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Review on Affinity chromatography in drug purification

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ABSTRACT: Affinity chromatography is a type of liquid chromatography for the separation, purification, or specific analysis of sample components. It is a separation technique in which the mobile phase carrying the mixture is allowed to move in contact with a selectively absorbing stationary phase. It uses a reversible biological interaction or molecular recognition called affinity, which refers to the attractive force exerted in varying degrees between atoms that cause them to remain in combination. Example: Enzyme with inhibitor, antigen with antibody, etc.

Key words: affinity, interaction, purification, separation, mobile phase, analysis, etc.

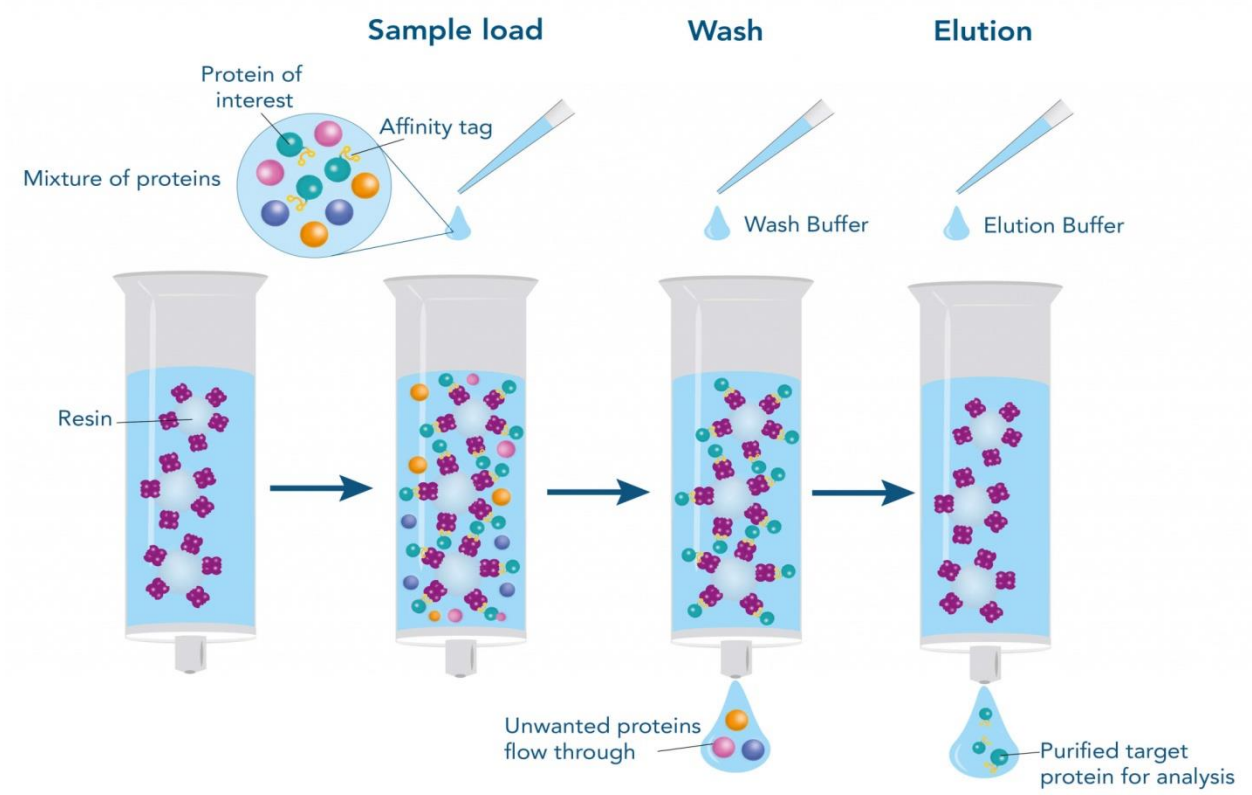
INTRODUCTION:

Affinity chromatography is a method of separating biochemical mixtures based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate or receptor and ligand. Affinity chromatography, also known as bioselective adsorption, is a protein purification technique. It is widely used as a means of separation and purification with specific properties. Biological macromolecules, such as enzymes and other proteins, interact with other molecules with high specificity through several different types of bonds and interaction. Such interactions include hydrogen bonding, ionic interaction, disulfide bridges, hydrophobic interaction, and more. The high selectivity of affinity chromatography is caused by allowing the desired molecule to interact with the stationary phase and be bound within the column in order to be separated from the undesired material which will not interact and elute first. The molecules no longer needed are first washed away with a buffer while the desired proteins are let go in the presence of the eluting solvent (of higher salt concentration). It was discovered by Pedro Cuatrecasas and Meir Wilcheck.

