



**University of Alberta
Department of Biochemistry**

NAME: KEY

CLEARLY WRITE YOUR I.D. # ON EACH PAGE INSIDE

University of Alberta
Department of Biochemistry
BIOCH 320
Midterm Examination II
Dr. J. Parrish

Time Limit: 80 minutes

March 20, 2014

INSTRUCTIONS

- Enter your **NAME** on this cover page and your **I.D.#** on each page inside.
- This exam has 10 pages with 44 marks worth 20% of your final grade.
- Ensure all multiple choice questions are completed on the scantron sheet.
- Ensure your scantron sheet has your **NAME** and **I.D.#**

Amino Acid		pK ₁	pK ₂	pK _R
Ala	A	2.35	9.78	
Arg	R	1.82	8.99	12.48
Asn	N	2.14	8.72	
Asp	D	1.99	9.90	3.90
Cys	C	1.92	10.70	8.37
Gln	Q	2.17	9.13	
Glu	E	2.10	9.47	4.07
Gly	G	2.35	9.78	
His	H	1.80	9.33	6.04
Ile	I	2.32	9.76	
Leu	L	2.33	9.74	
Lys	K	2.16	9.16	10.54
Met	M	2.13	9.28	
Phe	F	2.20	9.31	
Pro	P	1.95	10.64	
Ser	S	2.19	9.21	
Thr	T	2.09	9.10	
Trp	W	2.46	9.41	
Tyr	Y	2.20	9.21	10.46
Val	V	2.29	9.74	

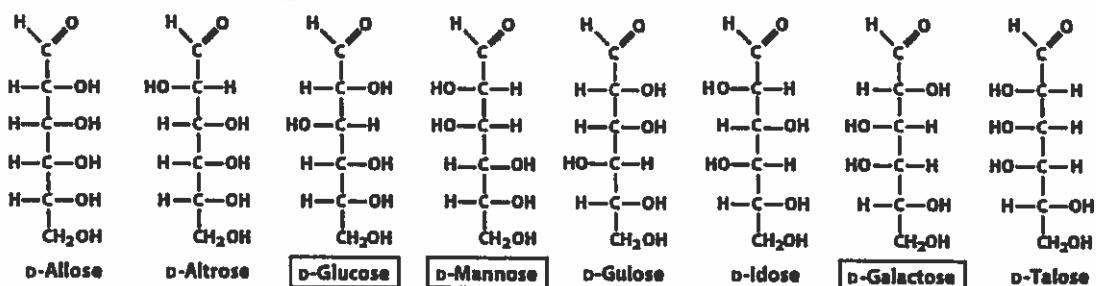
$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$\theta = \frac{k_3[E]}{k_d + [L]}$$

$$k_{cat} = \frac{V_{max}}{[E_T]}$$

$$V_0 = \frac{V_{max}[S]}{(\alpha)K_m + (\alpha')[S]}$$

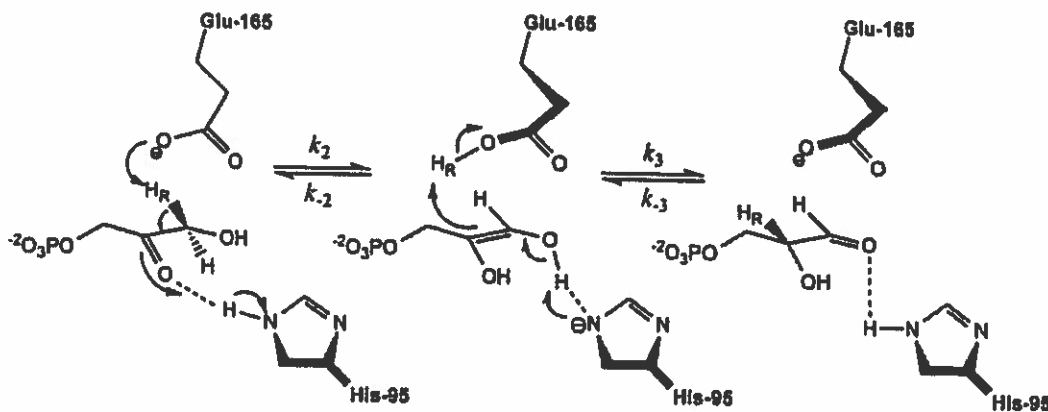
$$\text{Specificity constant} = \frac{k_{cat}}{K_m}$$



