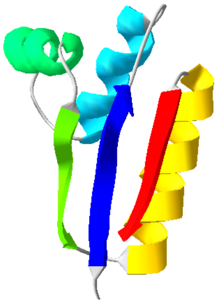
(1) Identify the secondary structure described in each of the following statements:

a. A coiled peptide chain held in place by hydrogen bonding between peptide bonds in the same chain
b. A structure that has hydrogen bonds between polypeptide chains arranged side by side

(2) Below, you can find the structure of the C-terminal domain of protein L7/12. It is coloured blue to red from its N- to C-terminus.

a. Provide the order of secondary structure elements by drawing a topology diagram.
b. Classify the secondary structure formed by the red, blue and green segments.
c. What type of domain is this? (Consider the four types we discussed in class).



(3) The primary determinant of the secondary structure of polypeptides is:

a. Hydrogen bonding between peptide groups
b. The hydrophobic effect
c. Covalent bond formation during the folding process
d. Attractive interactions between side groups of amino acid residues

(4) For each of the following residue pairs, identify the type of energetic interaction likely to occur between their side chains:

a. Leu and Val
b. Tyr and Thr
c. Asp and Arg
d. Cys and Cys

(5) Complete the following sentences:

a. Proteins possess quaternary structure only if ____.
b. The destruction of secondary, tertiary, or quaternary structures of a protein through conformational changes is by a process called ______________________.

(6) What kinds of interactions do NOT contribute to the tertiary structure of proteins?

- a. peptide bonds
- b. disulfide bridges
- c. Hydrogen bonds
- d. salt bridges
- e. Van der Waal's interactions

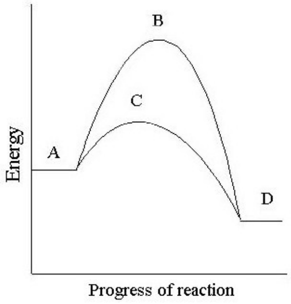
(7) Enzymes are potent catalysts because they:

- a. are consumed in the reactions they catalyze.
- b. are very specific and can prevent the conversion of products back to substrates.
- c. drive reactions to completion while other catalysts drive reactions to equilibrium.
- d. increase the equilibrium constants for the reactions they catalyze.
- e. lower the activation energy for the reactions they catalyze.

(8) The role of an enzyme in an enzyme-catalyzed reaction is to:

- a. bind a substrate analog that cannot be converted to product.
- b. ensure that all of the substrate is converted to product.
- c. ensure that the product is more stable than the substrate.
- d. increase the rate at which substrate is converted into product.
- e. make the free-energy change for the reaction more favorable.

(9) Identify each of the designated regions on the energy diagram for the conversion of a substrate to product with and without an enzyme.



(10) Consider an α-helix of 20 amino acid residues compared to a β-strand of 20 amino acids (which is of course part of a β-sheet).

- a. What is the length of this α-helix in Å?
- b. What is the length of the β-strand in Å?

(11) Each of the following reagents or conditions will denature a protein. For each, describe in one or two sentences what the reagent/condition does to destroy native protein structure.

- a. urea
- b. high temperature
- c. detergent
- d. low pH

(12) When a polypeptide is in its native conformation, there are weak interactions between its R groups. However, when it is denatured there are similar interactions between the protein groups and water. What then accounts for the greater stability of the native conformation?

(13) The sweet taste of freshly picked corn (maize) is due to the high level of sugar in the kernels. Store-bought corn (several days after picking) is not as sweet, because about 50% of the free sugar is converted to starch within one day of picking. To preserve the sweetness of fresh corn, the husked ears can be immersed in boiling water for a few minutes ("blanched") then cooled in cold water Corn processed in this way and stored in a freezer maintains its sweetness.

What is the biochemical basis for this procedure?

(14) Cell membranes contain proteins. Some of these proteins, involved in the transport of molecules across the membrane into the cell, span the entire membrane and are called trans-membrane proteins (or integral membrane proteins). The interior of the cell membrane is hydrophobic and nonpolar, whereas both the extracellular and intracellular fluid are water-based.

a. List 3 amino acid you would expect to find at the surface of the trans-membrane protein in the part that lies within the cell membrane. Explain your choice with one sentence.
b. List 3 amino acids you would expect to find at the surface of the trans-membrane protein in the part that lies outside the cell. Explain your choice with one sentence.

