

Chapter 11 Answers Enzymatic Catalysis

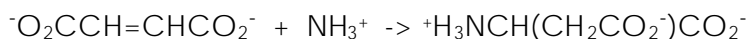
Book study questions: 2, 4, 5, 6 through 10 Book problems 1 (good question), 2 (not so much the drawing as the second part of the question. 3, 5, 8, 9, 12... Actually I like most of these questions. Also I will have a couple of general reactions and you will need to determine which is which. Finally if we don't get far enough, the protein regulation questions will not be on this test. Don't feel bad, we will get to them later.

1) Which of the following is not one of the in catalytic mechanism discussed.

- structural flexibility
- electrostatic effects
- general acid and general base catalysts
- hydrophobic effects ****
- covalent catalysts

2) What is meant by induced fit? *Induced fit implies a shape change in an enzyme upon interacting with its substrate*

3) The enzyme that catalyzes the following belongs to which enzyme class?



- oxidoreductase
- transferase
- hydrolase
- isomerase
- lyase *****

4) The rate of an enzymatic catalyzed reaction

- Increases as the temperature increases
- Decreases as the pH increases
- Is not affected by changes in enzyme concentration
- Is a direct function of the temperature
- None of the above *****

5) Describe the similarities and differences between organic/general chemical catalysts and biological catalysts.

You would need to talk about the specificity of the reaction for both the substrate (reactants) and the products (stereo specificity and lack of isomers) The regulation of enzymes as catalysis. The means of the reaction mechanisms no real strong acid or bases in these solutions rather the use of enzymes.

6) Given the sugar hexamer Nag Nam Nag Nam Nag Nam and the binding sites A, B, C, D, E and F of lysozyme, identify which of the following statements are true.

- a. Any segment of this sugar polymer having more than 3 sugars would bind with the same affinity as the hexamer.
- b. Ring strain on the first Nag aids in catalysis of the polymer.
- c. The carbonium ion intermediate is stabilized by the additional positive charges of surrounding amino acids.
- d. The hexamer would be cleaved between sugars 4 and 5 from the Nam end (left side) by a general acid catalysis mechanism.

7) What are the limitations of using NAG3 for mapping the binding interactions with lysozyme. How can you take advantage of these limitations?

- The true substrate for lysozyme is alternating NAG and NAM sugars not 3 NAG in a row. The NAG3 is too short to fit properly into the active site and does not fully react with lysozyme. This lower rate of reaction allows for the crystal structure of the enzyme and location/characterization of the active site.

8) The proposed mechanism for lysozyme requires that Asp 52 be charged and Glu35 be neutral at pH 5.

- Why - This permits one residue (glu35) the ability to donate a H and the other to act as a stabilizer of the carbonium ion intermediate

- What percentage of the asp side chain groups would be in the charged form? (Henderson Hasselbach) Assume the pKa of asp52 is 4.25.

pH = pK + log [A-]/[HA] - we use pH 5 because that is the listed pH above where it is optimally active at pH 5

$$5.0 = 4.25 + \log[\text{COO}^-]/[\text{COOH}]$$

$$0.75 = \log[\text{COO}^-]/[\text{COOH}]$$

$$5.6 = [\text{COO}^-]/[\text{COOH}] - \text{for every } 5.6 \text{ CoO}^- \text{ there is } 1 \text{ COOH,}$$

$$\text{so } \text{COO}^- + \text{COOH} = 6.6 \text{ and } 5.6/6.6 \times 100 = 84.8\% \text{ is in the } \text{COO}^- \text{ form}$$

- Do the same reaction for free glutamate pKa 3.8 and glutamate 35 with a pKa of 5.2

$$5.0 = 3.8 + \log[\text{COO}^-]/[\text{COOH}]$$

$$1.2 = \log[\text{COO}^-]/[\text{COOH}]$$

$$15.8 = [\text{COO}^-]/[\text{COOH}]$$

$$\text{for } pK_a = 3.8 : 94.0\% \text{ is in the } \text{COO}^- \text{ form}$$

$$\text{for } pK_a = 5.2 : 38.7\% \text{ is in the } \text{COO}^- \text{ form}$$

- describe how it is possible for glu35 in lysozyme to bind in the un-ionized form

The glu is in a hydrophobic environment. This leads to an increase in the pK so that the H is less likely to be dissociated. This is shown in the last problem where the pK is raised from 3.8 to 5.2 the major form of the Glu shifts from charged to neutral.

