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FACULTY OF MEDICAL SCIENCES

Affinity Chromatography

Content Outline

Principle & Working

•. It is based on highly specific biological interactions between two molecules, such as interactions between enzyme and substrate, receptor and ligand, or antibody and antigen.

•These interactions, which are typically reversible, are used for purification by placing one of the interacting molecules, referred to as affinity ligand, onto a solid matrix to create a stationary phase while the target molecule is in the mobile phase.

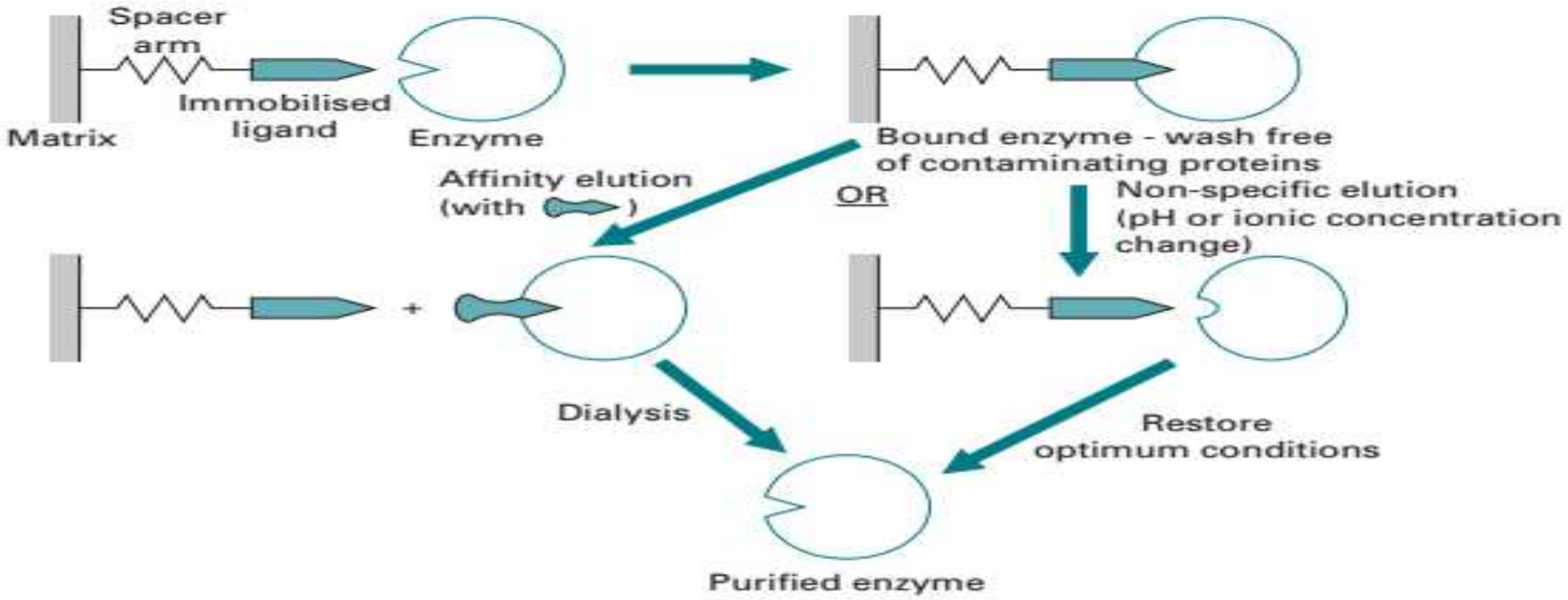
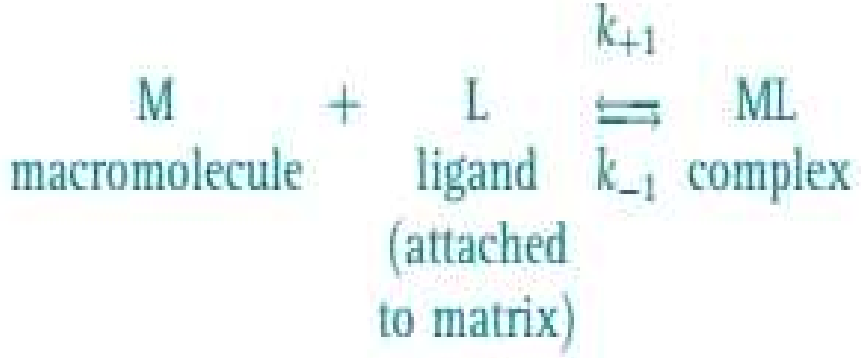


Fig. 11.10 Principle of purification of an enzyme by affinity chromatography.