1. If a protein is a soluble globular protein, which statement(s) do we expect to be true?
- The protein will contain a prosthetic group.
 - The protein will be an enzyme.
 - The protein will contain some amount of irregular structure.
 - Both A and B are true.
 - Both B and C are true.
2. A Ni^{2+} -binding protein that is 40% bound when $[\text{Ni}^{2+}] = 2 \mu\text{M}$ has a K_d equal to:
- $3.0 \mu\text{M}$
 - $4.0 \mu\text{M}$
 - $5.0 \mu\text{M}$
 - $8.0 \mu\text{M}$
 - $38.0 \mu\text{M}$

$$0.40 = \frac{2 \mu\text{M}}{K_d + 2 \mu\text{M}} \quad K_d = 3.0 \mu\text{M}$$

Use the following diagram to answer the next question. The enzyme mechanism below is for an enzyme that we did not cover in class.



3. Which of the following statements is true for the above mechanism?
- This enzyme is an oxidoreductase. ✗
 - Histidine acts as a general base in this mechanism. ✓
 - Histidine acts as a general acid in this mechanism. ✓
 - Both B and C are true.
 - All of the above statements are true.
4. Which of the following statements is FALSE for serine proteinases?
- The backbone NH of Ser 195 forms part of the oxyanion hole. ✓
 - The oxyanion hole stabilizes a negatively-charged intermediate. ✓
 - During the mechanism, His 57 acts as both a general acid and a general base. ✓
 - These enzymes are hydrolases. ✓
 - Asp 194 modulates the pKa of His 57. ✗

5. Which of the following is an example of an enzyme using covalent catalysis?
- A. Hexokinase.
 - B. Ribonuclease.
 - C. Chymotrypsin.
 - D. Both B and C.
 - E. All of these.
6. Decreasing the activation energy for a reaction will:
- A. Increase the rate of the forward reaction. ✓
 - B. Increase the rate of the reverse reaction. ✓
 - C. Decrease the rate constant for the forward reaction. X
 - D. Both A and B.
 - E. Both B and C.
7. Which of the following is characteristic of α -keratin?
- A. Secondary structure is largely helical. ✓
 - B. Contains a right-handed coiled coil. X
 - C. Contains an abundance of glycine residues. X
 - D. Both A and B.
 - E. All of the above.

Use the following information to answer the next two questions.

During purification of a protein, the composition was determined to consist of a mixture of two subunits: a 60 kDa α subunit and a 15 kDa β subunit. The intact protein is 240 kDa, and further work revealed that each subunit represented 50% of the total mass of the protein.

8. What is the ratio of the number of α subunits to β subunits in the intact protein? (α : β)
- A. 1:1
 - B. 1:2
 - C. 1:4
 - D. 2:1
 - E. 4:1
- 120kDa of each*
2 α subunits to 8 β subunits.
1:4
9. Which of the following are possible symmetry forms for this protein?
- A. C_2
 - B. D_2
 - C. C_4
 - D. D_4
 - E. Both B and C.

