Affinity Chromatography

Background Information

A major goal in the biochemistry or molecular biology laboratory is the isolation of a specific protein from complex mixtures. Such isolation procedures often involve several fractionation steps with the effectiveness of each step monitored by electrophoresis. Chromatography is a type of fractionation similar to electrophoresis in that proteins separate from each other as they are passed through a matrix. With chromatography, however, solvent flow (by gravity, pressure or capillary action), not an electric field**,** carries the proteins through the matrix. The separation occurs because proteins interact with various matrices in different ways. Chromatography matrices are designed to exploit the physical and chemical interactions of a protein of interest. For example, acidic proteins will interact with a basic matrix to a greater extent than basic or neutral proteins so a basic matrix can be used to separate acidic proteins (which will interact with the matrix and be retained) from non-acidic proteins (which will flow through the matrix). The acidic proteins retained by the matrix can then be removed or eluted by disrupting their interactions with the matrix. The major limitation of most types of chromatography is the lack of specificity for only one protein, for example a basic matrix will retain all acidic proteins to various extents and not just the specific protein of interest.

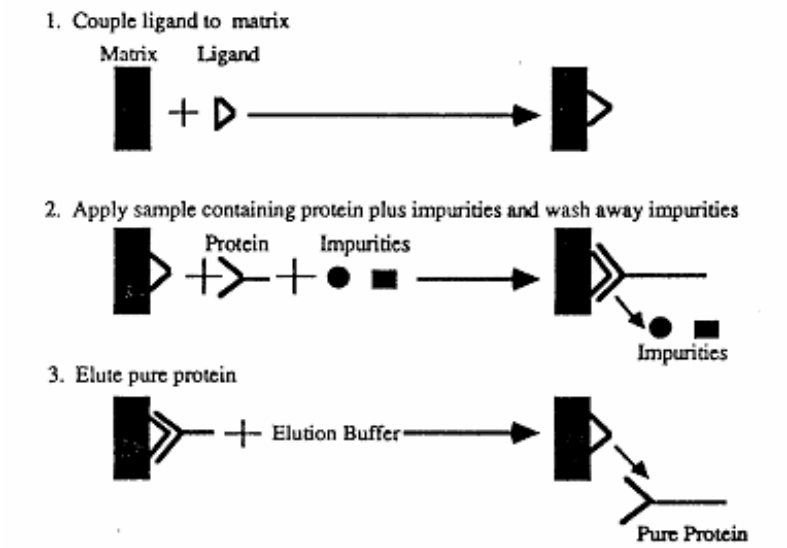
Affinity chromatography is a method that overcomes this limitation by exploiting the unique interaction of one molecule with a second, complementary binding molecule (ligand). It is one of the most useful and effective fractionations that can be applied and often proteins can be purified in single affinity chromatography step. Affinity chromatography applications are not limited to protein purification and some general types of molecules which have been isolated by affinity chromatography and their complementary ligands are listed in Table 1.

Table 1. Substances Isolated by Affinity Chromatography and the Complementary Ligand.

Substance Isolated by Affinity Chromatography	**Ligand**
Enzyme	Substrate, cofactor
Antibody	Antigen, Virus, Cell
Polysaccharide, glycoprotein	Lectin
Nucleic acid binding protein	Nucleic Acid
Hormone receptor	Hormone

The basic procedure for affinity chromatography is outlined in Figure 1. The complementary binding molecule (ligand) is first covalently coupled to an insoluble matrix, such as small agarose beads, and the matrix is poured into a column. An impure mixture containing the protein to be isolated is then applied to the column and allowed to interact with the immobilized ligand. In this step, the protein to be isolated binds specifically to the immobilized ligand while the remaining molecules in the mixture flow through the column. The protein of interest can then be eluted from the column under conditions which disrupt its interaction with ligand.

Figure 1. General Procedure for Affinity Chromatography



In this experiment you will be isolating horse serum albumin by affinity chromatography with Affi-Gel blue. Affi-Gel blue contains a reactive blue dye molecule (Cibacron Blue F3GA) linked to agarose beads. The albumin in serum binds specifically to this dye while other serum proteins do not. Thus, when horse serum is applied to a column of Affi-Gel blue, albumin is retained by the column and the remaining proteins flow through. Albumin is then eluted from the column with SDS which disrupts the interaction of albumin with the blue dye. The effects of this separation are then assessed by electrophoretic analysis of the column-bound material (albumin) and the column flow-through material (other serum proteins).