PRINCIPLE

The stationary phase is first loaded into a column with mobile phase containing a variety of biomolecules from DNA to proteins (depending on the purification experiment). Then, the two phases are allowed to bind. A

wash buffer is then poured through a column containing both bound phases. The wash buffer removes non-target biomolecules by disrupting their weaker interactions with the stationary phase. Target biomolecules have a much higher affinity for the stationary phase, and remain bound to the stationary phase, not being washed away by wash buffer. An elution buffer is then poured through the column containing the remaining target biomolecules. The elution buffer disrupts interactions between the bound target biomolecules with the stationary to a much greater extent than the wash buffer, effectively removing the target biomolecules. This purified solution contains elution buffer and target biomolecules, and is called elution.



INSTRUMENTATION

1. Matrix

- The matrix is an inert support to which a ligand can be directly or indirectly coupled.
- In order to for the matrix to be effective it must have certain characters:
- Matrix should be chemically and physically inert.
- It must be insoluble in solvents and buffers employed in the process
- It must be chemically and mechanically stable.
- It must be easily coupled to a ligand or spacer arm onto which the ligand can be attached.
- It must exhibit good flow properties and have a relatively large surface area for attachment.
- The most useful matrix materials are agarose and polyacrylamide.

2. Spacer arm

- It is used to improve binding between ligand and target molecule by overcoming any effects of steric hindrance.

3. Ligand

- It refers to the molecule that binds reversibly to a specific target molecule.
- The ligand can be selected only after the nature of the macromolecule to be isolated is known.
- When a hormone receptor protein is to be purified by affinity chromatography, the hormone itself is an ideal candidate for the ligand.
- For antibody isolation, an antigen or hapten may be used as ligand.
- If an enzyme is to be purified,a substrate analog, inhibitor, cofactor, or effector may be used as a the immobilized ligand.

STEPS INVOLVING IN AFFINITY CHROMATOGRAPHY

- Affinity medium is equilibrated in binding buffer.
- Sample is applied under conditions that favor specific binding of the target molecule(s) to a complementary binding substance (the ligand). Target substances bind specifically, but reversibly, to the ligand and unbound material washes through the column.
- Elution is performed specifically, using a competitive ligand, or non-specifically, by changing the pH, ionic strength or polarity. Target protein is collected in a purified, concentrated form.
- Affinity medium is re-equilibrated with binding buffer.

These events can be summarized into the following three major steps:

1. Preparation of Column

- The column is loaded with solid support such as sepharose, agarose, cellulose etc.
- Ligand is selected according to the desired isolate.
- Spacer arm is attached between the ligand and solid support.

2. Loading of Sample

- Solution containing a mixture of substances is poured into the elution column and allowed to run at a controlled rate.

3. Elution of Ligand-Molecule Complex

- Target substance is recovered by changing conditions to favor elution of the bound molecules.

APPLICATIONS

1. Protein purifying

Affinity chromatography has a large range of protein purifying applications. Extracellular and other receptor proteins can also be purified by affinity chromatography. It allows protein purification in a relatively short amount of time with a high yield.

2. Isolation of enzyme

Enzymes can be isolated by a host at different ligands fit for bioselective adsoprtion.For example, adenosine monophosphate (AMP) can be immobilized and used to bind those proteins exhibiting an affinity for AMP, ADP, or ATP.

3. Immobilized Metal Affinity Chromatography In Proteomics

It has been proved that the progress of proteomics is mostly determined by the development of advanced and sensitive protein separation technologies. Immobilized metal affinity chromatography (IMAC) is a powerful protein fractionation method used to enrich metal-associated proteins and peptides.

4. Affinity Chromatography To The Study Of Drug–Melanin Binding Interactions

Affinity chromatography using chromatographic stationary phases based on physically adsorbed or chemically bonded melanin provides a useful tool for studying the interactions of small molecules and metal ions with melanin

5. Purification of lectins by Biospecific affinity chromatography

Biospecific adsorbents which can be used for the purification of lectins are easily prepared by a one-step reaction between Epoxy-activated Sepharose 6 B and Lectin-specific sugars.

6. Purification of plasma proteins for therapeutic use

Affinity chromatography is a powerful technique for the purification of many proteins in human plasma. It is being used in the production of various licensed therapeutic plasma products, such as : Factor VIII, Factor IX, Von Will brand Factor, Protein C, Antithrombin III, and Factor XI.

Advantages

- High specificity
- Target molecules can be obtained in a highly pure state
- Single step purification
- The matrix can be reused rapidly.
- The matrix is a solid, can be easily washed and dried.
- Give purified product with high yield.
- Affinity chromatography can also be used to remove specific contaminants, such as proteases.

Limitation

- Time consuming method.
- More amounts of solvents are required which may be expensive.
- Intense labour
- Non-specific adsorption cannot be totally eliminated, it can only be minimized.
- Limited availability and high cost of immobilized ligands.
- Proteins get denatured if required pH is not adjusted.

CONCLUSION

Affinity separation, and especially affinity chromatography, has become popular tools for the selective purification and isolation of biological compounds, recombinant proteins, and biopharmaceuticals. Unlike other purification methods such as gel filtration and gel exclusion chromatography, affinity chromatography manipulates specific molecular properties and binding interactions between molecules to purify the desired protein.

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