

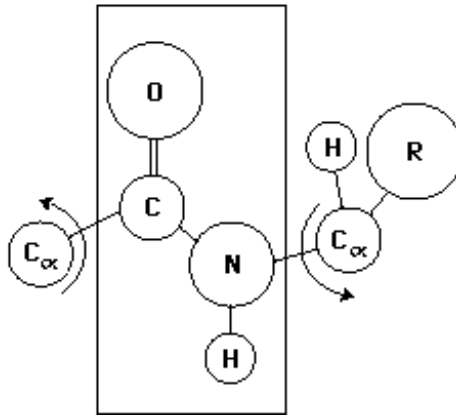
Multiple Choice Questions

1. The purity of an enzyme at various stages of purification is best measured by:
 - a. Total protein
 - b. Total enzyme activity
 - c. Specific activity of the enzyme
 - d. Percent recovery of protein
 - e. Percent recovery of the enzyme
2. Which would be best to separate a protein that binds strongly to its substrate?
 - a. Gel filtration
 - b. Affinity chromatography
 - c. Cation exchange
 - d. Anion exchange
 - e. Cation or anion exchange
3. How many hydrogen bonds can a water molecule potentially take in liquid form?
 - a. one
 - b. two
 - c. three
 - d. four
 - e. none of the above
4. How does the hydrophobic effect influence the structures of large molecules?
 - a. Nonpolar molecules are not easily solubilized in water and aggregate
 - b. Polar groups are oriented on the surface, interacting with the water
 - c. Nonpolar molecules can mask the polar characteristics of the hydrophilic molecules
 - d. a) and b)
 - e. a) and c)
5. The most important buffering system for maintaining proper blood pH is:
 - a. the charges on the amino acids
 - b. the bicarbonate buffer system of CO₂, carbonic acid, and bicarbonate
 - c. phosphate groups of serum phosphoproteins
 - d. none of the above
 - e. all of the above
6. Myoglobin binding of oxygen depends on:
 - a. the oxygen concentration (pO₂)
 - b. the hemoglobin concentration
 - c. the affinity of myoglobin for the O₂ (K)
 - d. a) and c)
 - e. a), b) and c)

7. In the α helix the hydrogen bonds:
- are roughly parallel to the axis of the helix
 - are roughly perpendicular to the axis of the helix
 - occur mainly between electronegative atoms of the R groups
 - occur only between some of the amino acids of the helix
 - occur only near the amino and carboxyl termini of the helix
8. In the binding of oxygen to myoglobin, the relationship between the concentration of oxygen and the fraction of binding sites occupied can best be described as:
- hyperbolic
 - linear with a negative slope
 - linear with a positive slope
 - random
 - sigmoidal
9. Which of the following is true about the properties of aqueous solutions?
- A pH change from 5.0 to 6.0 reflects an increase in the hydroxide ion concentration ($[\text{OH}^-]$) of 20%.
 - A pH change from 8.0 to 6.0 reflects a decrease in the proton concentration ($[\text{H}^+]$) by a factor of 100.
 - Charged molecules are generally insoluble in water.
 - Hydrogen bonds form readily in aqueous solutions.
 - The pH can be calculated by adding 7 to the value of the pOH.
- 10 The Henderson-Hasselbalch equation:
- allows the graphic determination of the molecular weight of a weak acid from its pH alone.
 - does not explain the behavior of di- or tri-basic weak acids
 - employs the same value for pK_a for all weak acids
 - is equally useful with solutions of acetic acid and hydrochloric acid
 - relates the pH of a solution to the pK_a and the concentrations of acid and conjugate base
11. Two amino acids of the standard 20 contain sulfur atoms. They are:
- cysteine and serine
 - cysteine and threonine
 - methionine and cysteine
 - methionine and serine
 - threonine and serine

