

Name \_\_\_\_\_

Student number \_\_\_\_\_

**University of Guelph**  
**Department of Chemistry and Biochemistry**  
**19-356**  
**Structure and Function In Biochemistry**

**Midterm Test, March 6, 1997.**

**Time allowed, 120 min.**

Answer questions 1-20 on the computer scoring sheet provided.

Only one option is correct for each of these questions.

Use pencil to fill in the circles representing your chosen answer; and erase cleanly if you make an error. Do not use ink or white-out on the computer scoring sheet.

You may mark your answer on the question paper for your own records, however in case of a discrepancy between question paper and the computer scoring sheet, the choice shown on the computer sheet will be taken as final.

Answer questions 21-25 directly on the question paper.

Questions 1-20 have a weight of 1 mark each

20 marks

Questions 21-25 have marks as indicated

20 marks

=====

40 marks total

Q.21-22	Q. 23-24	Q.25	subtotal
_____	_____	_____	_____
7	7	6	20

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Answer questions 1-20 on the computer scoring sheet.

Value 1 mark each

1. Identify the correct completion of the statement:

**Heme-Fe<sup>2+</sup> complex in solution**A) can't bind O<sub>2</sub>.**B) is easily oxidized to Fe<sup>3+</sup> by O<sub>2</sub>.**C) binds H<sub>2</sub>O with high affinity.D) binds CO<sub>2</sub> more tightly than O<sub>2</sub>.E) is a good transporter for O<sub>2</sub>.1) A **B** C D EWithout the protection due to being buried in globin, soluble heme-Fe<sup>2+</sup> is easily oxidized

2. Identify the correct completion of the statement:

**In the globin sequence, Histidine E7**

A) replaces glutamate (E) at position 7 of the polypeptide.

B) is the 5th ligand for Fe<sup>2+</sup>.**C) forces the 6th ligand into bent configuration.**D) pulls Fe<sup>2+</sup> out of the heme plane.

E) is present in myoglobin but not hemoglobin.

2) A B **C** D E

His E7 is just too far away to be ligand 6, but blocks access for "straight-on" binding ligands

His F8 is 5th ligand

- 3.
- Structure of globins is 70%  $\alpha$ -helix because**

A) the polypeptide lacks amino acids that prefer  $\beta$ -sheet.**B) amino acids that prefer  $\beta$ -sheet are scattered at random, but  $\alpha$ -helical amino acids are organized in clusters.**C)  $\alpha$ -helical amino acids face outwards but  $\beta$ -sheet amino acids face inwards.D)  $\alpha$ -helical amino acids face inwards but  $\beta$ -sheet amino acids face outwards.E) myoglobin and  $\alpha$ -globin are  $\alpha$ -helix but  $\beta$ -globin is  $\beta$ -sheet.3) A **B** C D E $\alpha$ -helix forming AAs must be grouped consistently to form the local majority

- 4.
- Which hemoglobin combination would be unexpected in a fetus 7½ months before birth?**

A)  $\alpha_2\varepsilon_2$ B)  $\alpha_2\gamma_2$ C)  $\zeta_2\varepsilon_2$ D)  $\zeta_2\gamma_2$ **E)  $z_2b_2$** 4) A B C D **E** $\beta$ -globin is not normally expressed until birth.  $\alpha$ -globin is just starting to replace  $\zeta$  at this time, and  $\gamma$  is just starting to replace  $\varepsilon$ , so the mixtures listed above are likely to occur

