

NAME:

I.D. #

1) Draw the structure at pH7 and give the name and 1 letter code for one amino acid whose **side chain: (10 pts)**

A) is hydrophilic

B) is a hydrogen bond donor

C) has a positive charge at pH7

D) is capable of absorbing ultraviolet light (280 nm)

E) contains sulfur

2) In a healthy individual blood plasma has a pH of 7.40. A change of as little as 0.2 of a pH unit can result in serious medical consequences. One of the ways that blood pH is maintained is through phosphate buffering. ( $pK_{a2}$  of the phosphate buffer = 6.7).

A) What is the ratio of  $HPO_4^{2-}$  to  $H_2PO_4^-$  in the blood at pH 7.4? **(3 points)**

B) Explain how a change of only 0.2 pH units in the blood could lead to serious medical consequences. Hint: this involves proteins but NOT haemoglobin. Try to be specific for full marks. **(3 points)**

D) If no precautions are taken, blood that has been collected from a donor and stored for some time becomes depleted of 2,3-bisphosphoglycerate (2,3-BPG). What is the role of 2,3-BPG and why would its loss from stored blood be a bad thing if this blood was used in a transfusion? **(4 points)**

3) A) You are given a blood sample containing four proteins. To check for a disease condition in the blood donor you need to purify all 4 proteins to **homogeneity**. Unfortunately, just as you start your experiments there is a power failure so that you can not use any techniques that require electricity. You need to process the sample as quickly as possible to preserve the proteins so you go ahead using **chromatographic techniques**. Based on the characteristics listed below, describe how you would separate these proteins. (7 points)

<u>Protein</u>	<u>Molecular weight (kDa)</u>	<u>pI</u>	<u>DNA-binding</u>
A	119	7.5	yes
B	121	8.0	no
C	120	3.9	no
D	245	7.5	no

B) Given the data listed below what are the **specific activities of both fractions** and what **fold enrichment** have you achieved? (3 points)

Purification step	Total protein (g)	Total activity (units)
1	100	40 000 000
2	0.2	8 000 000

4) Shown is the ribbon diagram of lysozyme (Berg *et al.*). Where on this protein would you most likely find the following amino acids? **Explain and be specific.** Use arrows to define locations precisely. (6 points)

A) Proline

B) Glutamic acid

C) Leucine

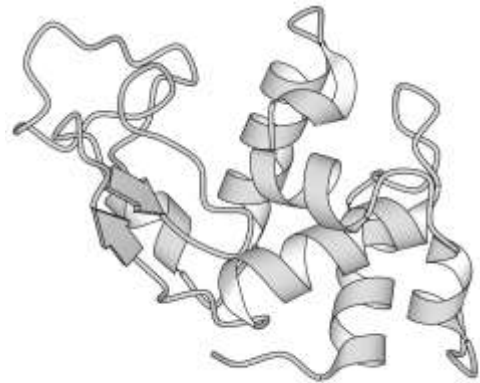


Figure 3-79  
Molecular Biology of the Cell, 6th Edition  
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5) The conversion of 3-phosphoglycerate (3PG) to 2-phosphoglycerate (2PG) during glycolysis is catalyzed by the enzyme phosphoglycerate mutase. This reaction has a standard free energy change ( $\Delta G^{\circ}$ ) of +1.1 kcal/mol.

A) Does this reaction occur spontaneously under typical cellular conditions, *i.e.*,  $[3PG] = 200 \mu\text{M}$  and  $[2PG] = 20 \mu\text{M}$ ? Show this mathematically. (Assume  $25^{\circ}\text{C}$  and  $R = 1.98 \text{ cal mol}^{-1} \text{ deg}^{-1}$ ) (3 points)

B) What would knowing the  $\Delta G$  for this reaction tell you about the rate of this reaction? (1 point)

C) Describe experimentally the correct way to determine a  $V_{\text{max}}$  for phosphoglycerate mutase. (3 points)

6) A) List any three types of noncovalent interactions that can contribute to the binding of substrate at the active site of an enzyme. **(3 points)**

B) Would you expect fewer, the same or more of these interactions between an enzyme and its transition state intermediate than between an enzyme and its substrate? Explain. **(2 points)**

7) In the space provided define any **two** of the following terms **including an example** if appropriate. **(4 points)**

A) homotropic effect

B) coenzyme

C) initial velocity

D) isoelectric point

8) What type of secondary structure (if any) would you expect each of these oligopeptides to adopt **at pH 7**? Explain and be specific. **(6 points)**

I)...glu-val-ala-ser-his-gly-gln-glu-ala-leu-ile-arg-leu-phe-lys...

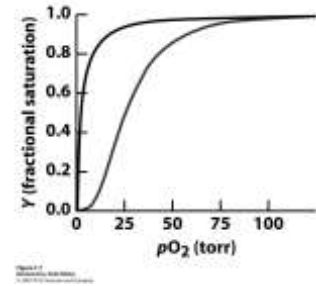
II)...asp-asp-asp-asp-asp-asp-asp-asp-asp-asp...

III)...asp-val-glu-ala-ser-ile-lys-ala-asn-val-ser...

Which of these oligopeptides most likely comes from lysozyme? Explain. **(2 points)**

9) On the following graph of oxygen binding plotted against the partial pressure of oxygen which line represents oxygen binding to myoglobin and which represents oxygen binding to hemoglobin? **(2 points)**

A) Explain why one line is hyperbolic while the other is sigmoidal. **(2 points)**



B) We have discussed two models (concerted and sequential) that account for the positive cooperativity seen when oxygen binds to hemoglobin. If you compare these two models, what differences would you expect to see in the haemoglobin populations at 50% oxygen saturation? **(3 points)**

10) A) The oxygen binding proteins leghemoglobin (from plants) and myoglobin (from animals) share only about 20% amino acid identity (indicating a relatively distant evolutionary separation), but have similar structures. Explain how two proteins with such divergent primary sequences can have similar structures and functions. **(3 points)**

B) In spite of what we see in question 10A, changing a single amino acid can dramatically affect the function of a protein. Using any example that we discussed in class, explain how a single amino acid substitution can alter hemoglobin's properties and lead to a disease state. **(3 points)**