Examination I Key

PHRM 836 – Biochemistry for Pharmaceutical Sciences II

September 26, 2012

Correct answers in multiple choice questions are indicated in RED and underlined.
Correct answers to essay questions are indicated in RED in comic book font.
In some cases and explanation is provided in BLUE/BLUE

MULTIPLE CHOICE. For problems 1 to 27, select from the list immediately following each question the single most correct choice to complete the statement, solve the problem, or answer the question. Mark that answer on your answer sheet. [3 points each]

1. What is the net charge of a peptide with the following sequence at a neutral pH?

   LKEFSLIKVDPGSIFMYRFKSDF The amino acid with a charged side chain are colored in blue.

   ① –3
   ② –2
   ③ –1
   ④ 0
   ⑤ +1 +4 from K, K, R, K and –3 from E, D, D.
   ⑥ +2
   ⑦ +3

2. Hemoglobin has an ion pair between a histidine residue and an aspartate residue. This ion pair breaks upon binding of oxygen to hemoglobin. As a result, the pK_a value of the histidine residue _____, and the pK_a value of the aspartate residue _____.

   ① decreases; decreases
   ② decreases; does not change
   ③ decreases; increases
   ④ increases; decreases
   ⑤ increases; does not change
   ⑥ increases; increases

   When the ion pair is intact, the neighboring negative charge of Asp makes His maintain its positively charged state better, which increases its pK_a. Likewise, the neighboring positive charge of His in the ion pair makes Asp stays in its negatively charged state better, which decreases its pK_a. When the ion pair breaks, the two pK_a values go back to its normal value. Therefore, the pK_a value of His decreases, and that of Asp increases.

3. The level of glycosylated hemoglobin (HbA_{1C}) is used clinically as an indicator of the concentration of _____ in the blood.

① cholesterol
② thrombin
③ platelet
④ glucose
⑤ ATP
⑥ proteins
⑦ prostaglandins

The glycosylation occurs nonenzymatically between the N-terminal amine group of hemoglobin and glucose in the blood.

4. Hsp90 is an important target for cancer therapy because _____.

① protein folding is very efficient in cancer cells.
② proteins do not form disulfide bonds effectively in cancer cells.
③ mutations in oncogenic proteins essential for cancer survival interfere with their folding.
④ cancer cells have higher temperature than normal cells.
⑤ cancer cells do not produce sufficient ATP.
⑦ Hsp90 is an important player in DNA replication.

Hsp90 is a chaperone that helps folding of other proteins. Due to the destabilizing mutations in oncogenic proteins, cancer cells are strongly dependent on the chaperone activity of hsp90.

5. Which of the following statements on serine proteases is NOT correct?

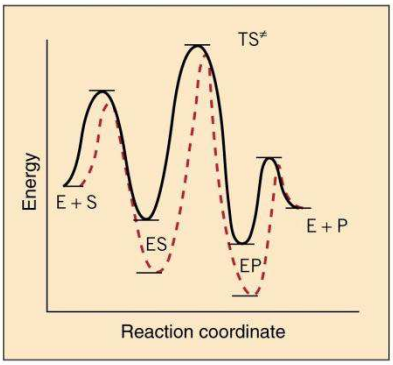
① A serine residue in the active site functions as a nucleophile for amide hydrolysis.
② The catalytic triad formed with Ser, His, and Asp is conserved.
③ Zymogens are activated by proteolysis.
④ Physiological functions of serine proteases include coagulation and digestion.
⑤ The chemical complementarity between the active site and the substrate determines the substrate specificity.
⑥ All serine proteases have a conserved structure similar to trypsin.

Some serine proteases including subtilisin have a structure distinct from trypsin but still have the typical catalytic triad, which is an example of convergent evolution.

6. The Hill coefficient for the binding of oxygen to myoglobin is 1.0. What can we learn from this?

① Oxygen binding to myoglobin has negative cooperativity.
② Oxygen binding to myoglobin has positive cooperativity.
③ Oxygen binding to myoglobin has no cooperativity.
④ One oxygen molecule binds to one myoglobin.
⑤ Myoglobin achieves 50% saturation when the partial pressure of oxygen is 1.0 atm.
⑥ The change in the net charge of myoglobin upon oxygen binding is 1.0.

7. The diagram shows the energy of a reaction catalyzed by a wild-type enzyme (black solid line) and a mutant enzyme (red dashed line). Which of the following statements is NOT correct?

