

**UNIVERSITY OF GUELPH CHEM 4540 ENZYMOLOGY**  
**Winter 2005 Quiz #2: March 24, 2005, 11:30 – 12:50**  
**Instructor: Prof R. Merrill**  
**ANSWERS**

**Instructions:** Time allowed = 80 minutes. Total marks = 30. This quiz represents 15% of the final grade. Please write all your answers in ink (not red ink). No examination materials may be removed from the examination room.

*All answers are to be written on the examination paper.*

**Part A. “Multiple-choice” questions; circle the letter corresponding to the best answer. Ten (10) questions x 1 mark per question = 10 marks total. No marks will be deducted for incorrect answers.**

1. Both water and glucose share an —OH that can serve as a substrate for a reaction with the terminal phosphate of ATP catalyzed by hexokinase. Glucose, however, is about a million times more reactive as a substrate than water. The best explanation is that:
  - A) glucose has more —OH groups per molecule than does water.
  - B) the larger glucose binds better to the enzyme; it induces a conformational change in hexokinase that brings active-site amino acids into position for catalysis.
  - C) water normally will not reach the active site because it is hydrophobic.
  - D) water and the second substrate, ATP, compete for the active site resulting in a competitive inhibition of the enzyme.
  - E) the —OH group of water is attached to an inhibitory H atom while the glucose —OH group is attached to C.
  
2. A sequential enzyme mechanism is:
  - A) one in which some substrate(s) must become bound to the enzyme and then some product(s) released before other substrate(s) become bound and other products released.
  - B) one in which all of the substrates must become bound to the enzyme before any product is released.
  - C) one in which one substrate reacts with the enzyme to form an acyl-enzyme intermediate.
  - D) one in which the order of substrate addition is obligatory.
  - E) none of the above
  
3. The equation for the analysis of binding data by fluorescence is:
  - A)  $\Delta F = \Delta F - K_L/[L]$
  - B)  $\Delta F = \Delta F_{\max} - K_L[L]/\Delta F$
  - C)  $\Delta F = \Delta F_{\max} - K_L/[L]$
  - D)  $\Delta F = \Delta F_{\max} - K_L (\Delta F/[L])$
  - E) none of the above.

4. Which of these is not a known function of vertebrate alcohol dehydrogenase:
- A) involvement in retinol oxidation.
  - B) participation in hydroxysteroid oxidation.
  - C) role in neuromediator metabolism.
  - D) defense against exogenous alcohols.
  - E) production of  $\text{NAD}^+$  for glycolysis.
5. How is substrate specificity determined by chymotrypsin?
- A) interaction of the active site amino acids with the substrate
  - B) binding of the N-terminus amino acid at the active site
  - C) covalent binding of a His residue to the substrate
  - D) conformational change upon binding of substrate
  - E) binding of the proper amino acid into a deep pocket on the enzyme
6. For an enzyme that has 2 equivalent binding sites per enzyme molecule the expression in terms of fractional saturation is:
- A)  $Y = [L]/([L] + K)$
  - B)  $Y = 2[L]/K + 2[L]/K^2/(1 + 2[L]/K + [L]/K^2)$
  - C)  $Y = 2[L]/([L] + 2K)$
  - D)  $Y = 2[L]/([L] + K)$
  - E) none of the above
7. Which of the following does not apply to the proximity effect in enzyme catalysis.
- A) intramolecular reactions are faster than intermolecular reactions
  - B) binding substrates increases their local concentration on the surface of the enzyme
  - C) entropy decreases upon binding of substrates to the enzyme
  - D) accelerates the reaction through the transient formation of an enzyme-substrate bond
  - E) increases the reaction rate by 1 - 2 orders of magnitude
8. An enzyme exhibits maximum activity at  $\text{pH} = 10.1$ . The enzyme also shows a fairly sharp decrease in its activity when the  $\text{pH}$  goes much lower than 9.5. One likely interpretation of this  $\text{pH}$  activity is that:
- A) the reaction relies on electrostatic catalysis
  - B) a Tyr residue on the enzyme is involved in the reaction
  - C) a Cys residue on the enzyme is involved in the reaction
  - D) the enzyme is found in alkaline bile secretions
  - E) the enzyme has a metallic cofactor
9. Metal ion catalysis is facilitated by any of several mechanisms, including:
- A) electrophilic activity which stabilizes negative charges on an intermediate
  - B) promoting formation of nucleophiles by affecting adjacent molecules
  - C) direct binding to substrate and increasing substrate:enzyme contacts
  - D) a and c
  - E) all of the above