10. Which statement below is true for proteins associated with assisted folding?
- A. Chaperonins help prevent aggregation of unfolded proteins. ✓
 - B. Chaperonins consume ATP to change the native state of some proteins. ✗
 - C. Protein Disulfide Isomerase is important for folding of most cytosolic proteins. ✗
 - D. Proline residues will become primarily cis peptide bonds when peptide prolyl cis/trans isomerase is active. ✗
 - E. More than one of these statements is true. ✗
11. Which of the following statements are true concerning ribonuclease A?
- A. This enzyme is a hydrolase. ✓
 - B. This enzyme contains disulfide bonds. ✓
 - C. This enzyme uses histidine residues as a general base in the mechanism. ✓
 - D. Both A and C are true.
 - E. All of the above statements are true.
12. Which of the following choices completes the following sentence? "As folding of a soluble globular protein proceeds..."
- A. ...the entropy of the polypeptide chain decreases. ✓
 - B. ...the number of hydrogen bonds in regular secondary structures increases. ✓
 - C. ...molten globule states may form with disordered hydrophobic cores. ✓
 - D. Both A and C.
 - E. All of the above.
13. What role does a general base play in the mechanism of hexokinase?
- A. It is required to extract a proton from water during the mechanism. ✗
 - B. It extracts a proton from the catalytic serine. ✗
 - C. It stabilizes the high-energy transition state. ✗
 - D. It accepts a proton during nucleophilic attack of the phosphate. ✓
 - E. It is required to "induce fit" the glucose. ✗
14. Which of the following is NOT an advantage of NMR relative to X-ray crystallography?
- A. Proteins can be in solution.
 - B. Useful for capturing changes or motions in a protein structure.
 - C. Routinely used for proteins that are 200 kDa or larger.
 - D. Both A and B.
 - E. Both B and C.
15. "Connections between sequential β -strands in a parallel β -sheet are left-handed."
- A. This statement is always true.
 - B. This statement is usually true.
 - C. This statement is rarely true.
 - D. This statement is never true.

16. If a protein has D_4 symmetry, this means:

- A. There are 4 subunits in the protein.
- B. There are 4 protomers in the protein.
- C. There are 8 protomers in the protein.
- D. There must be at least 8 subunits in the protein.
- E. Both C and D

17. Which of the following is FALSE for a Michaelis-Menten enzyme in the presence of an mixed inhibitor?

- A. Apparent K_m will always increase relative to the uninhibited enzyme. ✗
- B. Apparent V_{max} will always decrease relative to an uninhibited enzyme. ✓
- C. The relationship $\alpha' > 1$ will always be true. ✓
- D. As substrate concentration increases, so does the concentration of the enzyme/substrate/inhibitor complex. ✓

18. What amino acid residues can be modified by phosphorylation?

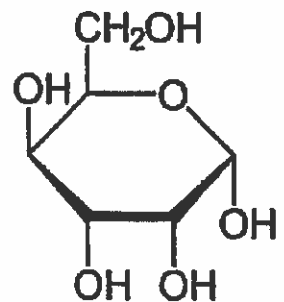
- A. Serine.
- B. Threonine.
- C. Tyrosine.
- D. Both A and B.
- E. All of these.

19. Lactose is a disaccharide with the systematic name β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucopyranose. Which statement regarding lactose is false? (1 mark)

- A. Lactose is a reducing sugar. ✓
- B. The chemical formula for lactose is $C_{12}O_{12}H_{24}$. ✗ $C_{12}O_{11}H_{22}$
- C. The anomeric carbon in the glucopyranose portion can mutarotate. ✓
- D. The glycosidic bond to the anomeric carbon in galactose would be on the same side of that ring as C6 in a Haworth projection. ✓

20. Which of the following statements is true regarding the structure shown on the right?

- A. This monosaccharide is an epimer of glucose. ✗
- B. This monosaccharide is gulose. ✓
- C. This monosaccharide is an epimer of galactose. ✓
- D. Both ~~C and D~~. Band C.
- E. All of these are true.



21. "The catalytic subunits of protein kinase A phosphorylate proteins at serine and threonine residues; the usual target sequence is [Arg-Arg-X-Ser/Thr-X], where X is a hydrophobic amino acid." Given this information, how many potential phosphorylation sites are there for this enzyme in the following sequence?

--QNRRTISLLRYYNKRWISQNLRHITHEREROSLLRAAQNRRVTISLLRELVISITHEKING--

- A. 1
- B. 2
- ~~C. 3~~
- D. 4
- E. More than 4

22. Which of the following statements is false regarding chymotrypsin activation?

- A. Chymotrypsinogen is converted into α -chymotrypsin by trypsin. ✗ π
- B. α -chymotrypsin has an active site that includes an oxyanion hole. ✓
- C. Ile 16 is able to form an ionic interaction once trypsin cleavage occurs. ✓
- D. Chymotrypsin activation is an irreversible process. ✓
- E. All of these statements are true.

Short answer questions.

1) Use the following information to answer the next two questions. A Michaelis-Menten enzyme is being studied where K_m for the substrate is $5 \mu\text{M}$ and the value of k_{cat} is 10 min^{-1} . An experiment performed with a substrate concentration of $100 \mu\text{M}$ has a V_0 measured to be 300 nM/min .