5. Plots of O
- <sub>2</sub>
- binding by myoglobin are made in terms of fractional occupancy
- q**
- .

**Which formula correctly represents q**

A) 
$$q = \frac{[MbO_2]}{[Mb] + [MbO_2]}$$
occupied divided by total

B) 
$$q = \frac{[Mb]}{[MbO_2]}$$

C) 
$$q = \frac{[MbO_2]}{[Mb]}$$

D) 
$$q = \frac{[Mb] + [MbO_2]}{[MbO_2]}$$

E) 
$$q = \frac{[MbO_2]}{[Mb][O_2]}$$

5) **A** B C D E

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6. **Binding of O<sub>2</sub> by hemoglobin is a sigmoidal function of pO<sub>2</sub> because**  
A) Four O<sub>2</sub> molecules bind to each hemoglobin simultaneously.  
B)  $\beta$ -globin has lower O<sub>2</sub> affinity than  $\alpha$ -globin.  
C)  $\beta$ -globin has higher O<sub>2</sub> affinity than  $\alpha$ -globin.  
D) hemoglobin has lower O<sub>2</sub> affinity than myoglobin.  
**E) the binding affinity of hemoglobin subunits increases as each O<sub>2</sub> is bound.**  
6) A B C D E
7. **In the Monod-Wyman-Changeux model of allosteric cooperative binding, e.g. of O<sub>2</sub> by hemoglobin, which one of the following is assumed?**  
**A) That all subunits change from T to R together.**  
B) Each subunit changes from T to R as it binds O<sub>2</sub>.  
C) All subunits bind O<sub>2</sub> simultaneously.  
D) high affinity sites are occupied first.  
E) O<sub>2</sub> can't bind until hemoglobin is in the R state.  
assumed on the basis of expected symmetry relationships  
7) A B C D E
8. **Which of the following is not observed in the T to R transition of hemoglobin subunits?**  
A) The dimer pair  $\alpha_1\beta_1$  rotates 15° relative to  $\alpha_2\beta_2$   
B)  $\beta_1$  moves closer to  $\beta_2$   
**C) Large structural changes take place in the contact between  $\alpha_1$  and  $\beta_1$  globins.**  
D) New hydrogen bonds form in the interface between  $\alpha_1$  and  $\beta_2$  globins.  
E) Histidine F8 moves closer to the plane of the heme ring system.  
No change in contact is seen within  $\alpha_1\beta_1$  and  $\alpha_2\beta_2$  pairs  
8) A B C D E
9. **Which of the following statements is correct with regard to 2,3-bisphosphoglycerate?**  
A) Binding 2,3-bisphosphoglycerate increases binding of O<sub>2</sub> at a given pO<sub>2</sub>.  
B) One mole of 2,3-bisphosphoglycerate binds per  $\beta$ -globin subunit.  
C) 2,3-bisphosphoglycerate is a normal intermediate in the glycolysis pathway.  
D) Binding of 2,3-bisphosphoglycerate favours the R state of hemoglobin.  
**E) 2,3-bisphosphoglycerate enhances O<sub>2</sub> release in the peripheral capillaries.**  
2,3-bisphosphoglycerate favours T-state, hence promotes O<sub>2</sub> release  
9) A B C D E
10. Individuals with  $\alpha$ -thalassemia have small amounts of  **$\beta_4$**  hemoglobin.  
**Which of the following correctly describes a property of  $\beta_4$  hemoglobin?**  
A)  $\beta_4$  hemoglobin can't bind O<sub>2</sub>.  
B)  $\beta_4$  hemoglobin has a tendency to crystallize.  
C)  $\beta_4$  hemoglobin binds O<sub>2</sub> with low affinity.  
**D) The binding of O<sub>2</sub> by  $\beta_4$  hemoglobin is more like myoglobin than normal hemoglobin.**  
E)  $\beta_4$  hemoglobin is replaced by persistent expression of  $\gamma$  globin in adult life.  
10) A B C D E

Name \_\_\_\_\_

11. **High [ATP] increases the rate of catalysis of aspartate transcarbamylase because**
- A) ATP is required in the catalytic process.
  - B) ATP causes release of active c subunits.
  - C) ATP hydrolysis provides energy for the reaction.
  - D) ATP is a positive homotropic allosteric effector.
  - E) ATP binding shifts the ATCase from the T to the R state.**
- 11) A B C D E
- Nasty! ATP is a positive effector, but it is not homotropic because it is not a substrate

12. @ **The sedimentation coefficient of ATCase decreases when phosphonylacetyl-L-aspartate (PALA) is added. This indicates that:**
- A) the ATCase molecule has increased in mass due to binding substrate analog.
  - B) the ATCase molecule is more open, increasing its friction factor.**
  - C) the ATCase molecule has split up into smaller subunits.
  - D) the ATCase molecule has closed, up making it appear smaller.
  - E) the ATCase molecule has decreased in mass.
- 12) A B C D E

Some topics were covered in more detail when Stryer was used as textbook (questions marked @). This question refers to the experimental method used to detect the structural change when ATCase shifts from T to R

13. @? **The  $c_6r_6$  organization of ATCase is dissociated into smaller subunits by covalent binding of *p*-hydroxymercuribenzoate. Which of the following is not true:**
- A) the  $c_3$  component is catalytically active.
  - B) the  $c_3$  component is under allosteric control.**
  - C) the  $r_2$  component can bind ATP or CTP.
  - D) the  $r_2$  and  $c_3$  components recombine into fully functional allosterically regulated ATCase.
  - E) a single c subunit can't bind substrate
- 13) A B C D E

14. @ **Which of the following is not true for enzymes which display negative cooperativity?**
- A) Substrate binding affinity is reduced at high [S].
  - B) Substrate binding affinity is enhanced at low [S].
  - C) Allosteric effects are based on the sequential model of Koshland, Nemethy and Filmer.
  - D) The binding or activity plot is sigmoidal.**
  - E) The binding energy for the first substrate molecule is higher than for the last substrate molecule.
- 14) A B C D E