(15) Gelatin is processed collagen that comes from the joints of animals. Collagen is a stable protein consisting of a triple helix – three polypeptide chains wound around each other. When gelatin is mixed with hot water, the triple helix structure unwinds and the chains separate becoming random coils that dissolve in the water. As the dissolved gelatin mixture cools, the collagen forms a matrix that traps water; as a result, the mixture turns into the jiggling semi-solid mass that is recognizable as Jell-O™. The directions on a box of gelatin include the following: "Chill until slightly thickened, then add 1 to 2 cups cooked or raw fruits or vegetables. Fresh or frozen pineapple must be cooked before adding". If the pineapple is not cooked, the gelatin will not set properly.

Pineapple belongs to a group of plants called Bromeliads. Pineapple contains a protease called bromelin. Proteases hydrolyze peptide bonds.

Explain (couple sentences) why pineapple must be cooked before adding to gelatin.

(16) Ingestion of methanol is a medical emergency. It is often treated by the administration of ethanol, which prevents the dangerous effects of methanol

metabolism. In the body, methanol is oxidized to formaldehyde (methanal), a toxic molecule that cannot be further oxidized and that damages proteins. Ethanol is oxidized by the same enzyme (alcohol dehydrogenase), to acetaldehyde, a metabolite that can be further processed. If ethanol and methanol are substrates for the same enzyme, how does ethanol prevent the oxidation of methanol?

(17) The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20°C by a factor of 10^{14}. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20°C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? (Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.)

(18) Give brief definitions or unique descriptions of the following terms:

(a) neutral drift
(b) sickle cell trait
(c) secondary structure
(d) ψ torsion angle
(e) domain
(f) quaternary structure
(g) prosthetic
(h) apoenzyme

(19) Peptide bonds are planar and adopt either *cis* or *trans* conformations. Draw a peptide bond in the energetically most favourable conformation.

(20) Consider the Isoleucylleucine dipeptide. Draw the structure of the dipeptide when the isoleucyl ψ torsion angle is 0º and the leucine ϕ torsion angle is 180º.

(21) The Ramachandran plot (ϕ vs. ψ torsion angle) shows the energetically favourable backbone conformations of proteins. Answer the following questions: For each of the following ϕ, ψ torsion angles

(a) Indicate the region where residues with α-helical conformations are located.
(b) If a Trp residue has $\phi = 60^\circ$, $\psi = -120^\circ$, is it in an energetically favourable conformation?
(c) If a Gly residue has $\phi = 120^\circ$, $\psi = -60^\circ$, is it in an energetically favourable conformation?

(22) Pauling predicted the structures of both α-helices and β-sheets from modeling studies. What physiochemical properties do α-helices and β-sheets share?

(23) Draw a β-sheet composed of two parallel β-strands that are each 4 residues in length. Indicate all H-bonds between backbone atoms. Note: Use R for each side chain.

(24) How are amino acid residues spatially distributed in soluble, globular

proteins?

(25) What are hydrogen bonds and how do they contribute to the folded state of polypeptides.

(26) Disulfide bonds confer additional structural stability to the folded state.
  (a) What types or groups of protein typically contain disulfide bonds?
  (b) Draw the structure of a disulfide bond.

(27) Draw a 2D representation (topology diagram) of the following structures:
  (a) parallel bundle
  (b) antiparallel barrel

(28) Several types of compounds or conditions promote protein unfolding or denaturation. Identify three classes of denaturants and their mechanism of action.

(29) What are the two major hypothesis that describe the interaction of enzymes and their substrates? How do they differ?

(30) The affinity of hemoglobin for dissolved O_2 is regulated by multiple different effector molecules.
  (a) Dissolved O_2 binding to Hemoglobin increases the protein's affinity for dissolved O_2. What type of effector molecule O_2 and what is the type of regulation.
  (b) 2,3 bisphosphoglycerate (2,3-BPG) binding to Hemoglobin increases the protein's affinity for dissolved O_2. What type of effector molecule is 2,3-BPG and what is the type of regulation.

