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Ion-Exchange Chromatography

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Overview

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Ion-exchange chromatography is a type of chromatography that separates analytes based on charge. A column is used that is filled with a charged stationary phase on a solid support, called an ion-exchange resin. Strong cation-exchange chromatography preferentially separates out cations by using a negatively-charged resin while strong anion-exchange chromatography preferentially selects out anions by using a positively-charged resin. This type of chromatography is popular for sample preparation, for example in the cleanup of proteins or nucleic acid samples.

Ion-exchange chromatography is a two-step process. In the first step, the sample is loaded onto the column in a loading buffer. The binding of the charged sample to the column resin is based on ionic interactions of the resin to attract the sample of the opposite charge. Thus, charged samples of opposite polarity to the resin are strongly bound. Other molecules that are not charged or are of the opposite charge are not bound and are washed through the column. The second step is to elute the analyte that is bound to the resin. This is accomplished with a salt gradient, where the amount of salt in the buffer is slowly increased. Fractions are collected at the end of the column as the elution occurs and the purified sample of interest can be recovered in one of these fractions. Another technique, such as spectroscopy, may be needed to identify which fraction contains the sample. Ion-exchange chromatography is especially useful in protein studies, to isolate proteins of interest that have a specific charge or size, as size can determine the number of interactions with the resin.

Ion-exchange chromatography is a more general separation technique than affinity chromatography, which is also often used in preparing protein samples, where an antibody is attached to a column to bind one specific analyte. A new affinity column must be purchased for each analyte, while the same type of ion-exchange column, often with different eluting conditions, can be used to clean up many proteins of the same charge. Ion-exchange chromatography can also be used in conjunction with other types of chromatography that separate based on other properties. For example, size-exclusion chromatography separates based on size and could be used before ion-exchange chromatography to choose compounds of only a given size.

Principles

Ion-exchange chromatography is governed by the principles of ionic chemical interactions that lead to reversible adsorption of the analyte on the stationary phase. The strength of the bonding between the sample and the solid support is governed by the number and types of functional groups on the sample and the resin. The loading buffer generally has low conductivity in order to promote interactions between the sample and the resin. Strong-cation exchange resins typically feature sulfonic acid functional groups while weak cation-exchange resins have carboxylic acids. Strong anionic-exchange resins contain quaternary amines while weak anion-exchange resins feature secondary or tertiary amines. The terms strong and weak refer to the acid/base properties of the column material and not how well it binds an analyte. Weak resins are charged over a smaller pH range than strong resins, but still bind the analytes very effectively, and could be a good choice if they are charged in the pH range needed. Before use, ion exchange columns are equilibrated at a pH using an equilibration buffer.

To elute the sample of interest, a salt gradient is used. Samples that are less tightly bound to the resin will elute first, as the salt will most easily disturb their ionic bonding to the column. Samples that are more tightly bound will elute later, when higher amounts of salt are used. Typically, a linear gradient of salt is used, where the salt concentration increases over time in a linear fashion. However, step gradients can also be used where the concentration of salt is stepped up over time.

One important consideration for ion-exchange experiments is the pH of the buffers. The pH needs to be maintained so that the analyte of interest is charged and will bind to the resin. For proteins, the charge is based on the protein's isoelectric point, where the protein is neutrally charged, and measured as pI. The pH in comparison to the protein pI gives information about the expected protein charge. In cation-exchange chromatography, raising the pH will cause the analyte to be less positively charged and less likely to interact with the negatively-charged resin. Similarly, in anion-exchange chromatography, lowering the pH will cause the analyte to be less negatively-charged and less likely to interact with the positively-charged resin. Thus pH adjustments can also be used to selectively elute analytes from the column. The use of pH adjustments instead of salts could be advantageous for separating two different types of proteins with different pI values.

Procedure

1. Preparing the Sample and the Column

1. In this demonstration, a mixture of 2 proteins will be separated on a cation-exchange column: hemoglobin and cytochrome C. Add 0.2 mL equilibration buffer (pH 8.1) to the protein sample and vortex to mix thoroughly. Centrifuge for 2 min to remove any froth.
2. Place the cation-exchange column in a test tube for 5 min to allow resin to settle. Clamp the test tube with the column onto a ring stand to make sure it is upright.
3. Open the top cap of the column, and then the bottom cap. Allow the buffer in the column to drip out under gravity into the test tube.

4. Wash the column twice with a column-volume (in this case the column volume is 0.3 mL) of equilibration buffer. Let the first column-volume (0.3 mL) of equilibration buffer drip out into the waste vial before adding the second column-volume. This step lets the column equilibrate with the equilibration buffer. Add the equilibration buffer slowly in this step to not disturb the resin.

