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Chromatography-based Biomolecule Purification Methods

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Abstract

In biochemistry, chromatography-based purification methods are employed to isolate compounds from a complex mixture. Two such methods used commonly by biochemists are size-exclusion chromatography and affinity chromatography. In size-exclusion chromatography, a column packed with porous beads separates components of a mixture based on size. On the other hand, affinity chromatography allows for a more specific separation of biomolecules by using a column that is composed of stationary phase, which contains target-specific ligands.

This video serves as an introduction to size-exclusion and affinity chromatography, as well as the concepts that govern them. A step-by-step procedure for the purification of a histidine-tagged protein by immobilized metal affinity chromatography is described. Applications for both of these chromatographic methods in biochemistry and biomedical research are also profiled.

"Chromatography" refers to a wide range of methods used to isolate a component from a complex mixture, an essential step before a biomolecule's properties and activities can be determined. Each chromatographic technique has a different mechanism for separation, depending on the sample matrix and target compound. This video will focus on the principles and operation of two methods common to biochemistry: size-exclusion and affinity chromatography.

Size-exclusion chromatography or SEC is based on the size of the compounds in the sample. A mobile phase containing the sample is added to a column with a porous material: the stationary phase. The molecules in the sample fall into 1 of 3 categories.

Molecules too large to enter the pores travel the shortest distance through the column. Any species with a molecular weight above this "exclusion limit" will exit the column at the same time. Molecules small enough to freely enter the pores will be retained the longest, and will exit the column together. The molecular weight allowing complete pore entry is the "permeation limit".

Only molecules between these limits will be separated from one another, as they spend varying amounts of time diffusing into and out of the pores. Smaller molecules are retained longer on the column because they spend more time in the stationary phase, whereas larger molecules within these limits exit earlier.

Molecular weights across 1 to 2 orders of magnitude fall within these limits. Columns are chosen with this in mind, or multiple columns can be used in series if there is a wide range of desired compounds.

Now that you've seen the theory of SEC, let's look at how it is carried out.

To begin the SEC procedure, the column must be equilibrated with deionized water and chromatography buffer. Once prepared, buffer containing the sample is injected onto the column. The buffer is then pushed through at a low flow rate. A detector monitors what exits the column to determine the presence of the desired analyte. Large molecules with molecular weights above the exclusion limit exit the column at the same time. Small fractions from the column are collected in tubes. Each fraction is tested for the quality of the target molecule by gel electrophoresis or other analytical techniques.

Now let's have a look at affinity chromatography or AC, one of the most efficient ways to purify proteins. Many biomolecules bind selectively to certain ligands-a property which AC utilizes by adhering a target-specific ligand to the stationary phase.

When the mixture flows through the column, the target molecules attach to the ligand, and the rest flow through. After the mixture has passed through the column, the target molecule can be collected through one of two elution methods based on specificity.

Biospecific elution can be said to a have a "normal-" or "reverse-role". In normal-role biospecific elution, an agent is added that competes with the adhered ligand to bind with the target biomolecule.

In reverse-role biospecific elution, an agent competes with the target to bind to the adhered ligand. The second elution type, *nonspecific elution*, lowers the target-to-ligand binding by changing the solution's pH, ionic strength, or polarity. If a protein does not bind to a ligand that can be immobilized, the protein can be expressed containing a "tag": short peptide sequences engineered to bind to the ligand.

One variety is immobilized metal ion affinity chromatography, "IMAC" for short, where an adhered metal ligand, like nickel or cobalt, binds to histidine residues on the modified protein. Through molecular biology techniques, target proteins are generated with repeating histidine residues, called a polyhistidine-tag, which binds to the metal via the imidazole side chain on histidine. Once bound, the protein can be collected with free imidazole via reverse-role specific elution and later used in a wide variety of downstream applications. Now that you've seen the theory of affinity chromatography, let's look at an IMAC procedure in the laboratory.

In IMAC, the stationary phase can be added directly to the mixture of mobile phase and sample, allowing the binding of the his-tagged protein. This slurry is then poured into the column, where the non-bound compounds drip into waste, while the slurry remains. The slurry container is rinsed to collect residual resin and sample, which is added to the column.

The resin is stirred to ensure the unbound components are free-flowing. Added wash buffer helps flush them away. Once all of the unbound components have been removed, the waste is replaced with a container to collect the target protein.

Buffer containing imidazole is added, stirred, and allowed to rest to unbind the target molecule. The imidazole binds to the metal, replacing and releasing the tagged protein. The freed protein is collected, and the imidazole step is repeated to ensure total collection. To further purify the sample, SEC can be run on the sample prior to analysis.

Now that we've seen the theory and procedure of these two techniques, let's look at some of the ways they're applied in the biochemical field.

A common reason to purify proteins is to study their role in disease. Cystic fibrosis is caused by defects in the cystic fibrosis transmembrane conductance regulator protein, or CFTR. After growing the protein with a his-tag in yeast, both affinity and size-exclusion chromatography allow the isolation of the protein, followed by the study of its function.

In some instances, the presence of a polyhistidine tag can change a protein's structure, thereby affecting its function. Another common tag is maltose-binding protein or MBP, which will bind to bound amylose in a column. Maltose is then used to release the complex. The MBP can then be cleaved and removed with SEC to produce the pure desired protein.

You've just watched JoVE's video on size-exclusion and affinity chromatography. It covered the theory of the techniques, went over general procedures, and covered some of the uses of the techniques.

Thanks for watching!