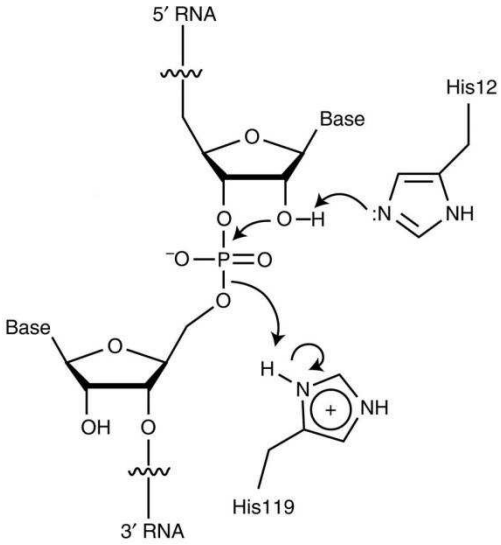


① The mutant binds the substrate (S) more tightly than the wild type
② The mutant binds the product (P) more tightly than the wild type
③ The substrate release from ES is slower in the mutant than in the wild type. The barrier from ES to E + S is higher in the mutant.
④ The product release from EP is slower in the mutant than in the wild type. The barrier from EP to E + P is higher in the mutant.
⑤ The mutant stabilizes the transition state better than the wild type. The energy of the transition state (TS*) is unchanged in the mutant.
⑥ The conversion from the substrate (S) to the product (P) is slower in the mutant than in the wild type. The barrier from ES to EP is higher in the mutant.

8. Ribonuclease A cleaves RNA through a transphosphorylation reaction shown below. In this catalytic mechanism, His12 functions as a _____, and His 119 functions as a _____.

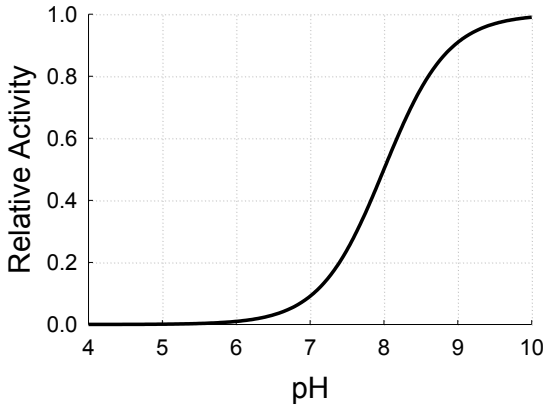
① general acid; general acid
② general acid; general base
③ general acid; nucleophile
④ general base; general acid
⑤ general base; general base
⑥ general base; nucleophile

His12 removes a proton from the 2'-OH group of RNA (general base), and H119 provide the leaving group with a proton (general acid).



9. The catalytic activity of an enzyme is determined at various pH. The enzyme is fully active at alkaline pH but loses its activity at acidic pH. The pH profile shown below tells us that the reaction is catalyzed by a _____ whose pK_a value is _____.

① general acid; 6
② general acid; 8
③ general acid; 10
④ general base; 6
⑤ general base; 8
⑥ general base; 10



A general base is functional only when it is not protonated. As the pH is decreases, the general based is protonated, and the enzyme loses its catalytic activity. According to Henderson-Hasselbach equation, when pH = pK_a, half of the enzyme is in the active form. In this case, 50% of activity is achieved at pH 8, which is the pK_a of the general base.

10. Which of the following statements is NOT correct on enzyme kinetics? The Michaelis-Menten equation is given for your information.

$$v_0 = \frac{V_{max}[\mathrm{S}]}{K_m + [\mathrm{S}]} = \frac{k_{cat}[\mathrm{E_t}][\mathrm{S}]}{K_m + [\mathrm{S}]}$$

① V_{max} is linearly proportional to the total enzyme concentration.
② V_{max}/K_m is linearly proportional to the total enzyme concentration.
③ When [S] << K_m, v_0 is linearly proportional to the substrate concentration.
④ When [S] >> K_m, v_0 is independent of the substrate concentration.
⑤ When [S] = K_m, v_0 is half of V_{max}.
⑥ k_{cat} is calculated by dividing V_{max} with the total substrate concentration.

$V_{max} = k_{cat}[E_t]$.
Therefore, to calculate k_{cat} from V_{max}, you need to divide V_{max} with the total enzyme concentration.

11. An enzyme catalyzes a reaction that involves two substrates A and B, which bind to the enzyme sequentially for catalysis. A kinetic study with a newly found inhibitor for this enzyme shows that following results. According to the results, the inhibitor is _____ with respect to A and _____ with respect to B.