12. An octapeptide composed of four repeating glycylalanyl units has:
- a. one free amino group on an alanyl residue
 - b. one free amino group on an alanyl residue and one free carboxyl group on a glycyl residue
 - c. one free amino group on a glycyl residue and one free carboxyl group on an alanyl residue
 - d. two free amino and two free carboxyl groups
 - e. two free carboxyl groups, both on glycyl residues
13. In a mixture of the five proteins listed below, which should elute second in size-exclusion (gel filtration) chromatography?
- | | |
|---------------------|-----------------|
| a. cytochrome c | $M_r = 13,000$ |
| b. immunoglobulin G | $M_r = 145,000$ |
| c. ribonuclease A | $M_r = 13,700$ |
| d. RNA polymerase | $M_r = 450,000$ |
| e. serum albumin | $M_r = 68,500$ |
14. The enzyme fumarase catalyzes the reversible hydration of fumaric acid to l-malate, but it will not catalyze the hydration of maleic acid, the cis isomer of fumaric acid. This is an example of:
- a. biological activity
 - b. chiral activity
 - c. racemization
 - d. stereoisomerization
 - e. stereospecificity
15. The first step in two-dimensional gel electrophoresis generates a series of protein bands by isoelectric focusing. In a second step, a strip of this gel is turned 90 degrees, placed on another gel containing SDS, and electric current is again applied. In this second step:
- a. proteins with similar isoelectric points become further separated according to their molecular weights.
 - b. the individual bands become stained so that the isoelectric focus pattern can be visualized
 - c. the individual bands become visualized by interacting with protein-specific antibodies in the second gel
 - d. the individual bands undergo a second, more intense isoelectric focusing
 - e. the proteins in the bands separate more completely because the second electric current is in the opposite polarity to the first current

16. In the diagram below, the plane drawn behind the peptide bond indicates the:



- a. absence of rotation around the C-N bond because of its partial double-bond character
 - b. plane of rotation around the C_α-N bond
 - c. region of steric hindrance determined by the large C=O group
 - d. region of the peptide bond that contributes to a Ramachandran plot
 - e. theoretical space between -180 and +180 degrees that can be occupied by the ϕ and ψ angles in the peptide bond
17. The major reason that antiparallel β -stranded protein structures are more stable than parallel β -stranded structures is that the latter
- a. are in a slightly less extended configuration than antiparallel strands
 - b. do not have as many disulfide crosslinks between adjacent strands
 - c. do not stack in sheets as well as antiparallel strands
 - d. have fewer lateral hydrogen bonds than antiparallel strands
 - e. have weaker hydrogen bonds laterally between adjacent strands
18. Experiments on denaturation and renaturation after the reduction and reoxidation of the -S-S- bonds in the enzyme ribonuclease (RNase) have shown that:
- a. folding of denatured RNase into the native, active conformation requires the input of energy in the form of heat
 - b. native ribonuclease does not have a unique secondary and tertiary structure
 - c. the completely unfolded enzyme, with all -S-S- bonds broken, is still enzymatically active
 - d. the enzyme, dissolved in water, is thermodynamically stable relative to the mixture of amino acids whose residues are contained in RNase
 - e. the primary sequence of RNase is sufficient to determine its specific secondary and tertiary structure

19. Myoglobin and the subunits of hemoglobin have:
- a. no obvious structural relationship
 - b. very different primary and tertiary structures
 - c. very similar primary and tertiary structures
 - d. very similar primary structures, but different tertiary structures
 - e. very similar tertiary structures, but different primary structures
20. When the linear form of glucose cyclizes, the product is a(n):
- a. anhydride
 - b. glycoside
 - c. hemiacetal
 - d. lactone
 - e. oligosaccharide

Written Answer Questions

1. Starch and cellulose are both polysaccharide compounds of 1,4-linked polymers of glucose. Why do these two polymers of glucose have such different properties? Discuss the chemical differences as well as the higher order structures of these polymers. Illustrate your answer with sketches.

Starch (include figure similar to Fig. 11-18, p. 367)

3 points

- $\alpha(1\rightarrow4)$ polymers with $\alpha(1\rightarrow6)$ crosslinks
- Produces a somewhat “globular” particle
- Use: energy storage

Cellulose (include figure similar to Figs 11-14 or 11-15, p. 365)

3 points

- $\beta(1\rightarrow4)$ linear polymer
- Very extended structure
- Chains capable of forming H-bonds with other chains and forming strong fibers
- Use: structural

2. You have isolated an unknown protein from thermophilic bacteria growing in one of the geothermal pools of Yellowstone National Park. Name three reagents you could use to cleave the protein to determine its primary structure. Describe the effect of each reagent on the protein and how you would use this information to reconstruct the protein sequence.

2 points for
each
reagent

Trypsin - cleaves after Lys, Arg
Chymotrypsin - cleaves after aromatic amino acids
CNBr - cleaves after Met
You could also include other reagents listed Table 7-2, p. 168

2 points

Use each reagent to produce smaller peptide pieces, determine the composition of each peptide, and analyze the overlaps to derive the sequence.

