

Chem 452 - Fall 2012 - Exam I

Potentially useful information:

pK_a values for ionizable groups in proteins: (α -carboxyl, 3.1; α -amino, 8.0; *Aspartic acid (Asp)* & *Glutamic acid (Glu)* side chains, 4.1; *Histidine (His)* side chain, 6.0; *Cysteine (Cys)* side chain, 8.3; *Tyrosine (Tyr)* side chain, 10.9; *Lysine (Lys)* side chain, 10.8; *Arginine (Arg)* side chain, 12.5)
 Minutes available for taking this exam = 50 min

1. Biochemistry is the study of the chemistry of life processes. From the biology courses you have taken, you no doubt have learned that biological systems are quite diverse, from the extremely small, single cell prokaryotes, such as bacteria, to large multicellular eukaryotes, such as yourself. In a paragraph, explain how evolution has made this course easier for you.

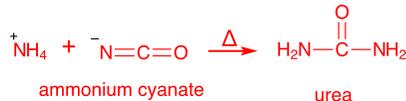
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(What I am looking for here is a concise argument that at the molecular level, all living systems look remarkably similar. Since biochemistry is the area of biology that studies life processes at the molecular level, it is therefore less important to be concerned with what organism(s) we are actually studying. This is due to the accepted theory of evolution that represents the core around which all biology is organized, and that theory states that all current life forms found on earth share a single common ancestor.)

(This was the "Question of the Day" for Lecture 1, Part 1.)

2. Ammonium cyanate is an inorganic salt.
 a. Write the chemical equation for the reaction that takes place when this salt is heated.

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- b. Explain the significance of this reaction.

The significance of this reaction is that it demonstrated that an organic molecule (urea) could be synthesized in the lab from an inorganic substance (ammonium cyanate). At the time there was a widespread belief that organic molecules could only be synthesized by living organisms. This reaction helped to demonstrate that this was not the case and that both living organisms and inanimate objects shared a common chemistry.

- c. Who, in 1828, was credited with recognizing the significance of this reaction? Frederich Wöhler

3. In their 1953 landmark article, published in the journal *Nature*, James Watson and Francis Crick proposed a 3-dimensional structure for DNA.
 a. In words, describe this structure. Be sure to include a description of the chemical components that make up a DNA molecule and the 3-dimensional arrangement of these components.

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A DNA molecule comprises two polynucleotide chains that are intertwined with one another to form a double-helix. Each polynucleotide chain is a linear polymer of nucleotide monomer. Each nucleotide contains a phosphate attached to a ribose sugar. The sugar is also attached to either a purine or pyrimidine bases. These are both aromatic, nitrogen-containing heterocyclic rings. There are two different purines that are used, adenine and guanine, as well two different pyrimidines, uracil and cytosine. The polymer chain is made by linking the deoxyribose sugar of one nucleotide to the deoxyribose sugar of the next using the phosphate, which forms a phosphodiester bridge between the two. The nucleotides then dangle as side chains from each of the sugars. When the double-helix forms, the phosphodeoxyribose main chains spiral about one another on the outside of the molecule, like the hand rail on a spiral staircase. The aromatic bases are packed to the inside and stacked on top of one another like the stairs of the spiral staircase. They also form hydrogen bonded to the aromatic base from the other chain that is located across from them. Adenine bases are always paired with uracil bases, while the guanine bases are always paired with cytosine bases.

- b. Describe three pieces of evidence that Watson and Crick used to support their proposed structure

- i. The X-ray fiber data of Maurice Wilkins and Rosalind Franklin indicated that the molecule was a helical. It also showed that the nucleotides were arranged in a stack on top of one another. There data also showed that the helix make one turn every 34Å and that there are 10 stacked nucleotides per turn.
- ii. The chemical analyses of Erwin Chargaff showed that the ratio of purines the pyrimidines was always 1 to 1, regardless of the source of the DNA, and in particular, the percentage of adenine was always equal to that of uracil, while the percentage of guanine was always equal to that of cytosine.

(Topics is related to the Homework Assignment 1 and the "Questions of the Day" for Lecture 1, Part 2)

- iii. They recognized that the phosphates were in their negatively charged, salt forms. This caused them to realize that the phosphates from the opposite chains should be placed as far apart from one another as possible.
- c. In their article, Watson and Crick made the following statement, which alludes to a structure/function relationship for their proposed model: “*It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.*” In a short paragraph, describe how the 3-dimensional structure of the DNA molecule makes it particularly well-suited to carrying out its biological role.
- It was known at the time that Watson & Crick made their proposal, that DNA represented the chemical form of genetic information. When a cell divides, it must somehow duplicate this genetic information so that each daughter cell may receive a copy. Watson and Crick’s proposed structure for DNA, with its two stranded double-helix and specific base-pairing between the strands in the double-helix, provided an elegant solution to this problem; if the two strands are separated from one another, then each contains a complementary form of the same information, which is contained in the sequence of bases on each of the strands. This genetic information can therefore be duplicated by separating the strands and then using each as a template to synthesize a new complementary strand.
- d. Since you have read Watson and Crick’s article, tell me how many pages in length it is. 1 page
4. We have also discussed the 3-dimensional structures of proteins.
- a. In this discussion, we described the 3-dimensional structures of proteins in terms of a hierarchy containing up to four levels of structure. Name each of these levels in order of increasing complexity. In words, describe the structural characteristics of each level. Also, identify the interactions, or bonds, that stabilize each level; your choices include, *covalent, charge/charge, dipole/dipole, hydrogen bonding, vander Waals interactions* and *hydrophobic interactions*. In addition, indicate the players that participate in these interactions; your choices include *polypeptide backbone* and/or *amino acid sidechains*.