Substrate concentrations		Inhibition
A	B	
Low	Low	Negligible
Low	High	Negligible
High	Low	Full
High	High	Negligible

① competitive; competitive
② competitive; uncompetitive
③ competitive; noncompetitive
④ uncompetitive; competitive
⑤ uncompetitive; uncompetitive
⑥ uncompetitive; noncompetitive

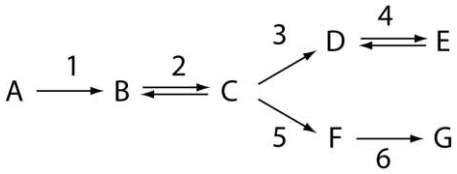
As the inhibition occurs when the enzyme is saturated with A, the inhibitor is uncompetitive with respect to A. However, the inhibition becomes negligible when the concentration of B is high, Therefore, the inhibitor is competitive with respect to B.

12. Individuals sensitive to alcohol have _____.

① a variant form of alcohol dehydrogenase with a greater k_{cat} value.
② a variant form of alcohol dehydrogenase with a greater k_{cat}/K_m value for NAD^+.
③ a variant form of alcohol dehydrogenase with a greater K_m value for NAD^+.
④ a variant form of aldehyde dehydrogenase with a greater k_{cat} value.
⑤ a variant form of aldehyde dehydrogenase with a greater k_{cat}/K_m value for NAD^+.
⑥ a variant form of aldehyde dehydrogenase with a greater K_m value for NAD^+.

The alcohol sensitivity is due to the insufficient conversion of acetaldehyde to acetate. The individuals with alcohol sensitivity have a sluggish aldehyde dehydrogenase. The greater K_m for NAD^+ results in a slower enzymatic activity with a given concentration of NAD^+. An enzyme with greater k_{cat} or k_{cat}/K_m would catalyze the reaction more efficiently.

13. The following diagram shows the metabolic pathway to generate E and G from the precursor A. Which step would be the most effective step for a feedback inhibition to control the synthesis of G?



① 1
② 2
③ 3
④ 4
⑤ 5 A irreversible commitment step
⑥ 6

14. Which of the following statements is NOT correct on non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenases (COX)?

① NSAIDs block cholesterol synthesis. NSAIDs block the synthesis of prostaglandin, which induces inflammation.
② NSAIDs irreversibly or reversibly inhibit COX.
③ Ibuprofen inhibits COX-1 and COX-2 nonspecifically.
④ The nonspecific inhibition of COX-1 and COX-2 may result in side effects.
⑤ Celecoxib is a COX-2 selective inhibitor.

15. Which of the following statements is NOT correct on aromatase?

① Aromatase is a cytochrome P450.
② Aromatase converts 17-β estradiol to testosterone. Aromatase converts testosterone to 17-β estradiol, which contains an aromatic ring structure.
③ Aromatase contains a heme group.
④ The catalysis by aromatase requires O_2.
⑤ The catalysis by aromatase requires NADPH.
⑥ Aromatase inhibitors are used to treat breast cancer.

16. What is the biochemical role of cytochrome P450 reductase?

① It reduces the product generated by cytochrome P450.
② It reduces $NADP^+$ to NADPH.
③ It provides cytochrome P450 with NADPH.
④ It provides cytochrome P450 with O_2.
⑤ It relays electrons from NADPH to cytochrome P450. Cytochromes P450 do not use NADPH directly. Cytochrome P450 receives electrons one at a time from cytochrome P450 reductase.
⑥ It reduces the size of cytochrome P450.

17. What would be the most likely product from the action of cytochrome P450 on $CH_3CH_2CH_2CH_3$.

① $CH_3CH_2CH=CH_2$
② $CH_3CH_2CH_2CH_2NH_2$
③ $CH_3CH_2CH_2CH_2OH$
④ $CH_3CH_2CH_2OH$
⑤ $CH_3CH_2CH_2NH_2$
⑥ $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$

Cytochromes P450 catalyze monooxygenation reaction, which results in an addition of a hydroxyl group to a substrate.

18. You are advised by a pharmacist not to drink grapefruit juice while taking a drug. What is the main reason for this?

① Grapefruit juice makes the drug taste bitter.
② Grapefruit juice may react with the drug and form a toxic intermediate.
③ Grapefruit juice may inhibit a cytochrome P450 and slow down the metabolism of the drug.
④ Grapefruit juice may induce a cytochrome P450 and slow down the metabolism of the drug.
⑤ Grapefruit juice may inhibit a cytochrome P450 and enhance the metabolism of the drug.
⑥ Grapefruit juice may induce a cytochrome P450 and enhance the metabolism of the drug.