(31) Draw a suitable Reaction Coordinate for a spontaeous overall reaction composed of two elementary steps in which the first step is rate determining. Label all elements of the plot.

(32) Write an overall reaction that has a second order reaction rate constant.

(33) Consider the following reaction mechanism.

$$A \xrightarrow{k_1} I_1 \xrightarrow{k_2} I_2 \xrightarrow{k_3} P$$

Draw a reaction coordinate consistant with the following information:

The overall reaction is spontaneous. The elementary reaction producing I_2 is not spontaneous. The rate constants for the individual steps have the following relationship, $k_1 = k_3 >> k_2$.

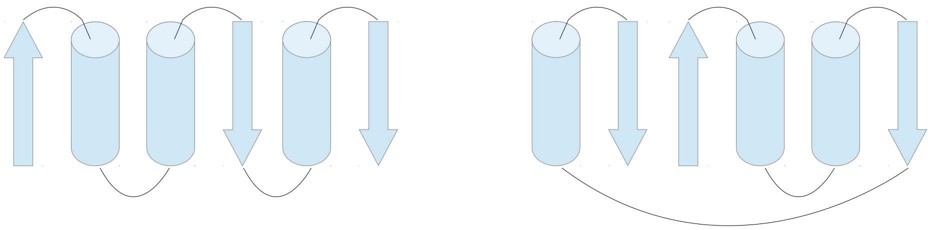
Answers:

(1)

a. α helix
b. β-pleated sheet

(2)

a. Either representation would be considered correct



Comment: The sequence of β-α-α-β-α-β must be correct as well as the orientation of the β-strands. For us, the spatial location of each secondary structure will not matter in the topology diagram.

b. Antiparallel β-sheet
c. α + β domain (because of the antiparallel β-sheet)

(3)

a. Hydrogen bonding between peptide groups

(4)

a. Leu and Val	Van der Waal's interactions
b. Tyr and Thr	Hydrogen bond
c. Asp and Arg	Salt bridge
d. Cys and Cys	Disulfide Bridge

(5)

a. Proteins possess quaternary structure only if <u>they consist of two or more polypeptide chains (subunits).</u>

b. The destruction of secondary, tertiary, or quaternary structures of a protein through conformational changes is by a process called <u>denaturation .</u>

(6)

a. peptide bonds

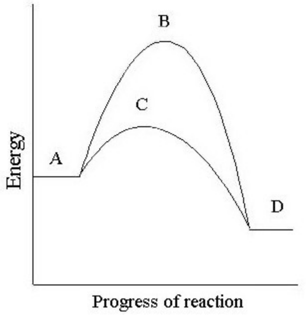
(7)

e. lower the activation energy for the reactions they catalyse.

(8)

d. increase the rate at which substrate is converted into product.

(9)



A energy of substrate

B activation energy without enzyme

C activation energy with enzyme

D energy of produc

(10)

a. The α helix makes 20 residues / 3.6 residues per turn = 5.56 turns, and thus is 5.56 turn * 5.4 Å per turn = 30 Å long.
b. In a β strand the distance between two adjacent Cα atoms is 3.5 Å, thus the β strand is 20 * 3.5 Å = 70 Å long.

(11)

a. Urea acts primarily by disrupting hydrophobic interactions.
b. High temperature provides thermal energy greater than the strength of the weak interactions (hydrogen bonds, electrostatic interactions, hydrophobic interactions, and van der Waals forces), breaking these interactions.
c. Detergents bind to hydrophobic regions of the protein, preventing hydrophobic interactions among several hydrophobic patches on the native protein.
d. Low pH causes protonation of the side chains of Asp, Glu, and His, preventing electrostatic interactions.

(12) In the unfolded polypeptide, there are ordered solvation shells of water around the protein groups. The number of water molecules involved in such ordered shells is reduced when the protein folds, resulting in higher entropy. Hence, the lower free energy of the native conformation.

(13) The activity of the enzyme that converts sugar to starch is destroyed by heat denaturation.

