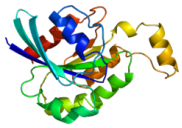
Chem 331 Biochemistry

Chapter 3

Learning Objectives, Study Guides



Learning Objectives

- Compare the 20 amino acids commonly found in proteins with each other in terms of their chemical properties.
- Describe the general properties of proteins and peptides.
- Analyze the different levels of protein structure.
- Describe several examples that illustrate the diversity of protein structures and functions.
- Be able to draw the basic structure of the amino acid and peptide bond – Do Not draw all of the side groups
- Memorize the three letter abbreviation for each amino acid
- Interpret, Analyze and Predict the chemical properties of amino acid side group
- Know the grouping based on these chemical properties
- Know the amino and carboxyl terminal pKa but only be familiar with side group general pKa
- Predict the impact of local chemical environment on pKa of amino acid side group
- Determine the charge state of an amino acid at indicated pH; including the pH at which the amino acid is a zwitterion and calculate the isoelectic point of a peptide
- Understand the importance and reactions of cysteine-cysteine bonds
- Explain the reactions of amino acids and their impact on proteins
- Evaluate the nature of the peptide bond on folding
- Understand the biologic activities of peptides
- Relate the sizes of proteins as described by the book
- Relate the two general classes of proteins to their respective structure and function
- Discriminate between the four levels of protein structure in terms of the "focus-level", bonds and roles each part of the amino acid/peptide backbone/aa side group plays in the structure
- Understand the primary amino acid sequence and why it is important
- Know the post-translational modifications which give proteins greater diversity – in chemical terms
- Summarize the main terms associated with primary structure including homology
- Analyze the peptide bond and how it impacts the predicted secondary structure – do not memorize bond angles but apply your understanding of the bond to how the two rotational angles impact structure
- Infer the limitation and use of the Ramachandran plot
- Describe the features of the peptide backbone that stabilizes and creates alpha helixes and beta-pleated sheets.
- Evaluate how some amino acids can alter the structure (stabilize or disrupt) secondary structures and some of the smaller features like beta turns.
- Describe both random coil and intrinsically unstructured domains
- Know the polarity and structural elements of secondary structure – include the impact of side groups and their relative location
- Compare the various specialty structures for their biochemical structural elements (keratin, silk, collagen…)
- Relate the change in function in collagen when primary acid, enzyme or enzyme co-factor is altered.
- Understand the thermodynamic forces and the process by which fold and maintain tertiary structure
- Comprehend the basic manner by which proteins interact to form quaternary structure
- In general terms understand the sources of starting material to purify proteins from
- Describe the factors to consider to stabilize and maintain a protein's native structure and activity
- Compare the mechanism to lyse/homogenize cells
- Distinguish between the two centrifugation methods to isolate organelles/partition lysed cells
- Know what fusion proteins are and why they are used in purification
- Explain the importance of GST and His tags and how they bind to a column chromatography
- Compare and contrast the purification methods of size exclusion, ion exchange, and affinity chromatography

- Explain how proteins are analyzed using native, and denatured electrophoresis and distinguish between the two methods
- Know HOW and why SDS, beta mercaptoethanol and heat are used in SDS PAGE applications
- Explain the use of antibodies in ELISA and westernblot
- Distinguish between monoclonal and polyclonal antibodies
- Understand and describe each set of a westernblot

Chapter 3 questions: 2, 4, 7, 8, 11, 14, 18, 19, 24, 29, 30, 31, 39, 40,

Study Notes from Dr P: *Understanding the chemistry of the side groups of amino acids is going to impact how you learn proteins, enzymes and other biochemical functions of the cell. But don't just memorize things. That is a waste of time. Instead look and interpret the side group's chemistry. Of course, you WILL need to memorize the three-letter abbreviation that is like a second language. You will need to know some of the chemical reactions like phosphorylation, reduction/oxidation, covalent modification as they will show up several times. Commit yourself to drawing a peptide with "R" for the side group. You should be able to look at an amino acid, its name or its three-letter abbreviation and describe all the biochemical features of the amino acid as a second language. We will see this knowledge coming up again and again this semester. On Protien structure: If you only focus on a description of collagen and the disease but don't focus on the MECHANISM – the amino acid sequence and why that sequence is biochemically important, you will not be detailed enough in your understanding.*

Purification - The main point of this section is to understand affinity tags/epitope tags, be able to know how to separate proteins and characterize a protein. Questions will range from simple tell about or factual issues of the chapter to a more complex question where you decide how to purify a protein from scratch using each of these tools.

Chapter Questions (not assigned for homework but to help you practice, don't turn in. BUT some may or will show up on the exam).

1) I you were conducting western blots and checking for a family of enzymes who's amino acid sequence is highly conserved, which type of antibody would you use to visualize as many of the enzymes at one time?
   a) Monoclonal antibodies
   b) Polyclonal antibodies ****
   c) Unclebodies

6) ß mercaptoethanol is used at high concentrations to:
   a) Cleave disulfide bonds ***** ***Note the high conc. At low conc it helps to prevent oxidation of proteins***
   b) Form disulfide bonds
   c) Act as a buffer
   d) Cleave peptide bonds

7) What is the purpose of the gel in SDS-PAGE?
   a) It serves as an electrical conductor
   b) Acts as a weak ion exchanger
   c) It serves as a means to physical separate the proteins based on size ***
   d) It serves to reduce the proteins onto their denatured form

8) Separation of proteins by gel filtration (SEC) chromatography take advantage of differences in:
   a) Isoelectric points of proteins
   b) The solubility of proteins
   c) The size of proteins ***
   d) The net charge of the protein
   e) all of the above

f) none of the above

9) A mixture of proteins was applied to a gel- filtration column. The exclusion range of the gel was 120,000 Da to 25,000 Da. What was the order of elution from the column? Was this a good choice for these proteins if you wanted to purify lactoglobin What if you wanted to purify Urease? WHY. (10 points)

Urease (pI = 5.1, molecular weight = 482,700)
Catalase (pI = 5.6, molecular weight = 242,500)
Lactoglobin (pI = 5.2, molecular weight = 37,100)
Hemoglobin (pI = 6.9, molecular weight = 64,500)

The largest protein will elute first regardless of pI. The charge of the protein does not enter into how SEC works. It is a red herring. The cut offs will result in Catalase and Urase both being excluded from the pores of the beads. Thus both proteins will co-elute and this is not a good gel to use for isolation of urase.

10) Many times western blotting is used to determine the difference between two proteins, which contain a high homology in their primary structure. Explain what type of antibody you would use and the general properties of that antibody.

Monoclonals are derived from specific short peptides or pieces of proteins, while polyclonal antibodies will be a mixture of many antibodies whose collective recognition will cover most if not all of the protein. Therefore, monoclonals will be selective for specific amino acid sequences.