15. **Which of the following statements is not true with regard to zymogen activation?**
- A) At low pH pepsinogen cleaves itself to remove a peptide that blocks the active site.
  - B) Cleavage of chymotrypsinogen by trypsin removes a peptide that blocks the active site.**
  - C) Cleavage of chymotrypsinogen by trypsin introduces a new positively charged  $\alpha$ -amino group at Ile 16 which helps position Asp 194 correctly.
  - D) Self-cleavage of  $\pi$ -chymotrypsin to form  $\alpha$ -chymotrypsin is not essential for activation.
  - E) Cleavage of chymotrypsinogen by trypsin positions Ser 195 and Gly 193 correctly for substrate binding.
- 15) A B C D E

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16. @? Which of the following statements best describes the working of natural protease inhibitors such as pancreatic trypsin inhibitor and a1-antitrypsin?  
**A) Very strong binding of the inhibitor increases the activation energy for the protease reaction.**  
B) The inhibitors bind covalently to the protease.  
C) Reactive groups in the inhibitor modify the protease irreversibly.  
D) The inhibitors lack the key amino acid that is the target for the protease so can't be hydrolyzed.  
E) The inhibitor is itself a protease that attacks and deactivates the target protease.  
16) **A B C D E**
17. Which of the following factors is not part of the intrinsic pathway for blood clotting?  
A) Factor XII                      B) Factor XI                      C) Factor IX  
D) Factor VIII                      **E) Factor VII**  
Factor VII starts the extrinsic pathway  
17) **A B C D E**
18. Which of the following blood clotting factors is not a serine protease?  
A) Factor XII                      B) Factor XI                      C) Factor IX  
**D) Factor VIII**                      E) Factor VII  
Factor VIII promotes binding of Factor X by the serine protease Factor IXa  
18) **A B C D E**
19. Which of the following substances is a positive allosteric effector of muscle phosphorylase *b*?  
A) ATP                                  B) Glucose                          C) Caffeine  
D) cyclic AMP                          **E) AMP**  
19) **A B C D E**
20. @ **Dicoumarol in spoiled hay causes hemorrhagic disease in cattle. Which of the following statements is not true?**  
A)  $\gamma$ -carboxylation of prothrombin is blocked the presence of dicoumarol.  
B) The abnormal prothrombin binds  $Ca^{2+}$  weakly.  
C) Without bound  $Ca^{2+}$ , prothrombin can't be activated.  
D) The  $Ca^{2+}$  binding sites are near the N-terminus of prothrombin.  
**E)  $Ca^{2+}$  binding is required for thrombin to bind its substrate, fibrinogen.**  
20) **A B C D E**

Dicoumarol is a natural fungal product with structure partly similar to Warfarin, and has similar effect as an antagonist of vitamin K. Prothrombin needs  $Ca^{2+}$  for its own activation, but once thrombin is activated, the N-terminal region containing  $\gamma$ -carboxyglutamate is removed. Hence thrombin does not use  $Ca^{2+}$  to bind its substrate, fibrinogen.

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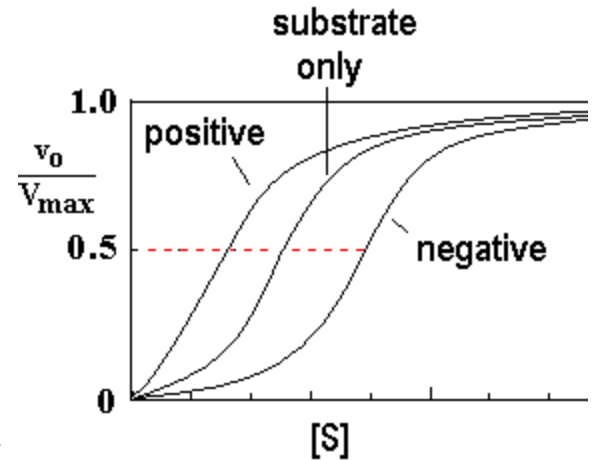
21. i) Briefly explain why the **positive homotropic effect** gives a sigmoidal curve for an allosterically regulated enzyme such as ATCase.  
ii) sketch the sigmoidal curve for substrate only, then add two curves showing the effect of adding a) an allosteric effector which has a **positive heterotropic effect**, and b) an allosteric effector with a **negative heterotropic effect**. Label these curves **positive** and **negative**.

The unoccupied ATCase has six substrate site and starts in T-state. The switch to R state involves rotation of upper  $c_3$  module relative to lower  $c_3$  module so is a global change affecting all subunits at once (MWC type process) (1/2)

As each substrate molecule binds, it affects internal forces in protein. At a certain point, the whole molecule switches to R. (1/2)

Any sites still unoccupied now have high affinity. (1/2)

As [S] increases, this increases the probability of T to R transition. The graph initially follows the low affinity hyperbolic curve, and the sigmoidal shape is due to the transition to the high affinity curve (1/2)



1 mark for the graph labelled as shown

3 marks

22. **What amino acid change is found in the polypeptide sequences of Hemoglobin S?**

**Glu** **A3** **b** **Val**  
\_\_\_\_\_ in normal HbA at position \_\_\_\_\_ of \_\_\_\_\_ globin is replaced by \_\_\_\_\_  
1/2 1/2 1/2 1/2

Why does this change lead to **aggregation of deoxyhemoglobin S** but **not the oxy form**?