3. A. Given the following peptides,

- i. RFEIKGHP
- ii. RVAHEIVQ
- iii. RVAKEIVR

4 points,
0.5 points
per residue

a. Draw the chemical structure of peptide (i) in solution at pH 7.

Compare your amino acid structure with those on pages 66-67. The N-terminal should be positively charged at pH 7, Arg and Lys should be positively charged, His should be neutral, the C-terminal should be negatively charged.

2 points for
each

b. What is the net charge of peptide (i) at pH 8.0? At pH 3.0?

At pH 8.0, net charge is +1; at pH 3.0, net charge is +3

1 point

c. If these sequences were found in a protein, which would you most expect to be in an α -helix and which in a β -sheet? Why?

2 points

(i) is in a β -sheet because it contains alternating hydrophobic and hydrophilic residues consistent with a β -sheet having a charged and a neutral side.

(ii) and (iii) are in α -helices because there is a hydrophobic residue about every 4 residues consistent with an α -helix containing a hydrophobic side.

B. The stability of an α -helix is determined not only by the intrastrand H-bonds, but also by the pH and the nature of its amino acid side chains. Predict whether the following will form ordered α -helices or not in solution. Give brief reasons for your predictions.

1 points

a. Polyleucine at pH 7.0

Yes, side chains are neutral so there are no charge repulsions

1 point

b. Polyarginine at pH 7.0

No, all side chains are positively charged therefore there will be charge repulsions

1 point

c. Polyglutamic acid at pH 1.5

Yes, at pH 1.5 all side chains will be neutral so there are no charge repulsions

4. With respect to what we learned in lecture, give one means by which short term high-altitude adjustment is accomplished in erythrocytes? Explain how this adjustment can help restore normal oxygen delivery. Use a sketch of fractional saturation (Y_{O_2} vs. pO_2) in your answer.

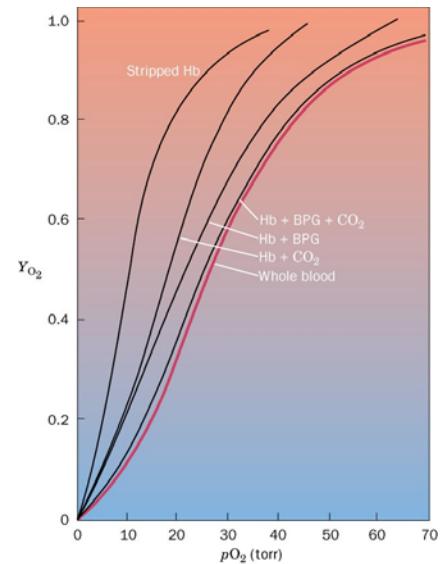
1 point

Short-term high altitude adjustments are accomplished by varying the concentration of BPG in the blood.

3 points

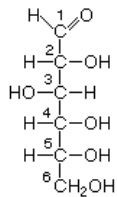
Higher concentrations of BPG allow for more oxygen to be released, i.e. less tightly bound, so more oxygen is delivered to the tissues.

3 points for the graph

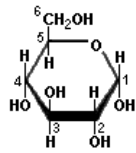


5. A. Draw the Fisher and Haworth projections of D-glucose and D-mannose.

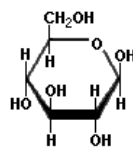
1 point for each drawing



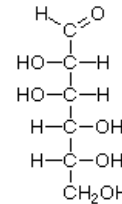
D-Glucose
(an aldose)



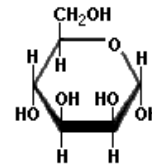
α -D-Glucose



β -D-Glucose



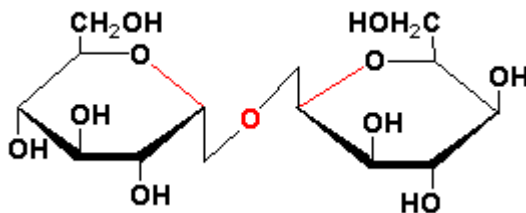
D-Mannose



Mannose

- B. α,α -Trehalose (α -D-glucopyranosyl (1 \rightarrow 1) α -D glucopyranose) is a major circulatory sugar that is used for energy in insects. Draw this sugar in Haworth projection.

2 points



6. A. Briefly discuss at least two ways that the availability of synchrotron radiation sources have impacted the field of structural biology.