Inhibition of a cytochrome P450 results in slow clearance of the drugs that require the same cytochrome P450 for metabolisom. Induction of a cytochrome P450 results in an elevated clearance of the drugs that are metabolized by the cytochrome P450. Grapefruit juice contains cytochrome P450 inhibitors.

19. Which of the following statements is correct about Na^+/glucose cotransporter?

① It is a symporter.
② It is an ATPase.
③ The direction of transport is determined only by the chemical gradient of glucose.
④ It is a Na^+ channel.
⑤ It is a primary active transporter.

Na^+/glucose costransporter is a secondary active transporter, which does not require ATP.

20. Which of the following transport actions is electronically neutral?

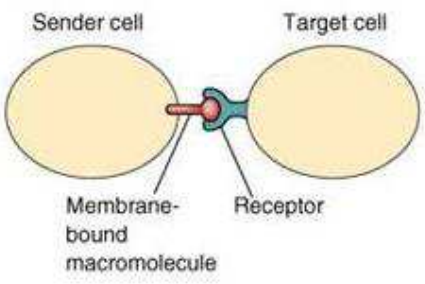
① ATP-ADP transporter Movement of one net charge (antiport of ATP^{4-} and ADP^{3-})
② Na^+/glucose cotransporter Movement of one net charge (symport of Na^+ and glucose)
③ Na^+/K^+ exchanging ATPase Movement of one net charge (antiport of 3 Na^+ and 2 K^+)
④ H^+/K^+ exchanging ATPase No movement of a net charge (antiport of H^+ and K^+)
⑤ valinomycin Movement of one net charge (uniport of K^+)

21. Overexpression of multidrug resistance protein (MDR) increases drug resistance in cancer cells because _____.

① MDR decreases drug concentration in cells by transporting drugs across the membrane.
② MDR metabolizes drugs by mono-oxygenation reaction.
③ MDR induces cytochrome P450s.
④ MDR rigidifies the membrane so that drugs cannot diffuse across the membrane easily.
⑤ MDR collapses the ion gradients.

MDR is a transporter that moves drug molecules across the membrane.

22. What is the mode of cell-cell communication shown below?



① Juxtacrine signaling The signaling molecule stays on the membrane of the sender cell.
② Endocrine signaling
③ Paracrine signaling
④ Synaptic signaling
⑤ Autocrine signaling

23. Which of the following second messengers functions at the membrane without diffusing into the cytosol?

① Ca^{2+}
② cyclic AMP
③ cyclic GMP
④ IP_3
⑤ diacylglycerol Diacylglycerol is a lipid, which is not soluble in water.

24. Which of the following descriptions on G-proteins is NOT correct?

① G-proteins function as transducing proteins.
② G-proteins are active in the GDP-bound form. G-proteins are active in the GTP-bound form.
③ G-proteins have an intrinsic GTPase activity.
④ G-proteins are activated through GTP-GDP exchange.
⑤ GTP-GDP exchange in G-proteins is induced by agonist-bound GPCRs.
⑥ ADP-ribosylation of G-proteins represses the GTPase activity.

25. G-protein-coupled receptors are commonly desensitized by _____.

① receptor internalization
② receptor degradation
③ glycosylation
④ proteolysis
⑤ phosphorylation
⑥ allosteric inhibition

26. Adenylate cyclase is activated by _____.

① α_s
② α_i α_i represses the activity of adenylate cyclase.
③ α_q α_q activates PI-PLCβ.
④ cAMP cAMP activates protein kinase A.
⑤ cGMP cGMP activates protein kinase G.
⑥ IP_3 IP_3 activates IP_3 receptor.

27. IP_3 receptor is a(n) _____.

① receptor tyrosine kinase
② G-protein coupled receptor
③ voltage-gated ion channel
④ ligand-gated ion channel
⑤ guanylate cyclase
⑥ serine/threonine kinase

ESSAY PROBLEMS. Write your answers to problems 28 to 30 in the space immediately below each problem.

28. [8 points] The following diagram shows the interactions between atovarstatin and the active site residues of HMG-CoA reductase. Please indicate what kind of chemical interaction (**H** for hydrogen bonding, **P** for hydrophobic interaction, or **E** for electrostatic interaction) between the drug and each residue contributes to the binding of the drug to the enzyme.