10. For the protease papain, the tripeptide Ala-Phe-Arg is a powerful inhibitor of the enzyme because:
- A) it binds to a site distinct from the active site (noncompetitive inhibitor)
  - B) it occupies the subsites,  $S_1$ ,  $S_2$ , and  $S_3$  and cannot undergo hydrolysis
  - C) it covalently modifies an active site residue within the enzyme
  - D) it is a transition state analogue for the enzyme
  - E) none of the above

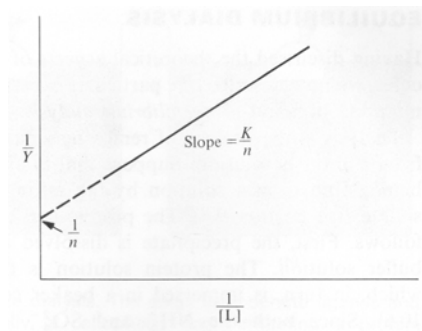
**Part B. “Short answer/” questions. Answer the following (six) questions with a short answer consisting of a few sentences or less (your answer can be in point form). Each question is worth 2 marks. Six (6) questions x 2 marks each = 12 marks total.**

11. Define the term “suicide substrate” and give an example.

A suicide substrate is a substrate that when acted upon by an appropriate enzyme is converted into a product that essentially irreversibly inactivates the enzyme usually by covalent modification.

An example given in class is that of difluoromethylornithine (DMFO) which reacts covalently with the enzyme, ornithine decarboxylase to form an inactive enzyme.

12. Sketch a Hughes-Klotz plot that is used for the analysis of binding data. Be certain to label the axes and indicate the parameters that can be obtained from the slope and y-intercept.



13. Define and explain the importance of “electrostatic interactions” in enzyme catalysis.

Electrostatic interactions involve the stabilization of the distribution of electrical charge in transition states by strategically positioned charged residues within the active site of an enzyme. These types of interactions are very important for stabilizing the transition state for a reaction and hence lead to large increases in the catalytic rate of the reaction. An example would be to locate a Lys residue near the oxygen atom of a carbonyl group to favour the formation of the tetrahedral intermediate for the reaction.

14. Why is the detection of reaction intermediates important for the understanding of enzyme reactions? How can these intermediates be identified?

In order to elucidate a reaction mechanism, one must first know the identity and structure of all the substrates, intermediates and products for that reaction. It is then necessary to determine the order of appearance and disappearance of those intermediates in the reaction pathway. Sometimes these intermediates are stable enough to be characterized by using conventional chemistry but often reaction intermediates are transient and unstable in nature. In order to identify and study these short-lived intermediates one must use approaches such as chemical trapping and various types of spectroscopy (NMR, absorbance, fluorescence, infrared, etc.).

15. Why is caution needed in order to interpret chemical modification experiments conducted on enzymes?

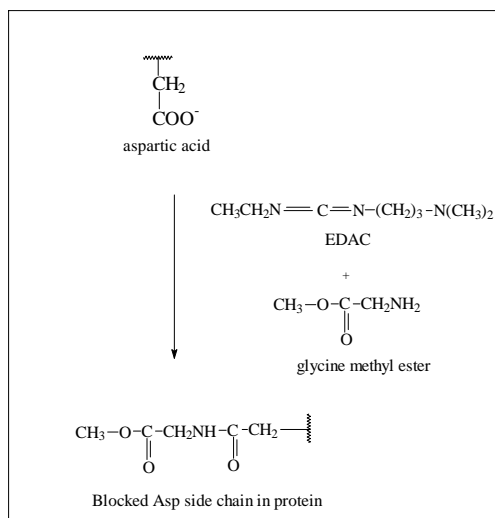
Caution is a necessary when interpreting chemical modification experiments because many amino acids are nucleophilic in nature and so that an electrophilic reagent will react with any number of side chains (residues) in proteins such as Cys, Lys, His, and Tyr. It is often a matter of relative reactivity of these amino acid residues with the reagent to be employed. The simple fact is that no reagent is completely specific for one type of residue. Another complication is the interpretation of the results since modification of a given residue type may compromise the structural integrity of the folded enzyme and not act to simply block a catalytic residue.