A) What is the concentration of enzyme under these conditions? Show all of your work. (4 marks)

1) V_{max} $V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$ $300 \text{ nM/min} = V_{max} \cdot \frac{100 \mu\text{M}}{100 \mu\text{M} + 5 \mu\text{M}}$

(2) $V_{max} = 300 \text{ nM/min} \cdot \frac{105}{100} = 315 \text{ nM/min}$

$k_{cat} = \frac{V_{max}}{[E_T]} \therefore [E_T] = \frac{V_{max}}{k_{cat}} = \frac{315 \text{ nM/min}}{10 \text{ min}^{-1}} = \boxed{31.5 \text{ nM}}$

~~2.15~~

$\boxed{0.0315 \mu\text{M}}$

(1) (1)

B) A competitive inhibitor is added to this experiment and the experiment repeated. With $100 \mu\text{M}$ substrate the measured velocity (V_0) is determined to be 250 nM/min . What is the value of α and/or α' ? (4 marks)

Competitive Inhibitor - No change in V_{max}

$250 \text{ nM/min} = \frac{V_{max} \cdot [S]}{\alpha \cdot K_m + [S]} = \frac{(315 \text{ nM/min}) \cdot 100 \mu\text{M}}{(5 \mu\text{M} \cdot \alpha) + 100 \mu\text{M}}$ calculations (2)

$5 \mu\text{M} \cdot \alpha + 100 \mu\text{M} = \frac{315}{250} \cdot 100 \mu\text{M}$

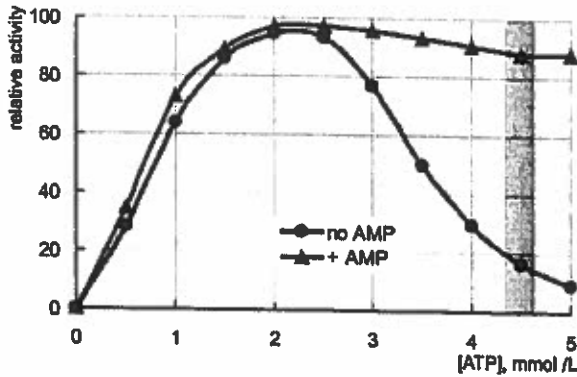
$\boxed{\alpha = 5.2}$ (2) no units (with units -1)

2) BRIEFLY explain the difference between uronic and aldonic acid derivatives for monosaccharides. (2 marks)

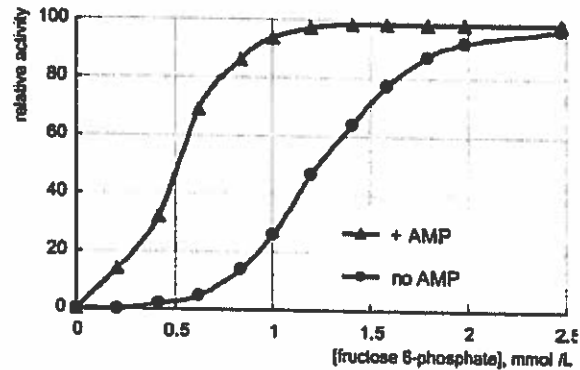
- (1) Uronic acid derivatives are oxidation (to carboxylic acid) of primary alcohols
- (1) Aldonic acid derivatives are oxidation (to carboxylic acid) of aldehyde (C1)

3) Briefly list the important differences between the symmetry and sequential models of allostery. (4 marks).

- (2) Each oligomer is in either T or R state in symmetry model, each subunit is either T or R state in sequential model.
- (2) Overall symmetry is maintained in symmetry model, not necessarily maintained in sequential model.



Experiment 1



Experiment 2

4) Above are two experiments performed to determine the effect of a heteroallosteric effector on the activity of phosphofructokinase ability to phosphorylate fructose 6 phosphate to fructose 1,6 biphosphate. Experiment 2 occurs at a concentration of 4.5 mM ATP.

