

Protein Purification/Characterization II

1. Nucleic acids (which are negatively charged and 'rod-like' in shape) can be separated by agarose gel electrophoresis readily. In this technique, a 'gel' is made that consists of a matrix material (agarose) that forms a sort of 'mesh' of hole through which the DNA molecules pass. Electric fields are used to separate macromolecules by size. The sample is loaded on the top of the gel and electrical current is passed through it such that the bottom electrode is positive and the top one is negative. Negatively charged DNA molecules at the top of the gel are driven away from the top towards the bottom. Small molecules make their way through the gel fastest and big molecules travel more slowly. The key to gel electrophoresis is to have all of the molecules being separated have a negative charge.

2. Proteins, however are usually globular in their native state and, without other modification, may be negatively, positively OR neutrally charged. Another type of electrophoresis employs polymers of acrylamide (called polyacrylamide) to form the mesh of the gel. This mesh has smaller holes than agarose and allows separation of smaller molecules like proteins. To separate proteins on the basis of size, the detergent sodium dodecyl sulfate (SDS) is added to the protein mixture, causing the proteins to denature, assume rod-like shapes, and be coated with the negative charge of the SDS. Consequently all proteins in the mixture obtain a negative charge and can be separated just like DNA on SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

3. During purification of proteins it is important to follow the purification process. At each step of the purification, a small sample of the protein extract is taken and the total amount of protein, and the amount of activity of the desired protein are measured. The specific activity of the protein in the tube is the amount of activity (in units) divided by the total mass of protein. The yield of the desired protein at any point in the purification process is the number of units of the desired protein at that point divided by the number of units that one started with. The purification level at any point is the specific activity at that point divided by the specific activity one started with.

4. Breaking large proteins down into smaller pieces is also important for studying them. Some reagents include cyanogen bromide (breaks bonds on carboxyl side of methionine residues in a protein) and enzymes known as proteases. One such protease is trypsin, which cleaves on the carboxyl side of lysine and arginine residues in a polypeptide. Thrombin is another enzyme that breaks peptide bonds. It cleaves them on the carboxyl side of arginine in a polypeptide.

5. Immunological techniques aid in identifying specific proteins. Antibodies (immune system proteins) recognize and bind to specific structures. The structures antibodies bind to are called antigens. Usually antibodies are targeted against specific protein structures. Since proteins differ from each other in their structure, molecules (like antibodies) that bind to specific structures will bind to specific proteins.

6. Antibodies are very useful in that they can be linked to fluorescent dyes, gold particles, or enzymes to help one visualize where an antibody has bound. They are useful in binding to specific cellular structures (in situ hybridization), to identify where within a cell or within a tissue a particular protein is located.

7. A technique that employs antibodies is western blotting. In this technique, a mixture of proteins is separated by SDS-PAGE. The proteins in the gel are transferred directly to a membrane, which is then treated with an antibody specific for one of the proteins. The membrane is washed to release unbound antibody and

then the antibody-protein complex is visualized. This can be by a fluorescent technique or more commonly by an enzyme linked to the antibody as above. In any case, the protein of interest is identified in this way.

8. MALDI-TOF is a mass spectrometric analysis instrument that facilitates determination of polypeptide masses with great accuracy. It employs a laser, which, when activated, causes a polypeptide sample to volatilize in the evacuated chamber of the instrument. Volatilization causes the molecules to both ionize and to break at peptide bonds. Masses are determined by the length of time it takes for the ions to travel through the chamber to the detector. The time it takes them to make the transit (Time of Flight= TOF) is proportional to their mass. Smaller fragments move faster than larger fragments. Using MALDI-TOF, it is possible to determine the sequence of amino acids in a polypeptide based on the analysis of the masses of the various fragments that arise from ionization.