1 point
each

1. High intensity light sources for use of small crystals
2. High intensity light sources to do time-resolved crystallography
3. Tunable wavelength sources to accomplish MAD phasing

(only need
2 of the 3)

B. Dr. Kitto and his group isolated a protein from a sea creature in 1968 that produces a gas when given aromatic amino acids. The protein is active for many years, but besides its long term stability relatively little is known about it - unknown sequence, unknown molecular weight. A new graduate student in the lab recently crystallized the protein and the X-ray tech on level five reported that "Kittoase" crystallizes in an orthorhombic space group P222 with unit cell dimensions of $65.0\text{\AA} \times 85.4\text{\AA} \times 117.7\text{\AA}$. The tech told the new student that there were probably four copies of the molecule in the unit cell, that he could measure Bragg reflections out to $0\ 0\ 55$, and that she should assume that the crystal was 45% solvent and could use an average density of 1.37 g/cm^3 to estimate the molecular weight of the protein.

a. What is the resolution limit for this crystal data set?

2 points

$$\text{Resolution limit} = \frac{117.7}{55} = 2.41\ \text{\AA}$$

b. What is the value that the student should come up with for the estimated molecular weight of Kittoase?

4 points

1. Volume of unit cell = $65.0 \times 85.4 \times 117.7\ \text{\AA}^3 = 653,000\ \text{\AA}^3$

2. Volume of protein content in unit cell
55% protein so $0.55 \times 653,000\ \text{\AA}^3 = 359,000\ \text{\AA}^3$

3. Volume of asym. Unit ($z = 4$)
volume of molecule $\approx 90,000\ \text{\AA}^3$

4. Mass of one molecule
 $m \approx \frac{1.37\text{ g}}{\text{cm}^3} \times \frac{1\text{ cm}^3}{10^{24}\text{\AA}^3} \times \frac{9 \times 10^4\ \text{\AA}^3}{\text{molecule}} = 1.23 \times 10^{-19}\text{ g/molecule}$

5. M.W. $\approx \frac{1.23 \times 10^{-19}\text{ g}}{\text{molecule}} \times \frac{6.02 \times 10^{23}\text{ molecules}}{\text{mole}} = 74,000\text{ g/mol}$

7. A. When a ^{14}N nucleus is placed in a magnetic field, it can assume any of three possible NMR energy states.

1 point

a. What is the nuclear spin (I) of ^{14}N ?

1

1 point

b. What are the allowed values of the magnetic quantum number "m" for ^{14}N ?

-1, 0, 1

B. What specific aspect of protein structure can be derived from a quantitative measurement of the nuclear Overhauser effect (NOE) between protons?

1 point

Identify interacting nuclei that are within $\sim 6 \text{ \AA}$ of each other

C. The Larmor equation states that the NMR frequency of ^1H nuclei is proportional to the magnetic field strength:

$$\nu = \frac{\gamma H}{2\pi}$$

However, the ^1H nuclei within a protein all have slightly different NMR frequencies when placed in a constant applied magnetic field. Briefly explain this apparent paradox.

2 points

The effective magnetic field of each nuclei varies due to local shielding effects, i.e. the unique chemical (or magnetic) environment of each proton.

D. When structures of protein determined by NMR methods are presented in the literature, it is common to show a superposition of a set of structures, such as shown below. What important point about the protein is illustrated by this set of structures?

2 points

Areas that are closely aligned are well defined by the NMR model, while those areas that show great variation are not well determined by NMR experiment.



7 E. Briefly contrast how the “phase problem” is overcome in the determination of structures by X-ray crystallography vs. overcoming the “assignment problem” in determining the structure of a protein by NMR methods.

2 points

The “phase problem” in crystallography needs to be overcome so that the measured amplitudes can be used to compute an electron density map. The “recovery” of the lost phase information is usually done by one of three methods:

1. MIR (multiple isomorphous replacement)
2. MR (molecular replacement)
3. MAD (multiple anomalous dispersion)

Using a good estimate of the phase with each measured amplitude allows for the direct visualization of the protein via its electron density map.

2 points

NMR is an indirect method of obtaining structural information. NOE measurements can provide a list of distance restraints to help obtain a model by simulated annealing methods. However, the NOE peaks must first be “assigned” to particular interacting nuclei. Assignments can usually be made by collecting and interpreting 2D or 3D NMR spectra.