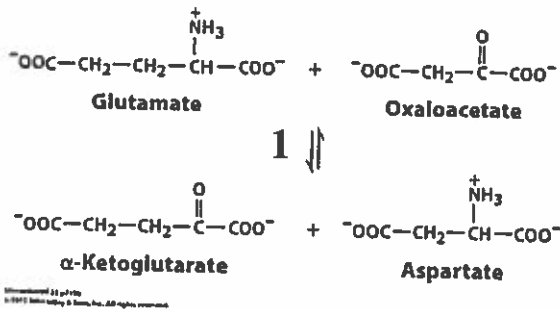
A) What does experiment 1 indicate is the effect of ATP on the enzyme? (2 marks)

ATP is a homoallosteric inhibitor (2) at high concentrations.
At low concentrations, it is a homoallosteric/homotropic activator (1)

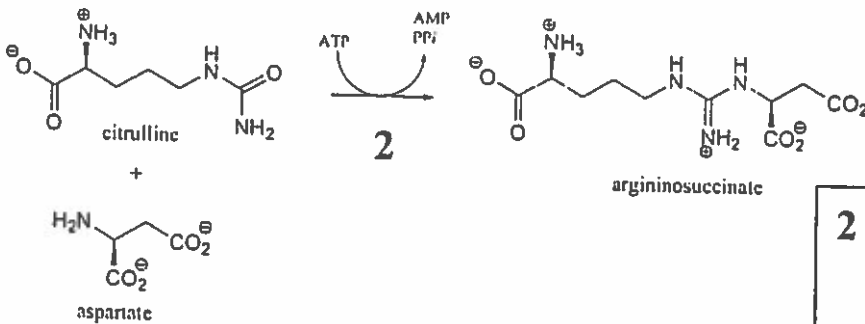
B) If the second experiment was carried out with [ATP]= 2mM instead, what difference(s) would you expect to see in the results? (2 marks)

The reaction containing no AMP will shift closer to the curve containing AMP, as ATP is not acting as an inhibitor at 2mM ATP. (2)

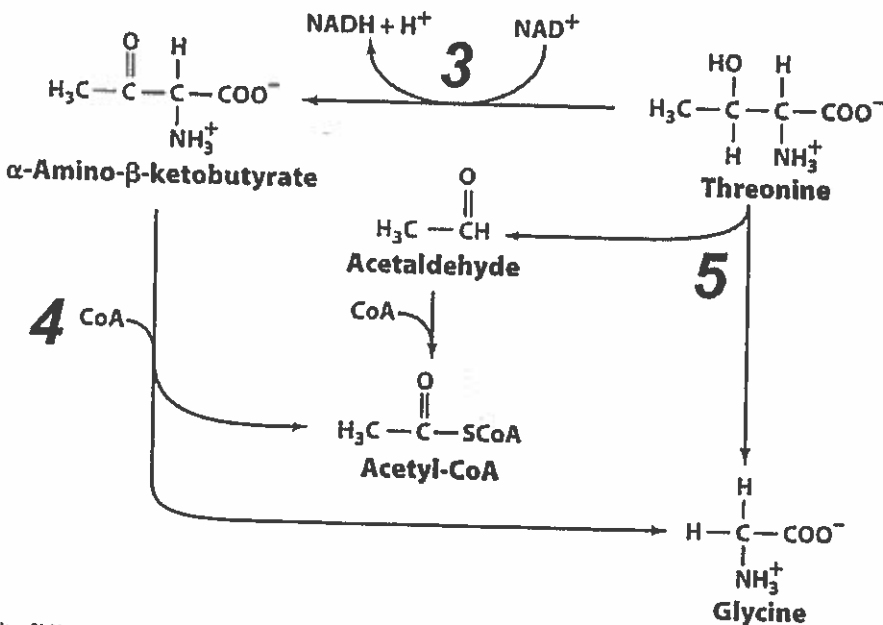
For each of the reactions shown below, correctly identify the enzyme class that catalyzes the reaction (just the name, it is not necessary to indicate the EC number). (1 Mark each)



1 Transferease (1)



2 Ligase (1)
(Synthetase 0.5)



3 Oxidoreductase (1)
(Dehydrogenase 0.5)

4 Transferease

5 Lyase
(Synthase 0.5)

Figure 21-14 part 1
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