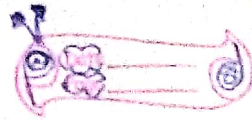
Affinity Chromatography



Affinity chromatography is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure. The substance to be purified is specifically and reversibly absorbed to a ligand (binding substance), immobilized by a covalent bond to a chromatographic bed material (i.e, matrix). Samples are applied under favourable conditions for their specific binding to the ligand. Substances of intrest are consequently bound to the ligand while unbound substances are washed away. Recovery of substances of intrest can be achieved by changing experimental conditions to favour desorption. The operation of affinity chromatography involves the following steps:-

→ Choice of an appropiate ligand
→ Immobilization of the ligand onto a support matrix
→ Binding of the molecules of intrest with ligand.
→ Removal of non-specifically bound molecules.
→ Elution of the molecules of intrest in a purified form.

A biospecific ligand that can be attached to a chromatography matrix covalently is one of the requirements for successful affinity purification.

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The binding between the ligand and molecules of inserts must be reversible to allow the molecules to be removed in an active form. After washing away the contaminants, the coupled ligand must retain its specific binding affinity for the molecules of intrest. Some examples of types of ligands that are usually used in affinity Chromatography are given below.

Typical biological interactions used in affinity Chromatography

Types of ligand		Target molecules or molecules of intrest
Enzyme		substrate analogue, inhibitor, cofactor.
Antibody		Antigen
Lectin		Polysaccharide, glycoprotein cell surface receptor, cell.
Nucleic acid	:	Complementry base sequence, nucleic acid binding protein
Avidin		Biotin
Calmodulin	-	Calmodulin - binding molecule
Poly (A)	-	RNA Containing Poly(U) Sequences
Glutathione	-	Glutathione-S-transferase or GST fusion proteins.
Proteins A + G	-	Immunoglobulins

For example - the Eukaryotic mRNA with poly (A) tail can be separated from other types of RNA molecules by Oligo (dT) - Cellulose affinity Chromato-graphy. Poly(A) tails form stable interaction with short chains of oligo (dT) that are attached to the support matrices. High salt is added to the chromatography buffer to stabilize the nucleic acid duplexes as only a few dT-A base pairs are formed. A low-salt buffer is used after non-polyadenylated RNAS have been washed from the matrix. This buffer helps to destabilize the double-stranded structure and elute the poly(A) RNAS from the resin.

