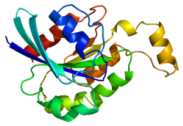
Chem 331 Biochemistry

Protein Purification

Learning Objectives, Study Guides



Learning Objectives

- 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 ***

*** if time permits coverage in class

Study Notes from Dr P: *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.