16. Discuss the biochemistry associated with ethanol ingestion by humans.

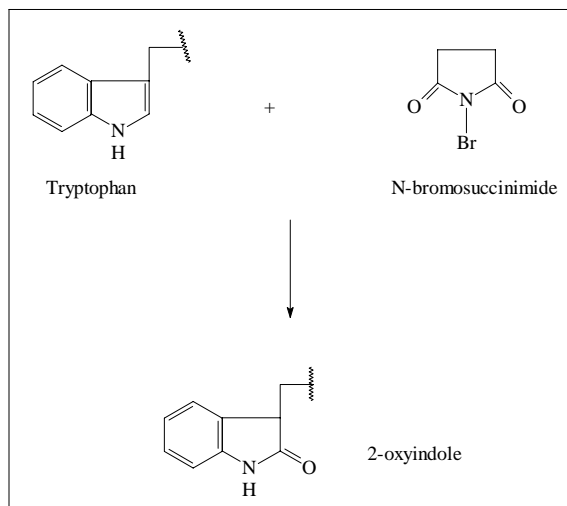
- intake of even moderate amounts of ethanol generates too much NADH
- many enzymes involved in gluconeogenesis and fatty acid oxidation are sensitive to product inhibition by NADH
  - during ethanol metabolism the results are fasting hypoglycemia and accumulation of hepatic triacylglycerols (fatty liver)
  - lactate may also accumulate due to inhibition of lactate gluconeogenesis
  - TCA cycle and GTP synthesis are inhibited by high NADH levels
  - much of the acetate made from ethanol escapes into the blood
  - acetaldehyde may also escape from the liver and forms covalent bonds with many proteins
  - hypoglycemia can affect the certain regions of the brain concerned with temperature regulation, which can lower the body temperature by 2°C
    - practice of feeding a person suffering from hypothermia brandy or whiskey is counterproductive
    - alcohol creates a sense of warming through vasodilation but this causes further heat loss

**Part C. Chemical Modification Questions. Answer the following two (2) questions. Give the reaction (amino acid residue, reagent and adduct/product) for the following. Be certain to draw structures. Two (2) x 2 marks each = 4 marks.**

17. EDAC (carbodiimide) with Asp



## 18. N-bromosuccinimide with Trp

**Part D. Problem Questions. Answer the following two (2) questions. Two (2) x 2 marks each = 4 marks.**

19. The enzymatic activity of lysozyme is optimal at pH 5.2 and decreases above and below this pH value. Lysozyme contains two amino acid residues in the active site essential for catalysis: Glu35 and Asp52. The pK value for the carboxyl side chains of these two residues are 5.9 and 4.5, respectively. What is the ionization state of each residue at the pH optimum of lysozyme? How can the ionization states of these two amino acid residues explain the pH-activity profile of lysozyme?

ANS: For the enzyme to be active, it is likely that Asp52 is unprotonated and Glu35 is protonated. When the pH is below 4.5, Asp52 becomes protonated, and when it is above 5.9, Glu35 is deprotonated, either of which decreases the activity of the enzyme.

20. What are transition state analogues and why do they generally show competitive inhibition for a given enzyme-catalyzed reaction? Why do they also exhibit high binding affinity to the enzyme?

The TS analogues are compounds that are structurally related/similar to the transition-state species (intermediate) for a given chemical reaction. However, these compounds are sufficiently different from the real transition state species that they do not react when bound to the enzyme to form a product(s). Thus, these TS analogues will compete with the substrate(s) and any corresponding intermediates that develop from the substrates during the reaction. Since enzymes are designed/have evolved to recognize the transition state species for a reaction, then they will also bind the TS analogues more tightly (higher affinity, lower  $K_D$  values) than either the substrates or the products.