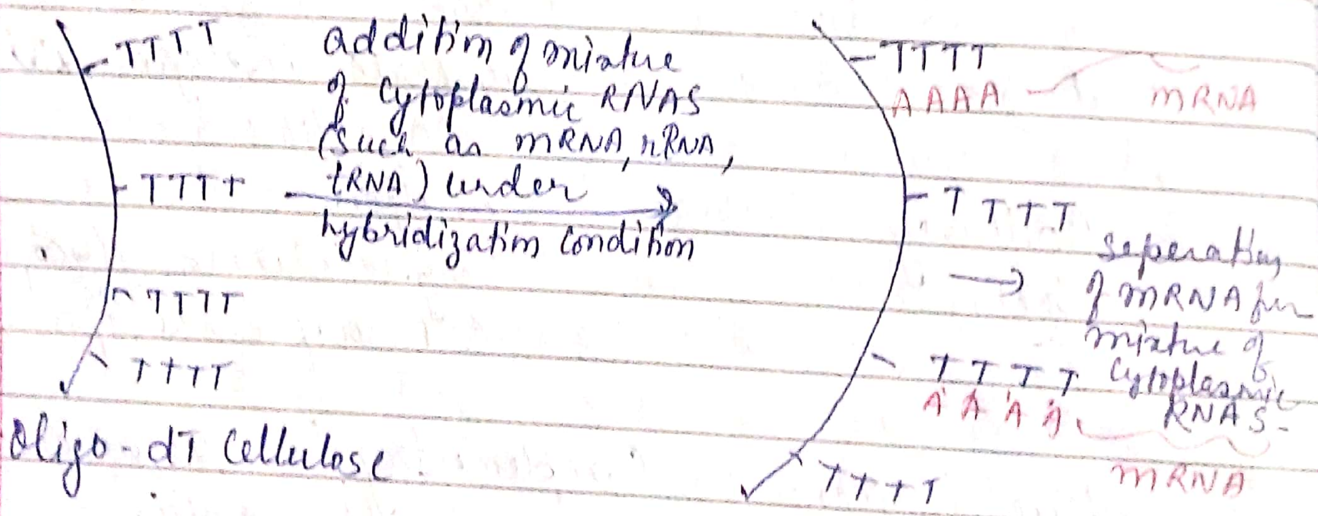

Fig:- Isolation of mRNA by affinity Chromatography. mRNA is isolated from the Cytoplasmic mixture of RNAS using the Oligo (dT) - Cellulose in a Column. Cytoplasmic RNAS such as tRNA & rRNA which are not bound to the matrix beads can wash away and then mRNA can be eluted from the column using a low-salt buffer.

Choice of ligand

Choice of a suitable ligand is the most important feature of affinity chromatography. A number of factors to be considered when selecting a ligand. These factors are:-

<u>Specificity</u> - The ligand should recognize only the molecule of intrest to be purified.

<u>Reversibility</u> - the ligand should form a reversible complex with the molecule of intrest to be purified

<u>Stability</u> - The ligand should be stable to the condition to be used for immobilization as well as the condition of use

<u>Size</u> - The ligand should be large enough such that it contains several groups able to interact with the molecules of intrest resulting in sufficient affinity.

<u>Affinity</u>:- Binding affinity is the strength of the binding interaction between a molecule of intrest to its ligand. Binding affinity is typically measured in terms of equillibrium dissociation constant (Kd). The smaller the Kd value, the greater the binding affinity of the ligand for its target. The equilibrium dissociation constant is the inverse of the equilibrium

association constant (ka). The interactions of a molecule of interest (M) and a ligand (L) can be described by the equation.

For this reaction, the equilibrium dissociation constant (kd) or equilibrium association constant (ka) is defined by

$$k_d = \frac{[M][L]}{[ML]}, \quad k_a = \frac{[ML]}{[M][L]}$$

DNA affinity chromatography

DNA affinity chromatography facilitates the purific[ation of] sequence-specific DNA binding proteins. In th[is] method a double stranded oligonucleotide of t[he] correct sequence is chemically synthesized an[d] linked to an insoluble matrix such as agarose. The matrix with the oligonucleotide attached is then used to construct a column that selectively binds proteins that recognize the particular DNA sequence. The whole process is explained in the following fig:-

DNA affinity chromatography

DNA affinity chromatography facilitates the purification of sequence-specific DNA-binding proteins. In this method a double-stranded oligonucleotide of the correct sequence is chemically synthesized and linked to an insoluble matrix such as agarose. The matrix with the oligonucleotide attached is then used to construct a column that selectively binds proteins that recognize the particular DNA sequence. The whole process is explained in the following figure 1.9.