9) The enzyme RNaseA is inhibited by treatment with iodoacetate. This observation shows the importance of which amino acid side chain is in the catalytic site of RNaseA?

Val

Lys

His ***
Ser,
Arg

10) The enzyme Ribonuclease A cleaves

- a) The P-O bonds on the 5' carbon of ribose in RNA ****
- b. The P-) bonds on the 3' carbon of ribose in RNA
- c. Peptide bonds
- d. The bases from RNA
- c. Phosphodiester bonds in DNA

11) Serine proteases form a covalent intermediate with their substrates. This intermediate is:

- a. an amide involving the carboxyl group of the serine
- b. an amide involving the amino group of the serine
- c. an ester involving the carboxyl group of the serine
- d. an ester involving the alcohol group of the serine *****
- e. none of the above

12) Which of the following is a NOT catalytic mechanism.

- a) structural flexibility
- b) electrostatic effects
- c) general acid and general base catalysts
- d) proximity effects
- e) covalent catalysts

13) Lysozyme utilizes glutamate and aspartate in it's reaction mechanism.

Which of the following statements about these amino acids is true?

- a) Since both amino acids are ionized, they act as a general base and extract a proton from the substrate
- b) The charged aspartate acts to stabilize the carbonium intermediate
- c) Glutamate is unusually protonated and can act to donate it's proton to the glycosidic bond of the substrate
- d) b and c
- e) all of the above
- f) none of the above

14) The ring strain/distortion found in the reaction catalyzed by lysozyme:

- a) is an obvious demonstration of induced fit
- b) results in a energy state which resembles that of the product
- c) increases the formation of the carbonium ion
- d) involves the free radical generation of oxygen which breaks the C=N bond
- e) includes the coordination of zinc which will activate the water for a nucleophilic attack

15) Which of the following enzymes would be inhibited by a serine modifying agent?

- a) chymotrypsin
- b) protien kinase C

- c) ribonuclease
- d) lysozyme

16) Zn^{+2} is often involved in

- a) stabilizing the negative charges found in ATP
- b) altering the pKa of histadine
- c) bound to aspartate
- d) the activation of water to make it behave more like an OH^-

17) A zymogen is

- a) an active by-product
- b) and active product
- c) an inactive precursor
- d) an active precursor
- e) none of the above

a and c

18) Draw a pH enzyme activity curve for an enzyme (NOT lysozyme) that you would expect to find if the amino acids were lysine (pKa = 10.8) and histadine (pKa = 6.0). Where histadine acts as a general base and lysine stabilizes a negatively charge transition state.

19) What would be the result of replacing a histadine with a serine residue in the catalytic triad of chymotrypsin?

20) Cyclic AMP dependent protein kinase (PKA):

- a) is activated by binding cAMP to its catalytic subunits.
- b) hydrolyzes cAMP to 5'AMP.
- c) has regulatory subunits which bind cAMP. ****
- d) Acts as an inhibitor of gene transcription.
- e) is a single subunit enzyme whose regulatory domain is adjacent to the catalytic domain

21)Protein kinases:

- a)transfer a phosphoryl group from one protein to another
- b)use AMP as a substrate
- c) (PKC and PKA) Ser, Thr or (PTK)Tyr ***
- d)transfer the alpha phosphate of ATP
- e)are located on the external surface of cells

22) True/ False: The extracellular portion of Src like kinases arethought to bind growth factors *False*

23) Which protein kinase is activated by calcium and lipids:

- a) protein kinase A.

- b) protein kinase B.
- c) protein kinase C. ****
- d) protein kinase D
- e) tyrosine protein kinase

- 25) An enzyme that inhibits its activity through a pseudo substrate does so by:
- a) listening to Phil Collin's side B of Susuidio.
 - b) binds a look a like substrate to the regulatory domain. ****
 - c) interacting the pseudo substrate at the catalytic site.
 - d) phosphorylating a different protein and thereby inactivating it.

26) Protein kinase activation of an enzyme is reversed by what?
Protein phosphatases

27) What stabilizes activation by protein kinase phosphorylation?
*Greater resonance stabilization of the products than the reactants, lower free energy of the products.
reaction is not likely to happen.*