

METHODS IN CELL BIOLOGY**EXAM II, MARCH 26, 2008**

1. DEFINITIONS (30 points). Briefly (1-3 sentences, phrases, word, etc.) define the following terms or answer question.
 - A. depot effect
 - refers to the slow release of an immunogen from the adjuvant
 - B. Hybridomas
 - a cell resulting from the fusion of two different cells
 - especially in reference to the production of monoclonal antibodies
 - C. What is potential problem with determining protein concentrations based on the A_{280} value?
 - contamination with other substances—especially nucleotides and nucleic acids
 - D. protein denaturation
 - refers to the loss of protein structure or function due to unfolding (ie, loss of secondary, tertiary, or quaternary structure)
 - E. How does autoradiography differ from fluorography?
 - autoradiography is the exposure of film due to direct interactions of radiation with film
 - fluorography is an indirect exposure of the film resulting from fluorescence generated via the radiation interacting with a fluor (low energy) or a fluorescent screen (high energy)
 - F. List 2 methods that separate proteins according to their surface hydrophobicity.
 - precipitation with ammonium sulfate (salting out)
 - hydrophobic interaction chromatography
 - G. Identify two methods to localize a protein within a cell using antibodies.
 - immunofluorescence
 - immunoelectron microscopy
 - H. monoclonal antibody
 - an antibody that recognizes a single antigenic determinant (epitope)
 - produced by hybridomas between single B-cell and myeloma cell
 - I. B-cell epitope
 - the amino acid residues (or other portions of a molecule) recognized by the antibody
 - generally 5-8 hydrophilic amino acid residues
 - J. Blocked amino terminus
 - refers to a modified amino terminus in a protein
 - can be natural or artificial
 - prevents the sequencing of peptide

2. (20 points) Both immunoprecipitation and immunoblotting can be used in conjunction with gel electrophoresis to identify specific proteins or subunits recognized by antibodies. Many times the two methods will provide the same information about a protein and can be used interchangeably. In other situations one of the methods will work better or be more appropriate. For each of the 5 situations below indicate whether immunoblotting or immunoprecipitation is usually the better method and briefly explain why. Also indicate whether the other method could be adapted in this situation and briefly discuss any special conditions or considerations that are necessary in order for that method to work.
- A. The antibody only recognizes the native conformation of the protein.
 - immunoprecipitation will work best since SDS-PAGE will destroy the epitope
 - if possible, native gels could be carried out in an attempt to preserve the epitope
 - B. The antibody only recognizes denatured protein (i.e., a linear epitope).
 - immunoblotting will be better since denaturing conditions may have an adverse effect on the antibody
 - it may be possible to denature the protein and remove the denaturing agent to allow for immunoprecipitation
 - C. The protein is composed of different polypeptide subunits.
 - immunoblotting will have an advantage since all of the subunits will be precipitated by the antibody and only a single subunit may be recognized in immunoblotting
 - D. The protein is only soluble in SDS or urea.
 - immunoblotting will be better since the SDS and urea will interfere with the antibody
 - adaptation to immunoprecipitation will involve removing most of the urea by dialysis or adding an excess of non-ionic detergent to remove the SDS
 - E. The protein is a very minor component of a crude protein mixture.
 - immunoprecipitation will be better since the antigen can be enriched from a large quantity of extract
 - other means would be needed to enrich the protein of interest
 - the immunoprecipitated protein could be analyzed by immunoblotting

3. (50 points) Answer two of the following essay questions.

A. Describe how differential solubility can be used to distinguish integral membrane proteins from peripheral membrane proteins. Indicate the solubilization conditions (i.e., types of solutions) and how membrane proteins may be affected.

Key points:

- solubilization of integral membrane proteins requires disruption of the lipid bilayer
- non-ionic detergents are typically used to solubilize integral membrane proteins
- generally these detergents are non-denaturing
- peripheral membrane proteins can be selectively solubilized using conditions that disrupt protein-protein interactions without disrupting the lipid bilayer
- low ionic strength and alkaline conditions are generally used since this weakens hydrophobic interactions
- these conditions can be harsh and denaturing
- the procedure consists of mixing the membrane fraction with the solubilizing solutions followed by centrifugation
- the pellet can be subjected to additional solubilization conditions

B. Rabbits and mice are the two most popular choices of animals for the production of antibodies. Compare these two animals for the production of antibodies and discuss the advantages and disadvantages of each model. Indicate any particular situations in which one animal would be preferable over the other.

Key points:

- rabbits are larger and obtain more antisera
- but need more immunogen
- rabbits relatively easy to maintain
- rabbits exhibit strong immune responses
- but also have high background levels
- mice require less immunogen
- but yield very small amounts of sera
- mice are easy to maintain
- a lot of inbred strains available
- mice are ideal for production of monoclonal antibodies

C. Both ion exchange chromatography and isoelectric focusing separate proteins according to the net charge of the protein. Briefly describe the basis of separation for each technique. As part of the answer define isoelectric point and describe how it determines the charge of the protein. Which method is better for determining the isoelectric point? Which method is more easily adapted to analytical work utilizing gels?

Key points:

- isoelectric point is defined as the pH at which the net charge on the protein is 0
- at pH below the isoelectric point proteins are positive and at pH above the isoelectric point the charge is negative
- in iec proteins are passed over a column with fixed charges and will bind according to their net charge at that particular pH
- proteins are generally elute with a counter ion such as NaCl
- more highly charged proteins (as determined by their isoelectric points) are eluted at higher concentrations than the less highly charged proteins
- in ief a pH gradient is established with a solution of carrier ampholytes and the application of an electric current
- proteins will migrate in this gradient until they reach a pH which equals their isoelectric point (ie, no net charge)
- ief is better for precisely measuring the isoelectric point
- ief is more readily adapted to gels and analytical work

D. The relative molecular weight of a protein can be determined by gel electrophoresis or gel filtration. Briefly describe the basis of separation for each technique and how molecular weights are calculated. Include information about the medium used and how they are similar or different. Identify potential problems with calculating the molecular weight for each method. Which technique generally has the greatest resolution?

Key points:

- both methods use a porous medium consisting of cross-linked polymers such as acrylamide or agarose (or derivatives)
- A pore size can be defined by the concentration of polymers and degree of cross-linking
- in gel filtration the medium is formed into small spheres and small solutes can more easily enter the matrix whereas larger molecules will be excluded from the matrix
- therefore larger molecules will pass through a column composed of the medium more rapidly than smaller molecules which are retained by the matrix
- in electrophoresis the medium is polymerized so that it is solid and the molecules are forced through the medium via electrophoretic force
- therefore smaller molecules will migrate through the matrix faster than larger molecules
- in both methods a standard curve is generated with proteins of known size and the mobility of the unknown protein is extrapolated to the relative molecular mass
- native gel electrophoresis is sensitive to the net charge and the shape of the protein
- SDS-PAGE minimizes the contribution of charge and shape and generally will estimate 'subunit' molecular weight accurately
- mobility in gel filtration is also affected by shape
- electrophoresis exhibits the higher resolution