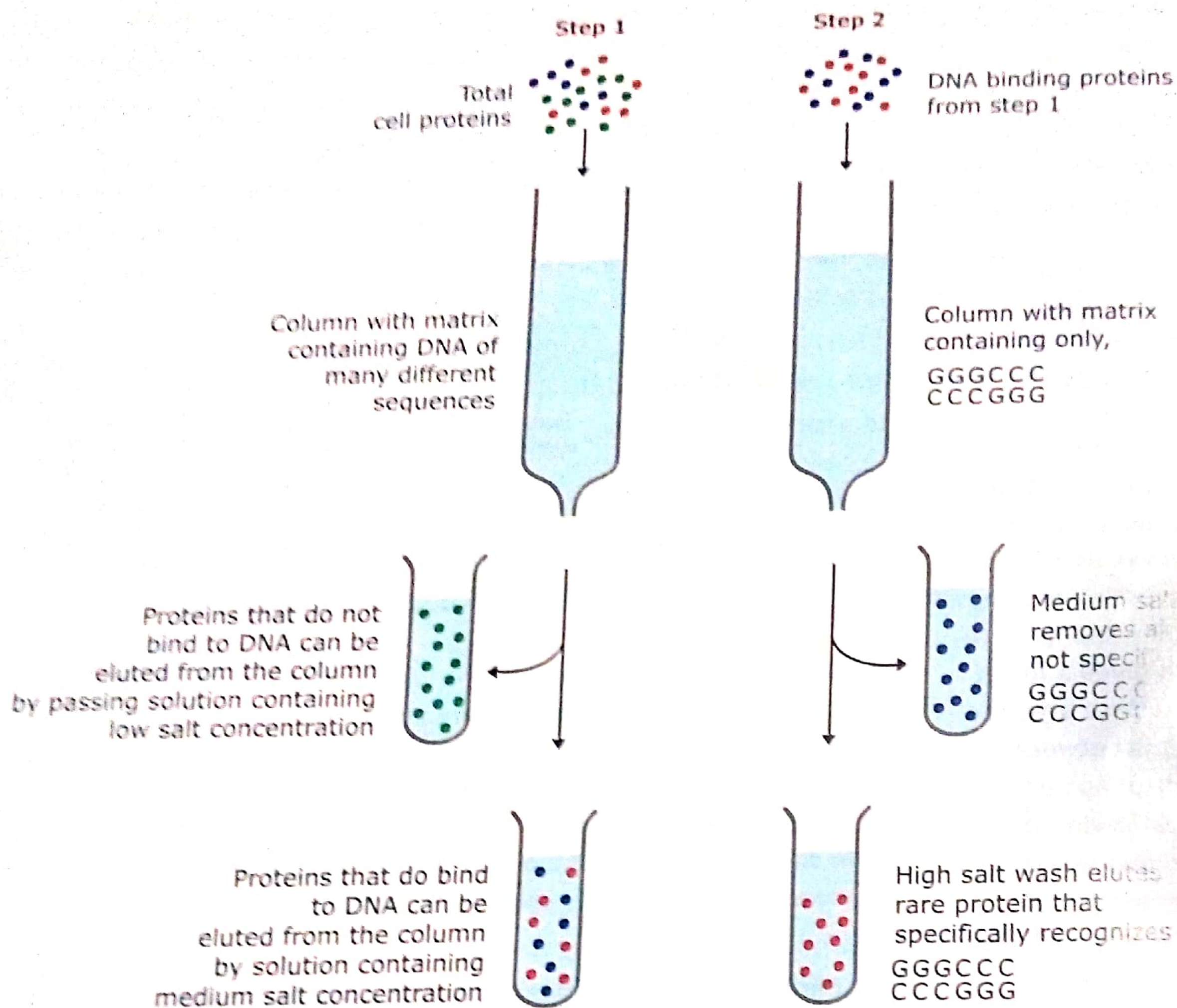

Figure 1.9 DNA affinity chromatography. In the first step, total cell proteins are passed through the column containing a huge number of different DNA sequences. In this process, the proteins that can bind DNA are separated from the remainder of the cellular proteins. Most sequence-specific DNA-binding proteins have a weak (nonspecific) affinity for bulk DNA and are, therefore, retained on the column. This affinity is largely due to ionic attractions, and the proteins can be washed off the DNA by a solution that contains a moderate concentration of salt. In the second step, the mixture of DNA-binding proteins is passed through a column that contains only DNA of a particular sequence. Typically, all the DNA-binding proteins will stick to the column, mostly by nonspecific interactions. These are again eluted by solutions of moderate salt concentration, leaving in the column only those proteins that bind specifically and, therefore, very tightly to the particular DNA sequence. These remaining proteins can be eluted from the column by solutions containing a very high concentration of salt.

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