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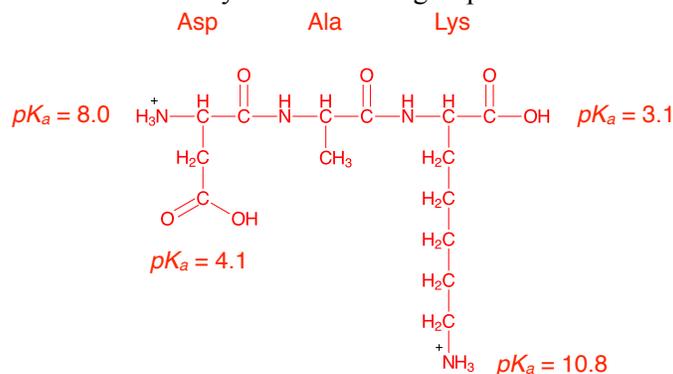
Name of the Level	Description	Interactions (Bonds)	The Players
Primary	The primary structure for a protein is represented by the sequence of amino acid in the polypeptide.	The <i>covalent</i> amide bonds bonds that form between the α -amino group of one amino acid residue and the α -carboxyl group of adjacent amino acid residue	<i>polypeptide backbone</i>
Secondary	The secondary structures comprise periodic structures that are found in proteins and includes the α -helices and β -sheets.	<i>hydrogen bonds</i> that form between peptide amide N-H (donor) and the peptide amide carbonyl oxygen (acceptor)	<i>polypeptide backbone</i>
Tertiary	The tertiary structure of a protein is its three-dimensional fold.	Non-covalent <i>charge/charge, dipole/dipole, hydrogen bonding, vander Waals interactions and hydrophobic interactions.</i>	Primarily the <i>amino acid side chains</i>
Quaternary	The quaternary structure of a protein is association of multiple polypeptides, each of which have one or more of the other levels of structure.	Non-covalent <i>charge/charge, dipole/dipole, hydrogen bonding, vander Waals interactions and hydrophobic interactions.</i>	Primarily the <i>amino acid side chains</i>

- b. Which interaction(s) identified above are weakened by the presence of water? (List them.)
charge/charge, dipole/dipole and vander Waals interactions (All of these interactions are electrostatic in nature and therefore, according to Coulomb's Law, are influenced by the dielectric constant of the surrounding medium. Water has a very high dielectric constant, which greatly diminishes the strength of these interactions.)
- c. Which interaction(s) identified above require the presence of water? (List them)
hydrophobic interactions (Hydrophobic literally means "water fearing", therefore, water needs to be present so there is something to fear. When nonpolar substances, which do not interact favorably with water, are exposed to water, the water will work to push them aside to minimize its contact with them. This is why oil and water separate from one another when mixed. Hydrophobic interactions are the driving force behind many of the important structure forming processes in biology, such as the formation of the DNA double-helix, the folding of a protein into its functional 3-dimensional form from a linear polypeptide, and the assembly of the mitotic spindle that is required during cell division.)
- d. Whereas DNA is made as a polymer with four different options for each nucleotide residue, a protein is made as a polymer with twenty different options for each amino acid residue. As you did for DNA in the Question 3c, in a short paragraph, describe how the 3-dimensional structures of proteins make them particularly well-suited to carrying out their biological roles.
 The four nucleotides that are used to make DNA have very similar chemical and physical properties. Consequently, DNA is chemically inert and its structure looks pretty much the same, regardless of the nucleotide sequence and the genetic information contained in that sequence. For example, DNA can be compared to books, which like DNA can contain a wide range of information, but the books themselves have very similar shapes. This allows us to easily store and access the information by arranging the books on shelves in a library. Proteins, on the other hand, are made of twenty different amino acids that have a very diverse range of chemical and physical properties. As a consequence, varying the sequence of amino acids in a protein can lead to a wide range of structures having different shapes and different functions. This makes them well suited to their role as the cellular work horses, where they must carry out a very wide range of activities, including, catalyzing chemical reactions, moving materials in and out of the cell and around the inside of a cell, transferring signals from one place to another, fighting off foreign invaders, and the list goes on.

5. A protease is an enzyme that breaks down proteins by catalyzing the hydrolysis of a peptide bond in the targeted protein. How might this protease bind the targeted protein so that its main chain becomes fully extended in the vicinity of the peptide bond that will be hydrolyzed?

