Biochemistry of Proteins CHEM 4630

February 12, 2013

Term Test-1

Answer all questions in the Exam Booklets. Put your name and student number on all exam booklets. You may use a non-programmable calculator. Draw <u>structures</u> and <u>diagrams</u> where appropriate.

The total number of marks is 54 and you have 75 minutes to complete the exam.

Answer question 1. It is worth 5 marks.

1. Draw the chemical structure at pH 7 of the <u>one</u> of the peptides that results from treatment of the following peptide with cyanogen bromide in 90% formic acid:

Gly-Ile-Pro-Met-Leu-Tyr-Gln

Answer question 2. It is worth 2 marks.

2. In the following peptide fragment, 4 atoms are labeled w, x, y, and z. Indicate the order of protonation of the atoms in strongly acidic solution.



Answer question 3 <u>OR</u> question 4. Each is worth 15 marks.

- 3. Outline a protocol for amino acid analysis of a protein and describe in chemical detail peptide hydrolysis by strong acid. What problem arises in amino acid analysis of proteins containing β-branched dipeptides? What can be done about this?
- 4. Describe in chemical detail the main steps of an Edman degradation indicating the role and importance of pH, buffers, and organic solvents.

Answer question 5. It is worth 6 marks.

5. A 100 micromolar solution of a protein comprising a single polypeptide chain has an absorbance in a 1 cm cuvette at 290 nm of 0.360 at pH 7 and an absorbance of 1.848 at pH 12. The molar extinction coefficient for tyrosinate at pH 12 is 2480 M⁻¹ cm⁻¹ and for Tyrosine at pH 7 is 0. How many tyrosine residues are in this protein? **For full marks show your work**. Draw the structure of tyrosinate.

Answer question 6 <u>OR</u> question 7. Each is worth 10 marks.

- Briefly describe Affinity Tag Purification of proteins mentioning some advantages and disadvantages. Be specific. Explain how metal-chelate affinity chromatography is used to purify proteins.
- 7. What is an osmolyte? Why is proline a good osmolyte? How does it work?

Answer question 8. It is worth 12 marks.

8. Describe the forces that are applied to a protein in a sedimentation velocity experiment with the use of the following equations:

$$F=m\omega^2 r; \quad F=-m_o\omega^2 r; \quad F=-fv$$

Explain Stokes Law: $f = 6\pi\eta r$

Explain why the eukaryotic ribosome sediments at 80 S (not 100 S) whereas its componenets sediment at 60 S and 40 S.

Briefly explain what information about the molten globule was revealed by sedimentation velocity analysis.

Answer question 9. It is worth 4 marks.

9. What are the sites in a protein charged by electrospray ionization in positive ion mode and negative ion mode?

Explain why oxidized lysozyme shows an electrospray ionization envelope with its tallest peak containing 10 positive charges whereas the reduced, unfolded protein shows an electrospray ionization envelope with its tallest peak having 17 positive charges.

Bonus Question: 2 marks maximum

10. Name the smallest protein known to fold into a stable 3D structure. How many amino acids does it contain? How was the protein discovered?