2. Running a Protein Sample Through a Cation-Exchange Column

1. Put the column in a 2 mL centrifuge tube labeled "Unbound 1". Carefully load 0.1 mL of the protein sample onto the top of the column.
2. Once the sample has been loaded onto the top of the column, wash it with 0.3 mL of equilibration buffer by adding the buffer at the top of the column and allowing it to drip all the way through. Repeat this step 4x (for a total of 5 washes) and collect each wash in a separate, 2-mL centrifuge tube. Label the tubes as "Unbound 1-5".
3. For the last 2 washes, centrifuge for 10 s at 1,000 x g to make sure all unbound species come off the column. Any sample that binds to the column should not be in these washes, but sample that does not bind will wash out unretained. Centrifuging provides more force to wash unbound materials off the column.
4. Put the column in a new 2-mL centrifuge collection tube labeled "Bound 1". Put 0.3 mL of elution buffer (high salt, pH 5.5) on top the column. Centrifuge the resulting eluent for 10 s at 1,000 x g.
5. Repeat the elution step 2 more times by placing the column in a new centrifuge tube, adding 0.3 mL, and centrifuging for 10 s. Label these "Bound 2 and 3".
6. Record any color changes or observations about the fractions. Hemoglobin is a colored protein that looks reddish-brown while cytochrome C is reddish colored.

3. Results: Cation-exchange of Hemoglobin and Cytochrome C

1. Cytochrome C has a pI of 10.5 and hemoglobin a pI of 6.8. Thus, when a pH 8.1 equilibration buffer is used, hemoglobin does not bind to the column because it is negatively charged. Cytochrome C is positively charged at this pH and does bind to the column because the pH < pI.
2. Hemoglobin is observed in the unbound fractions because it is not bound to the column.
3. Cytochrome C is observed in bound fractions, because it was bound to the cation exchange column.

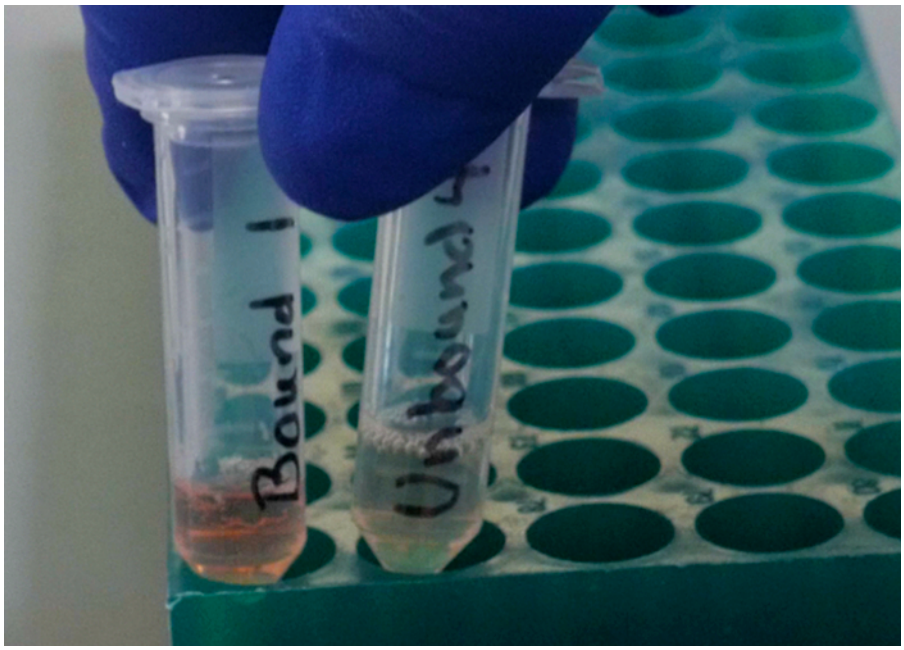

Figure 1. Picture of unbound fraction (hemoglobin) and bound fraction (cytochrome C).

Applications and Summary

Ion-exchange chromatography is widely used in biochemistry to isolate and purify protein samples. Proteins have many amino acids with functional groups that are charged. Proteins are separated based on net charge, which is dependent on pH. Some proteins are more positively charged while others are more negatively charged. In addition, peptide tags can be genetically added to a protein to give it an isoelectric point that is not in the range of normal proteins, making it possible to separate completely. Ion-exchange chromatography is useful for separating multimeric protein complexes, as different configurations would have different amounts of charge and different interactions.

Another major application of ion-exchange chromatography is water analysis. Anion-exchange chromatography can be used to measure the concentration of anions, including sulfates, nitrates, nitrites, fluoride, and chloride. Cation-exchange chromatography is used to measure the concentration of cations such as sodium, potassium, calcium, and magnesium. A type of ion-exchange chromatography is also used in water purification, as most water softeners filter out magnesium and calcium ions in hard water by binding them to a resin, which releases bound sodium. Heavy metals, such as copper or lead, can also be removed from water using ion-exchange chromatography.

Ion-exchange chromatography is also useful in metal purification. It can be used to purify actanides, such as plutonium, and remove it from spent nuclear reactor fuel rods. It can also be used to scavenge uranium and remove it from water or other environmental samples.