

Name _____

Student number _____

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

Midterm Test, March 3, 1998.

Time allowed, 90 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 21-24 directly on the question paper.

Questions 1-20 have a weight of 1 mark each

Questions 21-24 have marks as indicated

20 marks

20 marks

=====

40 marks

total

Q.21-22	Q.23	Q. 24	subtotal
9	7	4	20

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Answer questions 1-20 on the computer scoring sheet.

Value 1 mark each

1. **Thalassemia with two b-globin genes defective is not always lethal because:**
A) α hemoglobin functions adequately on its own.
B) The other β globin genes make adequate supplies of hemoglobin.
C) Fetal g-globins may continue to be expressed in adult life.
D) The body makes do with myoglobin provided no strenuous activity is undertaken.
E) This condition is never observed. 1) A B **C** D E

2. **The Bohr effect is the reduced affinity of hemoglobin for O₂**
A) Due to binding of 2,3-bisphosphoglycerate B) Due to high altitude.
C) Due to lower pH D) In fetal hemoglobin.
E) Resulting from the hemoglobin S mutation. 2) A B **C** D E
T state is favoured at lower pH

3. **ATP acts on aspartate transcarbamoylase:**
A) By providing energy for the reaction. B) By phosphorylating the substrate.
C) As a homotropic effector D) As a feedback inhibitor.
E) By binding and stabilizing the R state of the enzyme. 3) A B C D **E**

4. **Vitamin K is required for normal blood clotting because:**
A) Vitamin K binds to thrombin making it more active.
B) Vitamins make you more healthy.
C) Vitamin K is required for formation of γ -carboxyglutamate in the active site of thrombin.
D) Vitamin K is required for formation of γ -carboxyglutamate in prothrombin.
E) Vitamin K binds Ca^{2+} to neutralize the negative charge of fibrinogen. 4) A B C **D** E
 γ -carboxyglutamate is found in the N-terminal propeptide and is required for activation of prothrombin and other serine protease clotting factors; it is removed from the active thrombin.

5. **Which of the following does not increase activity in the cyclic AMP-protein kinase cascade?**
A) Adenylate cyclase B) Protein kinase A
C) Glycogen Synthase D) Phosphorylase kinase
E) Phosphorylase 5) A B **C** D E

6. **Caffeine acts as a stimulant by:**
A) Increasing synthesis of cyclic AMP B) Stimulating G-protein to bind GTP
C) Preventing breakdown of cyclic AMP D) Binding to protein kinase A.
E) Binding to a hormone receptor on the cell surface. 6) A B **C** D E

7. **The initial activation of trypsinogen requires:**
A) Enteropeptidase B) Pepsinogen
C) Chymotrypsinogen D) Chymotrypsin
E) Pancreatic trypsin inhibitor. 7) **A** B C D E

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8. **Which statement about 2,3- bisphosphoglycerate (BPG) is untrue?**
A) People who live at high altitude have higher BPG levels to enhance O₂ release in tissues.
B) BPG binds to a positively charged region between the β-globin subunits.
C) BPG binds more weakly to fetal hemoglobin, so O₂ binding is stronger.
D) BPG binding favours the R state of hemoglobin.
E) BPG is derived from intermediates of the glycolysis pathway. 8) A B C **D** E
9. **In the Monod-Wyman-Changeux model of allosteric cooperative binding, e.g. of O₂ 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 O₂.
C) All subunits bind O₂ simultaneously.
D) high affinity sites are occupied first.
E) O₂ can't bind until hemoglobin is in the R state. 9) **A** B C D E
10. **Which statement about Histidine F8 in the globin sequence is incorrect:**
A) changes position in the R state to T state transition
B) is the 5th ligand for Fe²⁺. **C) forces the 6th ligand into a “bent” configuration.**
D) pulls Fe²⁺ out of the heme plane. E) is found in myoglobin and all hemoglobins.
10) A B **C** D E
11. Plots of O₂ binding by myoglobin (Mb) are made in terms of fractional occupancy θ.
Which formula correctly represents Y?
A) $q = \frac{[MbO_2]}{[Mb]+[MbO_2]}$ B) $\theta = \frac{[Mb]}{[MbO_2]}$ C) $\theta = \frac{[MbO_2]}{[Mb]}$
D) $\theta = \frac{[Mb]+[MbO_2]}{[MbO_2]}$ E) $\theta = \frac{[MbO_2]}{[Mb][O_2]}$ 11) **A** B C D E
12. Which of the following correctly completes the statement
Binding of aspartate by ATCase is a sigmoidal function of [aspartate] because
A) Aspartate molecules bind to all catalytic subunits simultaneously.
B) As each aspartate binds, it changes the c-subunit it's bound to into the R-state.
C) Aspartate is a positive heterotropic effector for ATCase.
D) Each aspartate bound increases the probability that the whole c₆r₆ will convert to the R state.
E) Binding negatively charged aspartate favours the T-state. 12) A B C **D** E
13. **Which of the following is not a serine protease?**
A) Factor XIIa **B) Fibrin** C) Plasmin
D) Thrombin E) Tissue Plasminogen Activator 13) A **B** C D E

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21. Factor XIIIa plays a unique role in the blood clotting system. @ topic covered in more detail in 1998

Thrombin

i) Which serine protease activates factor XIII to give to XIIIa? _____

1 mark

ii) Once factor XIIIa is active, what reaction does it catalyze, and what effect does this have on the blood clot?

3 marks

Factor XIII is also known as transglutaminase. It catalyses reaction of lysine in one fibrin molecule with glutamine in a neighbouring fibrin molecule (1/2).

