Akal College of Nursing

Baru Sahib

Class Summary

**Date**: 11/12/15

**Time:** Section B 9-10am: Section A 11 to 12 am

**Class** B.Sc. Nursing First Year

**Subject:** Biochemistry

**Unit:** 6: Composition and metabolism of Amino Acids And Proteins

**Topics:** SEPARATION AND PURIFICATION OF PROTEINS

**Types of separation**

Separation based on protein solubility

-Dialysis and gel filtration

Separation on the basis of molecular charge

- Chromatography and electrophoresis

Separation on the basis of affinity binding

-Affinity chromatography

**SEPARATION BASED ON SOLUBILITY**

It is referred to as protein fractionation, the process by which various proteins form a mixture of proteins can be separated based on a property such as size or charge.

**DIALYSIS**

Proteins can be separated from the solvent due to their size by the process of dialysis

**PROCEDURE**

A partially purified protein, which is obtained as a result of salting out, is placed in a bag or tube that is made up of a semi-permeable membrane.

When it is placed in a container having buffered solution, the membrane allows the exchange of salt and buffer but not protein.

Thus it retains protein within the membranous bag while allowing the concentration of other solutes to change until they are in equilibrium with the solution outside the membrane

Since the larger molecules cannot pass through the pores of the membrane, they will stay in the sample chamber while the small molecules will easily diffuse across the membrane.

In time, the overall concentration of the molecules in the sample and the dialysate will change and equilibrium will be reached.

**Column chromatography**

After the initial fractionation steps we move to column chromatography.

The mixture of substances (proteins) to be fractionated is dissolved in a liquid or gaseous fluid called the mobile phase.

This solution is passed through a column consisting of a porous solid matrix called the stationary phase. These are sometimes called resins when used in liquid chromatography.

The stationary phase has certain physical and chemical characteristics that allow it to interact in various ways with different proteins.

**Common types of chromatographic stationary phases**

Ion exchange

Hydrophobic

Gel filtration

Affinity

**Ion exchange chromatography**

Ion exchange resins contain charged groups.

If these groups are *acidic* in nature they interact with positively charged proteins and are called cation exchangers.

If these groups are *basic* in nature, they interact with negatively charged molecules and are called anionexchangers.

Ion exchange chromatography

Proteins will bind to an ion exchanger with different affinities.

As the column is washed with buffer, those proteins relatively low affinities for the ion exchange resin will move through the column faster than the proteins that bind to the column.

The greater the binding affinity of a protein for the ion exchange column, the more it will be slowed in eluting off the column.

Proteins can be eluted by changing the elution buffer to one with a higher salt concentration and/or a different pH (stepwise elution or gradient elution).

Cation exchangers bind to proteins with positive charges.

Anion exchangers bind to proteins with negative charges.

**Gel filtration chromatography**

Also called size exclusion chromatography or molecular sieve chromatography.

The molecular mass of the smallest molecule unable to penetrate the pores of the gel is at the exclusion limit.

The exclusion limit is a function of molecular shape, since elongated molecules are less likely to penetrate a gel pore than other shapes.

**Affinity chromatography**

Many proteins can bind specific molecules very tightly but noncovalently.

How does it work?

Ligand - a molecule that specifically binds to the protein of interest.

**Electrophoresis**

The migration of ions in an electric field to separate molecules.

Many forms of electrophoresis-we will focus on polyacrylamide gel electrophoresis (PAGE).

**PAGE techniques** are often used determine the purity of proteins.

**Sodium dodecyl sulfate (SDS-PAGE)**

Native protein is unfolded by heating in the presence of β-mercaptoethanol and SDS.

SDS binds to the protein so that it stays in solution and denatures.

Large polypeptides bind more SDS than small polypeptides, so proteins end up with negative charge in relation to their size.

Thus, we can separate the proteins based on their mass.

Submitted by

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