Exam 3 Spring 2017 Dr. Stone 8:00 Name_____

There are 100 possible points on this exam.

$v_o = \frac{V_{max}[S]}{K_m + [S]}$ $\quad rate = \frac{kT[S]}{h} e^{-\Delta G^{\ddagger}/RT}$ $\quad V_{max} = k_{cat}E_t$ $\quad \Delta G^{\circ\prime} = -RT \ln K'_{eq}$

$rate_{forward} = k_{forward}[reactants]$ $\quad K_{eq} = \frac{[products]}{[reactants]}$

$rate_{reverse} = k_{reverse}[products]$

CH_3COOH Ka = 1.78×10^{-5} $\quad$ $H_2P0_4^{-2} = 3.98 \times 10^{-13}$

H_3P0_4 Ka = 7.25×10^{-3} $\quad$ H_2CO_3 Ka = 1.6×10^{-4}

$H_2P0_4^-$ Ka = 1.38×10^{-7} $\quad$ HCO_3^- Ka = 4.68×10^{-11}

For the reaction of water with carbon dioxide: $K_{eq} = 1.69 \times 10^{-3}$

R = 8.315 J/mole*K $\quad$ k = 1.381×10^{-23} J/K $\quad$ h= 6.636×10^{-34} J*sec.

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1. (10 points) Chymotrypsin (Mr 21,600) degrades peptides. Under saturation conditions (0.4M), the substrate glycyltyrosinylglycine(GYG), is cleaved at a rate of 1.43 moles/min, k_{cat} is 100 sec^{-1}, and Km is 108 mM. The rate of the uncatalyzed reaction is 1.2 µmoles/min. Show your work.

A. What is the enzyme concentration?

B. What substrate concentration will give a velocity that is 1/4 of Vmax?

C. What is the difference between the activation energy for the reaction catalyzed by chymotrypsin and the activation energy for the uncatalyzed reaction? Calculate the value that the activation energy is changed by the enzyme.

D. What are the products of this reaction?

Each Multiple Choice Question is worth 2 points, circle the best answer on this exam:

2. Why is SDS (sodium dodecyl sulfate) added to the acrylamide gel for the electrophoresis of proteins?
A. To provide ampholytes to enhance the separation of charged proteins
B. To denature the proteins
C. To add a uniform amount of negative charges
D. To add a uniform amount of positive charges
E. To denature the polyacrylamide
F. None of these
G. More than one of these, **circle all** correct answers

3. Which of the following is true about the Edman degradation system of sequencing polypeptides?
I) The Edman degradation system is carried out on a machine called an Edmanator.
II) The Edman degradation system will work on any size polypeptide.
III) In the Edman degradation system the amino-terminal residue is labeled with 1-fluro,2,4-dinitrobenzene and the polypeptide is hydrolyzed with 6M HCl to its constituent amino acids.
IV) In the Edman degradation system the amino-terminal residue is labeled with phenylisothiocyanate, cleaved with trifluroacetic acid, purified and identified in each successive cycle.

A. I and II B. II and IV C. I, II and III D. IV only E. none of these

4. Why is a peptide treated with dithiothreitol and iodoacetate prior to SDS PAGE?
A. This breaks the crosslinks in collagen
B. This oxidizes the disulfide bonds to form iodosulfoxides
C. This oxidizes tryptophan so it can be measured by UV absorbance
D. This reduces disulfide bonds and acetylates the resultant thiol groups
E. None of these

5. What is isoelectric focusing used for?
A. Determine the pI of a protein
B. Determine the pKa of a protein
C. Separate proteins based on their molar mass
D. Separate proteins based on their hydrophobicity
E. None of these
F. More than one of these, circle **all correct** answers

6. What effect does carbonic anhydrase have on the equilibrium concentrations of carbon dioxide, water and carbonic acid?
I. Carbon dioxide increases
II. Carbon dioxide decreases
III. Carbonic acid increases
IV. Carbon dioxide does not change

A. I and III B. II and IV C. I, II and III D. IV only E. None of these

7. Which of the following binding constants is for a protein that has the lowest affinity for its ligand?
A. $K_a = 1.0 \times 10^{8}$
B. $K_d = 1.0 \times 10^{-10}$
C. $K_a = 1.5 \times 10^{-9}$
D. $K_d = 2.0 \times 10^{8}$
E. None of these, they are all the same

8. A simple plot of V_0 versus [S] is superior to a double-reciprocal plot ($1/V_o$ versus 1/[S]) for:
A. determining V_{max}.
B. determining the type of inhibition.
C. determining the K_m.
D. detecting allosteric regulation.
E. none of these

9. In competitive inhibition, increasing concentrations of the inhibitor will have the following effect on the kinetics of the enzyme:
A. V_{max} will stay the same.
B. K_m will decrease.
C. The reaction will cease because the inhibitor binds irreversibly.
D. K_m / V_{max} will stay the same.
E. None of these, or more than one of these, circle all correct answers