The amino group of lysine displaces the amide N of glutamines and forms a new amide bond with it. (1/2)



This causes the clot to harden (1/2) and become more resistant to being dissolved by plasmin (1/2)

22. Prothrombin and the zymogen forms of several serine protease blood clotting factors, e.g. factor X, have an unusually large propeptide which is important in the activation process.

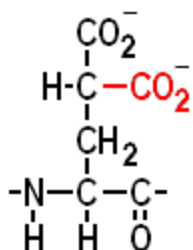
g-carboxyglutamate

What modified amino acid is found in this propeptide? _____

1 mark

How does this modified amino acid participate in the activation mechanism?

**γ-carboxy
glutamate**



g-carboxyglutamate promotes Ca^{2+} binding because each of these dicarboxylic side chains can provide 2 ligands for Ca^{2+} (1/2). Ca^{2+} prefers a 6-ligand arrangement, so it can serve to bridge the zymogen form of the clotting factor to a negatively charged surface (1/2)

When clotting factors are dispersed in solution, their concentration is so low that the activating reaction does not propagate from molecule to molecule (1/2).

When the zymogens bind to a surface, they become concentrated on that surface, allowing the activating reaction to spread through the cascade (1/2).

Explain why blood clots in contact with glass, which has a negatively charged surface.

Glass carries negative charge due to silicates, and may have sufficient bound Ca^{2+} on its surface, so that it readily acts as a concentrating surface (1).

What natural negatively charged surface triggers blood clotting in the body?

Platelets release negatively charged phospholipids which form bilayer surfaces and act as good ligands for Ca^{2+} (normal phospholipids are neutral) (1).

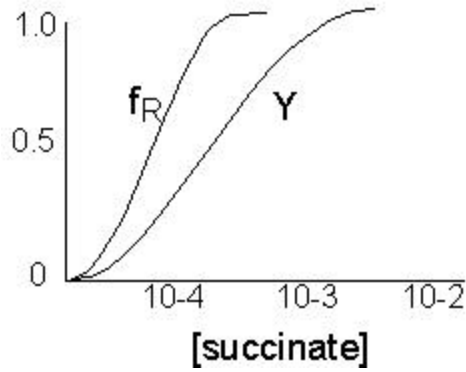
4 marks

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@ This topic was covered in more depth in 1998

23. The diagram on the right shows a plot of f_R , the fraction of ATCase molecules in the R allosteric state, as a function of [succinate]. Also plotted is Y, the fraction of substrate sites occupied by succinate.



What instrumental method was used to measure f_R ?
ultracentrifuge

1 mark

What property of ATCase changes on adopting R state to allow this measurement?

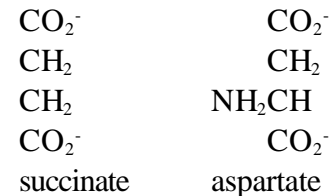
The R state is more open, which increases the frictional resistance to sedimentation; hence the sedimentation rate decreases on switch to R state.

1 mark

Why was the experiment done with succinate instead of aspartate, the natural substrate?

Succinate is similar to aspartate with the exception of the amino group.

Without the amino group, succinate can't react, so it forms a stable complex. Aspartate would react to give products, which are then released from the enzyme.



1 mark

What is the significance of the fact that the curve for f_R lies to the left of the curve for occupancy, Y? What conclusion might be drawn if the two curves were superimposed?

3 marks.

The f_R curve indicates what proportion of molecules have switched to R (1/2). The fact that the curve lies to the left of the fraction of sites occupied indicates that molecules of ATCase switch states before all substrate sites are occupied (1/2). This means that some of the sites are still vacant when the ATCase goes to high affinity R state, so the remainder of the substrate will bind in the high affinity mode (1/2). This is characteristic of MWC or concerted type cooperative behaviour. (1/2)

If the two curves were superimposed, this would imply that change to R state occurs exactly as substrate sites are occupied (1/2). This would be characteristic of KNF or sequential type cooperative behaviour (1/2)

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

carbamoyl phosphate

1 mark

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24. The arrangement of subunits in hemoglobin is best described in terms of two globin pairs, $\alpha_1\beta_1$ and $\alpha_2\beta_2$ (subscripts identify different subunits rather than showing stoichiometry).

What structural changes are noted in the change from T to R state of hemoglobin?

How is the orientation of $\alpha_1\beta_1$ to $\alpha_2\beta_2$ changed? _____

The pair $\alpha_1\beta_1$ rotates 15° relative to the pair $\alpha_2\beta_2$

1 mark

Which combination is least affected by change from T to R?

$\alpha_1\beta_1$ $\alpha_1\beta_2$ $\alpha_1\alpha_2$ $\beta_1\beta_2$ (circle one)

Which combination alters its hydrogen bonding pattern on change T to R?

$\alpha_1\beta_1$ $\alpha_1\beta_2$ $\alpha_1\alpha_2$ $\beta_1\beta_2$ (circle one)

Which combination moves further apart on change T to R?

$\alpha_1\beta_1$ $\alpha_1\beta_2$ $\alpha_1\alpha_2$ $\beta_1\beta_2$ (circle one)

Which combination moves closest together on change T to R?

$\alpha_1\beta_1$ $\alpha_1\beta_2$ $\alpha_1\alpha_2$ $\beta_1\beta_2$ (circle one)

Which combination contains the 2,3-bisphosphoglycerate binding site?

$\alpha_1\beta_1$ $\alpha_1\beta_2$ $\alpha_1\alpha_2$ $\beta_1\beta_2$ (circle one)

Which combination contains salt bridges that help restore the molecule to the T state?

salt bridges = ion pairs

$\alpha_1\beta_1$ $\alpha_1\beta_2$ $\alpha_1\alpha_2$ $\beta_1\beta_2$ (circle one)

3 marks