

Chapter 5 Chapter questions Proteins Primary Structure

Study exercises 2 through 5 and chapter problems 1 through 5 and 11 (interesting thought problem)

1) If you were conducting western blots and checking for a family of enzymes whose 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) β 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 physically 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 takes 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 lactoglobulin? 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)

Lactoglobulin (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 Urease 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 urease.

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.

11) A protein that is normally an alpha helix at low pH is distorted and curves or bends at a neutral and higher pH. Why? The only unique feature about the primary sequence is that every fourth or third amino acid is glutamate. **Looking at the repetition of acidic amino acid, glycine, one would predict that the side chain would line up on one side on top of each other. While side chains are not involved in the manner in which a protein forms its secondary structure, in this case it would be easy to imagine how at low pH when the carboxyl group of the glycine side chain is charged this stack of negative charges would repel each other and thus bend. While at the same time when the pH is raised below the pKa of the side chain there would be no repulsion and thus no effect on the alpha helix.**