10. The steady state of an enzyme-catalyzed reaction is reached when
A. the rate of enzyme-substrate formation is constant.
B. the concentration of enzyme-substrate complex equals the concentration of product.
C. the rate of disappearance of reactant is constant.
D. the concentration of the enzyme-substrate complex is constant over time.
E. none of these

11. An enzyme-catalyzed reaction was carried out with the substrate concentration initially a thousand times greater than the K_m for that substrate. After 6 minutes, 1% of the substrate had been converted to product, and the amount of product formed in the reaction mixture was 12 mmol. If, in a separate experiment, one fourth as much enzyme and three times as much substrate had been combined, how long would it take for the same amount (12 mmol) of product to be formed?
A. 24 min B.1.5 min C.72 min D. 4.5 min E. 8 min

F. None of these, the correct answer is_____

12. What chemicals are used to only determine the N-terminus amino acid of a peptide?
A. 1-fluro-2,4-dinitrobenzene, 1.0M HCl
B. phenyl isothiocyanate, 1 M trifluoracetic acid
C. dithiothreitol, iodoacetate
D. performic acid
E. Non of these

13. (6 points) Match the following techniques with how they are used to study proteins. Write the number on the blank line.

A. SDS PAGE_____
B. mass spectroscopy_____
C. Edmund degradation_____
D. X-ray crystallography_____
E. nuclear magnetic resonance ____
F. circular dichroism spectroscopy _____

1. measure amounts of protein secondary structures
2. determine protein structure using diffraction patterns and a computer program
3. determine exact mass of proteins and the sequence of peptides
4. determine structure of a protein in solution
5 determine molar mass of denatured proteins by comparing to standards with know molar masses
6. determine the sequence of peptides

Short Answer section. Give specific details.

14. (6 points) A common theme in biochemistry is “shape change leads to function change” describe one step in the Power cycle of muscle contraction that is an example of this theme.

15. (10 points) About 20% of the carbon dioxide that is produced in the tissues (from burning fuel) is transported on the hemoglobin molecule.

A. How is carbon dioxide transported on hemoglobin? (Give specific molecular details.)

B. How many carbon dioxide molecules can bind to each hemoglobin molecule?

C. How does the other 80% of carbon dioxide move from the tissues to the lungs?

D. During hyperventilation, too much carbon dioxide leaves the lungs, what effect will this have on the function of hemoglobin? Will the hemoglobin deliver more or less oxygen to the tissues? Explain your answer.

16. (10 points) Fully describe the enzyme mechanism that your group presented during the parade of mechanisms. Be sure to include the types of catalysis that are involved. Be specific and give details.

17. (12 points) We studied Chymotrypsin (Mr 21,600) in detail in lecture.

A. What three amino acids are part of the catalytic triad?

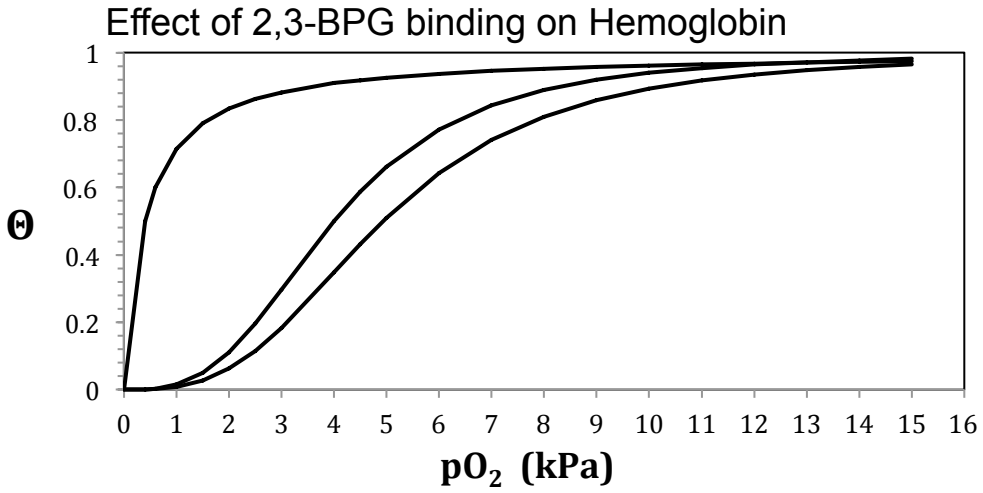
B. What does the catalytic triad do in the enzyme mechanism?

C. What is the function of the hydrophobic pocket?

D. What is the function of the oxyanion hole?

18. (12 points) At high altitudes a person can become light headed, fatigued and nauseous. These effects are exacerbated upon exertion, and can be very dangerous. This effect is due to a lower concentration of oxygen in the air at high altitudes. The body adjusts by making additional red blood cells, so more oxygen can be delivered at the tissues (each hemoglobin delivers about 30% of the oxygen that is bound to it.) But that takes several days, and who wants to wait precious vacation days before having fun on the slopes? The more immediate response (about 8- 12 hours) is for the red blood cells to increase their concentration of 2,3BPG (by diverting glucose in the glycolysis pathway.) The following graph shows the binding curves for three concentrations of 2,3 BPG.