(14)

a. The inner part of the cell membrane is hydrophobic / nonpolar, therefore we would expect to find nonpolar amino acids such as Ala, Val, Leu, Ile, Phe, Met, Pro, Trp at the surface of the protein in this region.
b. The extracellular fluid is water, i.e. hydrophilic / polar, therefore we would expect to find polar and charged amino acids such as Ser, Thr, Tyr, Cys, Asn, Gln, Asp, Glu, His, Lys, Arg at the surface of the protein in this region.

(15) When pineapple is cooked, the high temperature causes heat **denaturation** of the protease bromelin contained in pineapple. Thereby, the protease becomes **inactive**. Without cooking, the protease in fresh or frozen pineapple is still active and **hydrolyzes the gelatin – i.e. collagen** which is a protein. Thereby the primary structure of collagen is destroyed. Hydrolyzed gelatin consists of amino acids and short oligopeptides which can no longer form a gel because the peptide fragments are too small.

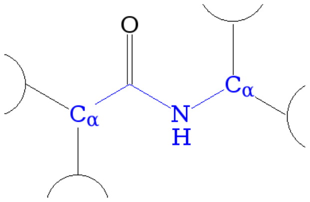
(16) Methanol and ethanol **compete for the active site** of alcohol dehydrogenase, they are **both substrate** for this enzyme. If **ethanol is present in excess** over methanol, it will "win this competition" thereby preventing methanol from being oxidized. (Instead methanol will be secreted from the body.)

(17) 5 min * 10^{14} = 5 10^{14} min = 8.333 * 10^{12} h = 3.47 10^{11} days = 9.5 * 10^{8} years

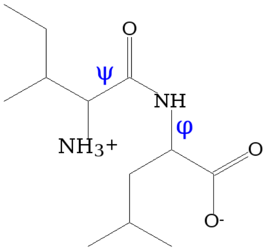
(18)

a. Random mutations in a well adapted protein that do not affect function.
b. Disease characterized by one normal and one mutant allele encoding HbS.
c. Regular, repeating backbone conformation in a protein.
d. Torsion angle defined by the N – C_a – C – N atoms.
e. Groupings of secondary structures that adopt the same conformation in unrelated proteins.
f. Spatial arrangement of subunits and their side chains in a multi subunit protein.
g. a cofactor that is permanently associated with its protein
h. an inactive protein or enzyme in the absence of its cofactor

(19) The trans conformation is the most stable peptide bond conformation.

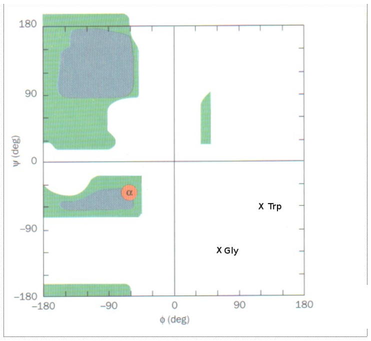


(20) The isoleucyl psi angle is defined by the N – C_α – C – N atoms.
The leucyl phi angle is defined by the C – N – C_α – C atoms.



(21)

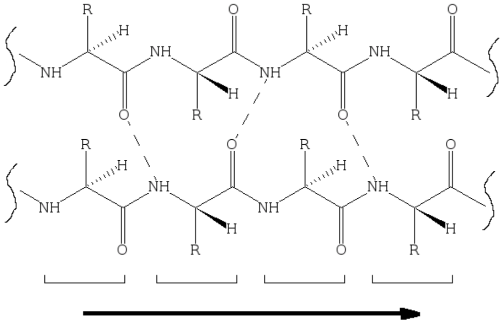
a. α-helical residues have ϕ, ψ torsion angles of roughly -60º, -45º.
b. This is an energetically unfavourable conformation.
c. This is an energetically unfavourable conformation for all residues except Gly.



(22) Secondary structures

(1) adopt favourable backbone conformations,
(2) satisfy the hydrogen bonding potential of the backbone and
(3) have optimal van der Waal's packing interactions.

(23) The arrow shows the direction of the individual strands and the four residues in each strand are indicated (not required but added for clarity). Hydrogen bonds are shown as dashed lines.



(24) There are three classes of amino acid residues (Voet & Voet); polar charged, polar uncharged and non-polar.

Polar charged residues – occur almost exclusively on the surface.