4 marks

Aggregation is due to Val A3 in one  $\beta$ -globin fitting into a nonpolar recess near Leu F4 in the other  $\beta$ -globin (1/2 for principle, 1/2 for identifying F4)

In deoxyhemoglobin S, the hemoglobin molecules are in T state and Val A3 and the Leu F4 recess line up to allow formation of a continuous strand (1/2). When hemoglobin S is oxygenated, it switches to R-state and the relative orientation of the two  $\beta$ -globins changes so that they no longer align (1/2).

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23. Aspartate is one substrate for aspartate transcarbamoylase (ATCase); name the other:

**carbamoyl phosphate** (½)

Name the **product** of the normal reaction \_\_\_\_\_ carbamoylaspartate \_\_\_\_\_ (½)

Briefly explain why it is useful to the organism that **ATP binding activates ATCase** while **CTP binding should inhibit** the reaction.

3 marks

ATCase is the first step in the synthesis of pyrimidines, and CTP contains the pyrimidine cytosine (½).

Therefore if CTP levels are already high, pyrimidine synthesis is unnecessary (½)

ATP represents the state of the purines, and if purine levels are high but pyrimidines are not, then pyrimidine synthesis should start (½)

High ATP levels also indicate good energy status so the cell may grow and divide, and thus require pyrimidines for DNA and RNA synthesis (½).

24. Outline briefly how **cleavage of fibrinogen by thrombin** allows formation of a fibrin clot.

What additional changes **convert the fibrin from soft clot to hard clot**? **What factor** is responsible for this change, and **how is it activated**?

4 marks

Fibrinogen is a symmetrical linear molecule with negatively charged globular domains at the centre and at each end. (½) Under these conditions it remains soluble as single molecules.

Thrombin cleaves off fibrinopeptides A and B from the N-terminals of the A $\alpha$  and B $\beta$  chains of fibrinogen (½). Fibrinopeptides carry a high negative charge, and when they leave, the central domain of fibrin becomes positive (½). Fibrin molecules can then bind to each other in an end-to-middle (brick-like) fashion (½). This assembles the fibrin into fibres causing blood to gel (½).

The hard clot is induced by Factor XIIIa (½) which causes Gln side chains in one fibrin molecule to become cross linked with Lys side chains in another (½).

Factor XIII is activated by thrombin (½) @

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25. Draw the sequence of steps in the **cascade for stimulation of muscle glycogen phosphorylase** activity by the **hormone epinephrine**.

3 marks

Epinephrine  $\Rightarrow$  *Adenylate cyclase*

(A  $\Rightarrow$  B implies that B is activated by A)

ATP  $\rightarrow$  cyclic AMP  $\Rightarrow$  Protein kinase A  $R_2C_2$   $\rightarrow$  Protein kinase A  $R_2.cAMP_4$

(1)

+

*Protein kinase A C subunit* (1)

Phosphorylase b kinase + ATP  $\rightarrow$  *Phosphorylase b kinase*  
inactive active + ADP

ATP +  $\rightarrow$  ADP +

Phosphorylase b *Phosphorylase a*  
T-state inactive R state active

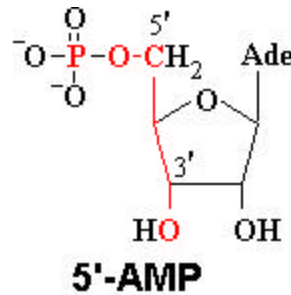
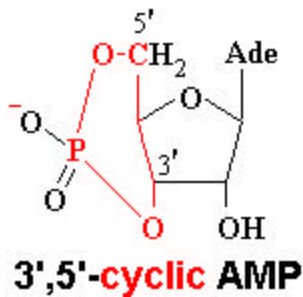
(1)

Draw the structures of 3',5'-cyclic AMP and ordinary 5'-AMP **showing how the phosphate is linked to the ribose in each case**; the adenine portion can be represented as Ade.

3',5'-cyclic AMP:

5'-AMP

2 marks



How does **caffeine** elevate the level of glucose in the blood?

1 mark

Caffeine is an inhibitor of cyclic nucleotide phosphodiesterase, which destroys cyclic AMP by hydrolysis of the 3' bond, converting it to 5'-AMP. (1/2)

Since cyclic AMP is not being destroyed, the stimulating effect of epinephrine becomes more prolonged than usual.