Objective: To isolate albumin from horse serum by affinity chromatography with Affi-Gel blue, and to analyze the isolated protein by electrophoresis.

Procedure

I. Preparation of the Affi-Gel blue column. The Affi-Gel blue is supplied as a slurry of the agarose-ligand matrix and a buffer containing stabilizing preservatives. This slurry must first be poured into the column and equilibrated (washed) with column buffer to remove the preservative.

1. Secure the column in a vertical position with the narrow, plugged end down. This may be accomplished by clamping the column to a ring stand (optional) or taping the column to a shelf. The porous disc in the bottom of the column will serve to retain the Affi-Gel blue matrix while allowing solutions and proteins to freely flow through the column.

2. Place a beaker under the column to catch the buffer that will flow through and remove the bottom closure. Thoroughly mix the slurry of Affi-Gel blue to make sure that it is suspended uniformly. With the macropipetor, quickly transfer 2 ml of the slurry to your column, allowing the buffer to drip through and into the beaker.

3. As the Affi-Gel blue is settling you will observe two layers forming: a bottom blue layer of Affi-Gel blue and a top layer of buffer. The buffer will flow through the column, leaving the Affi-Gel blue behind. When the level of the buffer approaches the top of the Affi-Gel blue layer, fill the column with column buffer and allow the solution to flow through the column to wash it. Repeat the washing at least one time but you may keep adding more buffer as needed to prevent the Affi-gel from becoming dry. If the experiment must be interrupted at any time, the column should be filled with buffer and the column sealed with the bottom closure to prevent it from becoming dry. The column should not be allowed to run dry during the experiment.

II. Applying the sample.

1. Label 3 microcentrifuge tubes #1, #2, #3, and 2 test tubes "flow through" and "bound". Remove 20 µl of the serum sample and place it into tube #1. Keep the remaining tubes close at hand while running the column.

2. After the column has been washed, allow the buffer to drain to within 1-2 mm of the top of the Affi-Gel blue. Quickly transfer the 0.5 ml of the serum sample into the column. Immediately place the tube marked "flow through" under the column and collect the solution containing protein not bound to the column that flows through.

III. Washing the column

When the serum sample has entered the column, remove the tube marked "flow through" and refill the column with column buffer. Allow the washings to drip into your beaker to be discarded. Repeat the washing by refilling the column with buffer.

IV. Eluting the column

Allow the final wash solution to drain to within 3 mm of the top of the Affi-Gel blue. Quickly transfer 1 ml of elution buffer into the column with your pipetor. Immediately place the tube marked "bound" under the column and collect the solution containing albumin.

V. Sample preparation

Add 10 µl of water to the reserved serum sample in tube #1. Transfer 20 µl from the tube marked "flow through" to tube #2. Transfer 20 µl from the tube marked "bound" to tube #3. Add 20 µl sample buffer to each of the tubes #l-#3 and tap the tubes with the tip of your index finger to mix. Boil the samples.

VI. Electrophoresis

1. Load 10 μl of the following sample solution into the sample wells:

Sample Well #	Sample Solution
1,5	Standard proteins
2,6	Protein sample from tube #1
3,7	Protein sample from tube #2
4,8	Protein sample from tube #3

2. Electrophorese until the bromphenol blue has migrated to within 1 cm of the positive electrode end of the gel.

3. Stain and destain the gel as described in section IV. Record your results noting the presence or absence of serum albumin in each sample.

Questions to Think About

1. Describe the results of your albumin purification including a description of the relative mobility of the purified protein. Did your affinity chromatography column remove all of the albumin from the serum? How pure was the affinity isolated protein?

2. An antiserum contains many other proteins in addition to antibodies and is therefore an impure mixture. Design an affinity chromatography experiment to purify the antibodies that react with human serum protein. Would baboon or cow serum proteins serve as a good ligand in this experiment?

3. Hemophiliacs are deficient in the production of a serum blood clotting factor called Factor VII. Assuming that ligands are available for any substance that you could imagine, how could hemophiliacs benefit from affinity chromatography?