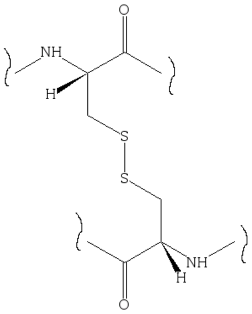
Polar uncharged residues – typically occur on the surface of proteins though they can be found in the hydrophobic core where they are involved in hydrogen bonding interactions that counter their polar nature.

Non-polar residues – fill the hydrophobic core of proteins and are frequently found on the surface of proteins.

(25) Hydrogen bonds are short, electrostatic interactions between a potential acid and a potential base. The interaction distance is less than the sum of the van der Waal's radii of the atoms and is said to have partial covalent bond character. Hydrogen bonds make minor contributions to the stability of the folded state of proteins (10-20% of the total). However, hydrogen bonds are critical for selecting the single folded state corresponding to the native fold. The native fold minimizes the number of unsatisfied H-bonds in the hydrophobic core.

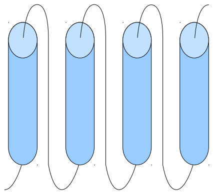
(26) a. Disulfide bonds typically occur in two types of proteins:
  (1) proteins that are secreted into the relatively more oxidizing extracellular medium
  (2) small proteins that do not have a sufficiently large hydrophobic core to stabilize the folded state

b.

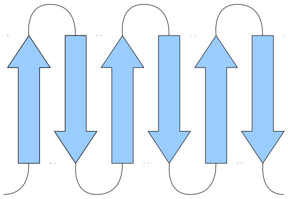


(27)

a. Only α-helices form bundles. The helices are parallel in a parallel bundle.



b. Only β-sheets of at least 6 strands form barrels. The strands are antiparallel in an antiparallel barrel.



(28) Any three of the following
(1) pH extremes – alter ionization states and H bonding
(2) detergents – associate with nonpolar residues and disrupt hydrophobic core
(3) water soluble organics – interfere with hydrophobic forces
(4) chaotropic salts – increase solubility of nonpolar substances and disrupt hydrophobic core
(5) heat – kinetic energy overcomes energy of folded state

(29)

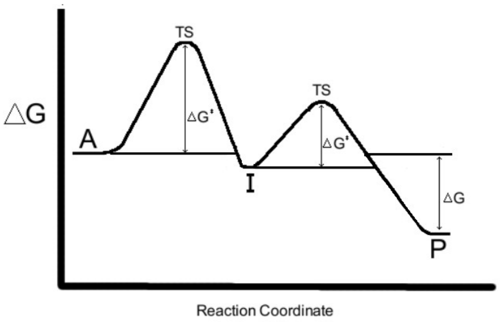
(i) Lock & Key Hypothesis – suggests enzymes have rigid, preformed active sites that do not undergo changes upon substrate binding.
(ii) Induced Fit Hypothesis – suggests enzymes have flexible active sites that adapt their shape to the substrate they bind.

The two models differ with respect to the shape of the active site prior to substrate binding and whether or not the active site undergoes conformational changes upon substrate binding. Experimental evidence suggests most enzyme active sites are largely preformed and only undergo subtly conformational changes upon substrate binding.

(30) a. O_2 is a homotropic activator and is a cooperative regulator of Hb activity.
b. 2,3-BPG is a heterotropic acitivator and is an allosteric regulator of Hb activity.

(31) The reaction is spontaneous as the relative energy of 'P' is lower than that 'A'.

The 1st elementary reaction is rate limiting as its activation energy barrier ($\Delta G^{\ddagger}$) is larger than that of step two.



(32) A reaction will have a second order rate constant if the reaction rate depends upon the concentration of two reactants.

$A + B \text{ -----> } P$ or $2A \text{ -----> } P$

(33) The overall reaction is spontaneous as the relative energy of 'P' is lower than that of 'A'. The elementary reaction producing I_2 is not spontaneous as the relative energy of 'I_2' is higher than that of 'I_1'.

The reaction rate constants k_1 and k_3 are equal as their activation energy barriers are equal. The reaction rate constant k_2 is lower than that of k_1 and k_3 as its activation energy barrier is much larger.