In a folded protein, the stretches of the polypeptide backbone that find themselves in the strands that make up β -sheets are nearly fully extended, with hydrogen bonding between the strands. A protease could take advantage of this by adding the portion of the polypeptide in the target protein that is to be cleave as an additional strand to an existing β -sheet located near the active site of the protease.

6. Draw the *chemical structure* of the tripeptide **Asp-Ala-Lys** in its fully protonated (acidic) form. The pK_a values for the various ionizable groups on this tripeptide can be found on Page 1. If you do this correctly, you should be able to identify four ionizable groups.



- a. What is the net charge on the fully protonated structure that you have drawn? +2

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(This is Problem 17 from the Chapter 2 assigned problems.)

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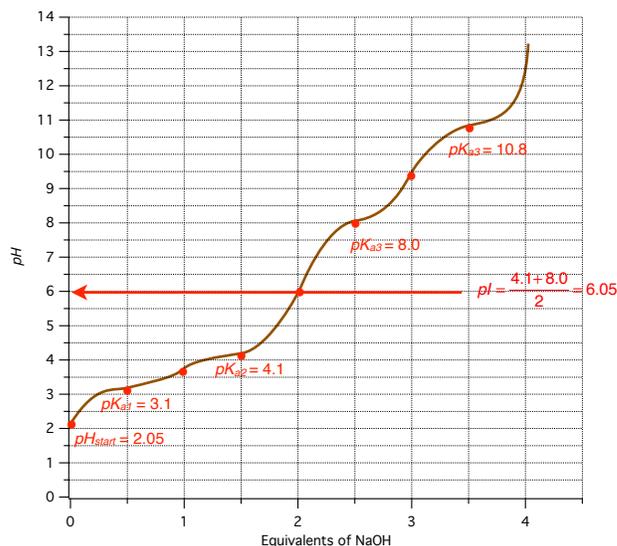
- b. Estimate the pH for a 0.10 M solution of this fully protonated tripeptide.

(This is similar to Problem 7 from the assigned problem for Chapter 1. It is also similar to Question 2 from Quiz 1)

$$[H^+] \approx \sqrt{K_a \cdot C} = \sqrt{(10^{-3.1}) \cdot (0.1 \text{ M})}$$

$$\approx 8.91 \times 10^{-3} \text{ M}$$

$$pH = -\log(8.91 \times 10^{-3}) = 2.05$$



- c. Sketch a titration curve for the titration of all four ionizable groups with NaOH.
- d. From your sketch, estimate the pI for the tripeptide. (The pI is the pH at which the net charge on the peptide is zero. $pI = \underline{6.05}$)
- e. If I ask this same question each year, but change the sequence I use each time I ask it, how many years will it take me to exhaust all of the possible choices for the tripeptide sequence? 8000 yr

$$20 \times 20 \times 20 = 8000 \text{ yr}$$

7. The 3-dimensional, tertiary fold of a protein is determined by its amino acid sequence.
- a. Who is credited with demonstrating this using the protein *Ribonuclease A*?

Christian Anfinsen

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- b. *Ribonuclease A* contains four disulfide bonds; in words, describe how disulfide bonds are formed.

The disulfide bonds form between the sulfhydryl (thiol) groups of two cysteine residues. This is an oxidation reactions in which an equivalent of a dihydrogen is removed (H_2) along with the formation of the a covalent bond between the two sulfur atoms. The effect of forming disulfide bonds is to cross link the polypeptide chain. This helps to stabilize the tertiary fold of the protein.

- c. The individual, whom you identified in part a, obtained quite different results when reduced and denatured *Ribonuclease A* was reoxidized while still exposed to 8M urea compared to when it reoxidized after first removing the 8M urea. In the first case, only 1% of the enzymatic activity of the native protein returned compared to 100% in the second case. Why were the outcomes so different depending on presence or absence of urea during the reoxidation step.

In the first case, the disulfide bonds were allowed to form when the polypeptide was still in the unfolded state. The presence of the urea keeps the polypeptide in the unfolded state. Since there are a total of 8 cysteine residues involved, there are 105 different permutations of how the disulfide bonds could form. (To see how this works, starting with one of the cysteines, there are 7 possible ways to form the first disulfide bond. After this bond forms, this leaves 6 free cysteines. Again, picking one there are now 5 possible ways to form the second disulfide bond, then 3 different ways to form the third and only one way left to form the last. Therefore, there are $7 \times 5 \times 3 \times 1 = 105$ different ways to form the disulfide bonds for the unfolded polypeptide, and only one of these, or $1/105 = 1\%$, is correct. The 99% of polypeptides containing the incorrect pairings of cysteines are unable to adopt the correct tertiary fold that is needed for there to be a functional enzyme.

If, on the other hand, the polypeptide is allowed to fold first into its correct tertiary structure, then each the cysteines will find themselves next to their correct partner, and only the correct set of disulfide bonds will form.

(This is Problem 31 from the assigned problems for Chapter 2)

8. Extra credit:

- a. Ask the one question that you were dying to answer on this exam, but which I failed to ask. You will receive up to 1 points for asking a clever and insightful question.

+3

- b. You will receive up to 2 points for answering your question correctly.

100/100 +3