S684 _____H____OH and C=O_____

K692 _____E (or H)__NH_3^+ and COO^-____

D690 _____H____COO^- and OH_____

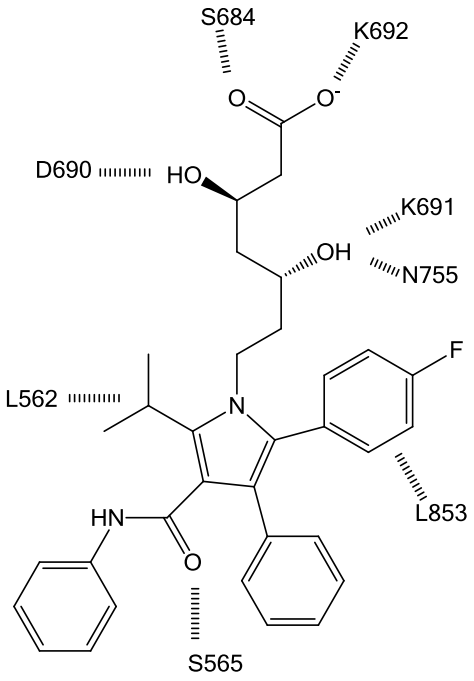
K691 _____H____NH_3^+ and OH_____

N755 _____H____$CONH_2$ and OH_____

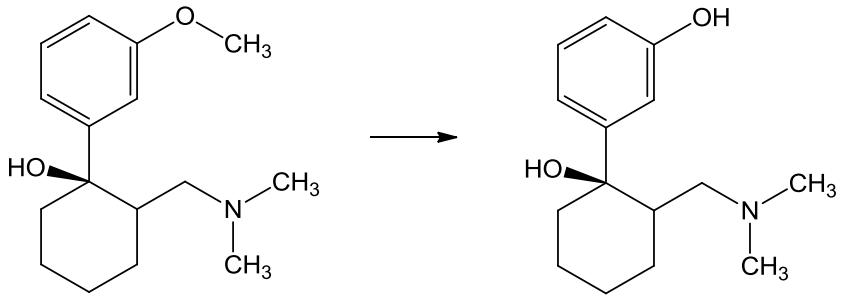
L562 _____P____aliphatic and aliphatic____

L853 _____P____aliphatic and aromatic____

S565 _____H____OH and C=O_____



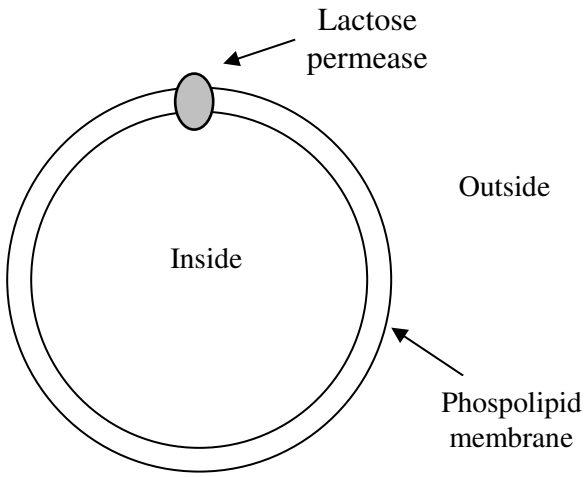
29. [5 points] Tramadol is a drug used to treat moderate to moderate-severe pain. To exert its full efficacy, tramadol needs to be converted to desmethyltramadol. How would this conversion occur in human body?



Tramadol **Desmethyltramadol**

This conversion is a dealkylation reaction, which cytochromes P450s catalyze.

30. [6 points] Bacterial lactose permeases transport lactose by using proton electrochemical gradient (symport of H^+ and lactose) under their physiological conditions. Purified *E.coli* lactose permease is used to reconstitute its activity in a vesicular phospholipid membrane as shown below. Discuss the direction of movement of lactose and the change of pH inside and outside under each given artificial condition.



a. pH(outside) < pH(inside) and [lactose](outside) = [lactose](inside).

Lactose moves inside.
pH inside decreases.
pH outside increases.

The concentration gradient of the proton drives the transport. As the proton concentration is higher outside (lower pH), both H^+ and lactose move inside.

b. pH(outside) = pH(inside) and [lactose](outside) >> [lactose](inside).

Lactose moves inside.
pH inside decreases.
pH outside increases.

The concentration gradient of lactose drives the transport. As the lactose concentration is higher outside, both H^+ and lactose move inside.

c. pH(outside) = pH(inside) and [lactose](outside) = [lactose](inside), but the inside has a negative membrane potential.

Lactose moves inside.
pH inside decreases.
pH outside increases.

The membrane potential drives the transport. As the inside has a negative membrane potential, the movement of the positive charge of H^+ inside is favorable, and both H^+ and lactose move inside.