	1.91
18.4	1.85
13.5	1.80
12.5	1.78
7.30	1.46
4.60	1.17
2.04	0.779

a) Estimate values for the parameters Vmax [mM/min) and Km [mM]. (2.5 marks)
b) For this system, what would you expect the initial reaction velocity to be at a lactose concentration of 15.0 mM? (2.5 marks)

9. A diagram of the enzyme active site for chymotrypsin is shown. The amino acids Gly 193, Ser 195, His 57 and Asp 102 form part of the active site. The amino acids Ser 195, His 57 and Asp 102 comprise what is known as the catalytic triad. A portion of a natural peptide substrate from R to R' is depicted with the residue phenylalanine occupying the hydrophobic pocket of the enzyme.



Chymotrypsin is a serine protease enzyme. The K_M for the reaction of chymotrypsin with N-acetylvaline ethyl ester is 8.8 X 10^{-2} M, and the K_M for the reaction of chymotrypsin with N-acetyl tyrosine ethyl ester is 6.6 X10^{-4} M.

a) Which substrate has a higher apparent affinity for the enzyme? (1 mark)
b) Propose a reason for the difference in affinity based on the shape of each of the substrates (see active site figure, chymotrypsin cleaves on the C-side of aromatic residues). (4 marks)

10. The Ping-Pong enzyme mechanism can be represented by the equations below where A and B arc substrates and P and Q are products. Using the steady-state approximation, derive an equation for the rate of formation of Q.

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} P + E'$$

$$E' + B \xrightarrow{k_2} E + Q$$