A. Label each line for for zero, 5mM and 8 mM 2,3-BPG.



B. What does BPG do to hemoglobin? (How does it interact, what is stabilized?)

C. Compare the amount of oxygen delivered to the tissues at each concentration of BPG at sea level, the partial pressure of oxygen gas in the lungs is 12 kPa, the partial pressure of oxygen In the tissues is 4kPa.

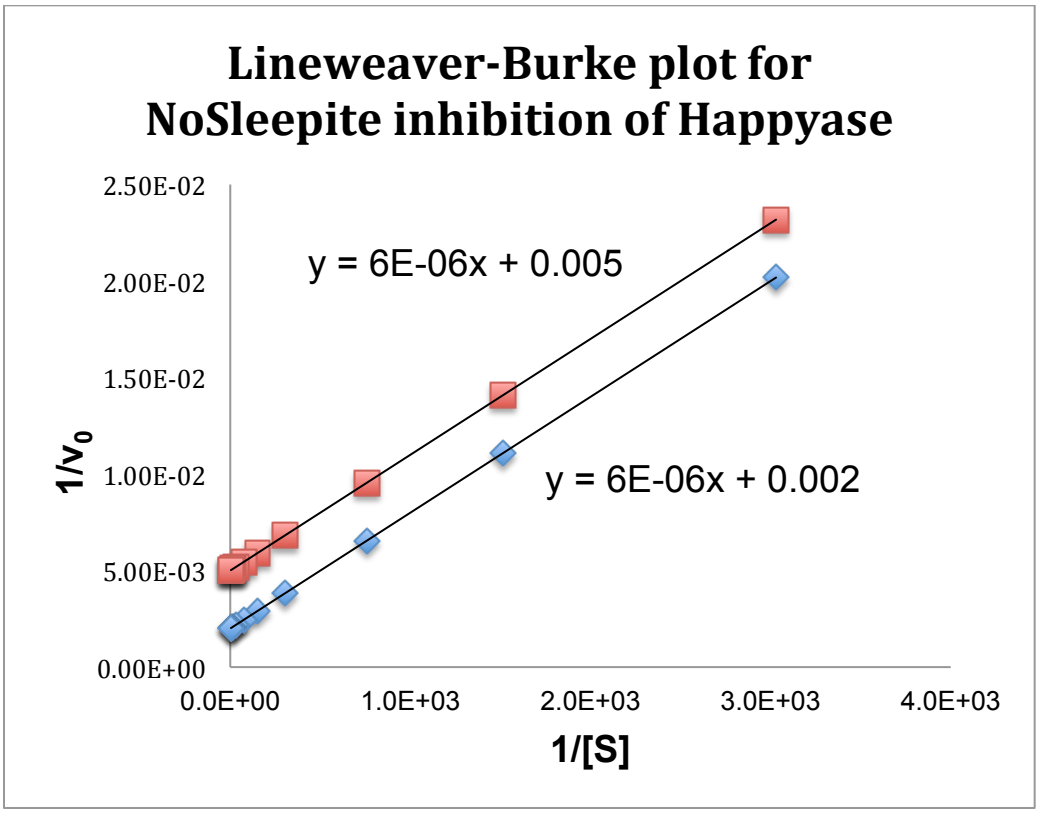
i. 5 mM 2,3-BPG _____ ii. 8 mM 2,3-BPG _____

D. Compare the amount of oxygen delivered to the tissues at each concentration of BPG at 9800 ft (the summit Kirkwood), the partial pressure of oxygen gas in the lungs is 7.3kPa.

i. 5 mM 2,3-BPG_____ ii. 8 mM 2,3-BPG _____

E. Fetal hemoglobin does not have beta chains, instead it has two gamma subunits and two alpha subunits. This fetal tetramer (HbF) does not bind 2,3-BPG. The “0 mm BPG” curve represents the oxygen binding to HbF. Why does the fetus require this type of binding curve?

19. (12 points) Happyase is a naturally occurring enzyme that reacts with sugar to form molecules that act like the active ingredient in chocolate. With Happyase, all is grand. However, Happyase is inhibited by a compound called Nosleepite. An experiment was done to measure the effect of Nosleepite on Happyase and the date is shown in the following graph. (You need to determine which line is for the experiment with Nosleepite.) The velocity of the reaction is measured in mmol/sec, the concentrations of the substrate (Sugar) are micro Molar.



A. Complete the following table

Experiment	Km	Vmax
Happyase		
Nosleepite + Happyase		

B. What type of inhibition is this?

C. Does the inhibitor bind to the enzyme, the enzyme-substrate complex, or both?