

Application of Hydrophobic Interaction Chromatography to Purification of Green Fluorescent Protein

Abstract: Chromatography is a technique used to separate molecules that may be present inside a cell. They may be parts of the cell or macromolecules, such as ribosomes, mitochondria, cell membranes, or DNA. Chromatography can also be used to separate proteins from other cellular material. For example, the Green Fluorescent Protein, GFP, can be separated from the bacterial extract by using a type of hydrophobic interaction chromatography.

Introduction

Chromatography is a technique used to separate molecules based on how they tend to cling to or dissolve in various solids, liquids and gases. The word originates from the Greek word for color (chroma) and writing (graphein). A Russian botanist coined the term to describe the technique he used when he separated plant pigments. There are 5 major types of liquid chromatography techniques as listed in Table 1.

Table 1. Different chromatography techniques used in separation of biomolecules and their definitions.

Liquid Chromatography Technique	Definition
Size-Exclusion (or Gel Filtration)	Gel media contains pores. Separation occurs when molecules are included or excluded from the pores.
Ion-Exchange	Negative or positively charged functional groups are bound to a support matrix. A charged molecule binds to an exchanger of the opposite charge.
Hydroxyapatite	Unique interaction of both electrostatic and formation of calcium coordination complexes.
Affinity	A ligand is selectively bound to a solid matrix. Unbound contaminants are washed away.
Hydrophobic Interaction	Exposure of hydrophobic and hydrophilic regions on the molecule of interest are manipulated in various salt concentrations.

The method used to isolate the green fluorescent protein, GFP, from jellyfish is called HIC or hydrophobic interaction chromatography. The gene for GFP has become an important tool for researchers as a reporter gene on other genes of interest. GFP has also been expressed in whole organisms as a marker for genetically manipulated genes.

Chromatography and GFP

How does this work to separate GFP from the other proteins and macromolecules that are floating around in the bacterial lysate? First, you'll need to understand a little about what makes up a protein. Proteins are made up of amino acid chains (primary structure). While the chain is being formed, the amino acids interact with each other to twist and turn, what scientists call "fold" into the protein's secondary structure such as sheets and helices. Next, these formed secondary structures also interact and further fold and compact the protein into its tertiary structure. That means some of those original amino acids can be buried on the inside of a protein while some are on its outside. Usually, the hydrophobic (or dislike of water, uncharged) regions are buried on the inside of the protein while the hydrophilic (or water loving, charged) regions are on the outside happily interacting with the water molecules in its environment (Figure 1).



Figure 1:
 • charged aa's outside
 • hydrophobic inside

When a protein is in a high salt solution, the salt interacts with water molecules, causes a conformational (shape) change and reveals the hydrophobic regions of the protein. These hydrophobic regions then interact with the functional group on the column matrix (Figure 2). This attractive force between the hydrophobic regions of two molecules is also seen when you try to mix oil and water. The hydrophobic oil droplets cling to each other pushing the water away from it, not easily mixing with the water. As the GFP is loaded onto the column packed with matrix beads with hydrophobic groups, GFP “sticks” to the beads without forming covalent bonds, while hydrophilic bacterial proteins elute (wash) out.

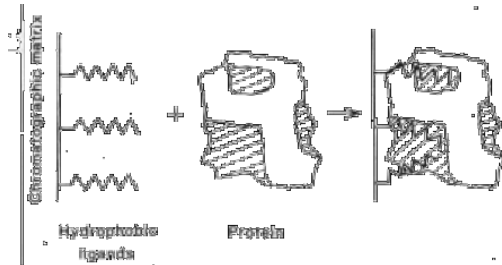


Figure 2: Functional groups on the beads of the column matrix interact with protein hydrophobic regions to bind.

To remove the GFP from the column, you need to decrease its affinity to the column, or get the GFP to “unstick.” This is done by gradually lowering the salt concentration within the column (as you add your medium salt wash buffer followed by the very low salt elution buffer). As you shine the UV light to your column, you can track the green band of GFP gliding down your column into your sample tube.

Reference:

Power Point presentation by Dr. Susan Bernhard at SCCBEP's 8th Annual Summer Workshop Series.

<http://www.biorad.com>

Bio-Rad supplies a variety of chromatography matrices.

<http://biotech.icmb.utexas.edu/search/dict-search.html>

Biotech Life Science has several sites on chromatography definitions.

http://www.accessexcellence.org/AB/GG/prot_Sep.html

Access Excellence site graphically describing chromatography methods.

chromatography

An analytical technique used to separate molecules based on how they tend to cling to or dissolve in various solids, liquids and gases. The word originates from the Greek word for color writing. First used by a Russian botanist who was describing the separation of plant pigments using a column.

column

The support (plastic, glass, steel, etc.) that holds the matrix and allows directional flow of sample within a liquid environment.

enzyme

A protein that facilitates a specific chemical reaction.

fluorescence

A phenomenon shown by certain substances when they encounter ultraviolet radiation. The substance absorbs high frequency wavelengths and emits it at a lower frequency light. This emission stops as soon as the high frequency radiation is removed. For example, GFP absorbs the higher frequency blue light emitted by aequorin, undergoes a chemical reaction, and emits the lower wavelength green light.

green fluorescent protein (GFP)

A protein found in jellyfish that fluoresces, or emits a green visible light when excited by UV light with a wavelength of 395 nanometers. It can function as a biological marker when attached to other proteins. The structure of the protein is cylindrical with the glowing component, an amino acid complex called a *fluorophore*, in the middle of it.

hydrophobic interaction chromatography (HIC)

A chromatography technique that uses a column to separate proteins by eluting with buffers of decreasing salt concentrations. Since no organic solvent is used, biological activity of the protein(s) has a much higher probability of being retained.

hydroxyapatite (hydroxylapatite)

The primary mineral component of bone. It has the chemical formula of $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ and in crystal form is needle-shaped and is arranged with other needles in a rosette. It melts above 1100°C and cannot be dissolved in water. A form of the mineral is used by molecular biologists in certain column chromatography laboratory techniques to separate single-stranded nucleic acids from double-stranded ones (the mineral tends to hang on to the double-stranded molecules while letting the single-stranded ones through the column unimpeded), or to separate different proteins.

hydrophilic

Used to describe molecules or portions of a protein that are attracted to water.

hydrophobic

Used to describe molecules or portions of a protein that repels from water.

lysis (to lyse)

The destruction of a cell's membrane or wall, which breaks open the cell, spills its contents, and kills it. The lysate is the contents that are collected from the lysed cell.

macromolecule

A molecule with a molecular weight in excess of 1,000 daltons.

matrix (or media)

The support inside a column that contains functions to interact and separate the molecules within a sample.

molecule

The result of two or more atoms combining by chemical bonding.

protein

A covalent chain of amino acids whose sequence was predetermined by its gene.

protein structure

primary structure

The covalent chain of amino acids.

secondary structure

The folding of a chain of amino acids due to ionic interactions between them. Forms structures like sheets and helices.

tertiary structure

The further folding of a protein due to hydrophobicity and ionic interactions between secondary structures.