•The technique requires that the material to be isolated is capable of binding reversibly to a specific ligand that is attached to an insoluble matrix.



•Under the correct experimental conditions, when a complex mixture containing the specific compound to be purified is added to the immobilised ligand, generally contained in a conventional chromatography column, only that compound will bind to the ligand. All other compounds can therefore be washed away and the compound subsequently recovered by displacement from the ligand.

Materials and applications

Matrix

An ideal matrix for affinity chromatography must have the following characteristics:

• possess suitable and sufficient chemical groups to which the ligand may be covalently coupled, and be stable under the conditions of the attachment;

• be stable during binding of the macromolecule and its subsequent elution;

• interact only weakly with other macromolecules to minimise non-specific adsorption;

• exhibit good flow properties.

In practice, particles that are uniform, spherical and rigid are used. The most common ones are the cross-linked dextrans and agarose, polyacrylamide, polymethacrylate, polystyrene, cellulose and porous glass and silica.

Ligand

The chemical nature of a ligand is dictated by the biological specificity of the compound to be purified. To prevent the attachment of the ligand to the matrix interfering with its ability to bind the macromolecule, it is generally advantageous to interpose a spacer arm between the ligand and the matrix.

The optimum length of this spacer arm is six to ten carbon atoms or their equivalent. Some spacers are purely hydrophobic, most commonly consisting of methylene (CH_2) groups; others are hydrophilic, possessing carbonyl (CO) or imido (NH) groups. Spacers are most important for small immobilized ligands but generally are not necessary for macromolecular ligands as their binding site for the mobile macromolecule is well displaced from the matrix.

Ligand	Affinity
5'-AMP	NADP-dependent dehydrogenases, some kinases
Avidin	Biotin-containing enzymes
Lysine	rRNA
Cibacron Blue F3G-A	Nucleotide-requiring enzymes, coagulation factors
Concanavalin A	Glycoproteins containing a-D-mannopyranosyl and a-D-glucopyranosyl residues

Practical procedure

The procedure for affinity chromatography is similar to that used in other forms of liquid chromatography. The buffer used must contain any cofactors, such as metal ions, necessary for ligand–macromolecule interaction. Once the sample has been applied and the macromolecule bound, the column is eluted with more buffer to remove nonspecifically bound contaminants. The purified compound is recovered from the ligand by either specific or non-specific elution. The non-specific elution causes change in the the state of ionisation of groups in the ligand and/or the macromolecule that are critical to ligand–macromolecule binding or due to to a disruption of the ligand–macromolecule interaction; 1M NaCl is frequently used for this purpose

Application

Many enzymes and other proteins, including receptor proteins and immunoglobulins, have been purified by affinity chromatography.

Messenger RNA, for example, is routinely isolated by selective hybridisation on poly(U)-Sepharose 4B by exploiting its poly(A) tail. Immobilised single-stranded DNA can be used to isolate complementary RNA and DNA.

Test your Understanding

The use of insulin hormone to purify its receptor is an example of

a. Ion exchange chromatography
b. Affinity chromatography
c. Gel filtration chromatography
d. Ligand mediated chromatography

Affinity chromatography separation is based on

a. specific interaction between the analyte and the ligand
b. the flow-through time of the analyte
c. molecular weights
d. the duration of the analyte

What is the length of spacer arm used in affinity chromatography?

a. 6-10 carbon chain
b. 20-50 carbon chain
c. 50 carbon chain
d. None of the above

Gradient elution refers to

a. Elution of sample from column by keeping composition of mobile phase constant
b. Elution of sample from column by gradually changing the composition of mobile phase over time
c. plot between elution volume and absorbance
d. None of these

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.