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Mass Spectrometry & Purification Techniques

Short Communication

Brief Note on Principles of Affinity Chromatography

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ABSTRACT

Affinity chromatography is a technique that is essentially dependent on the interaction between the purified molecule and the solid phase that allows the separation of contaminants. Lectins are carbohydrate-binding proteins that can be purified by affinity chromatography. Also, the presence of several molecular lectin forms in the preparation can be isolated. Immobilized lectins are useful in purifying affinity proteins. Immunoaffinity chromatography immobilises an antibody or antigen on a support to purify the protein from which the antibody was produced.

Keywords: Affinity chromatography; Immunoaffinity; Immobilises; Antibody

DESCRIPTION

Monoclonal antibodies are very useful as immunoadsorbents for purifying antigens. The immobilisation of a monoclonal antibody on the appropriate material on the column creates support for the antibody to drag to the produced protein with high selectivity. Affinity chromatography containing DNA which is very specific and important technique for purifying DNA-binding proteins involved in transcription, replication, and recombination. The success of affinity chromatography depends on the conditions used in each chromatography step. Therefore, protocol optimization is essential to achieve optimal protein purification with maximum yield.

Fundamental principles of affinity chromatography

Separation of the protein of interest using affinity chromatography depends on the reversible interaction between the protein to be purified and the affinity ligand bound to the chromatography matrix [1]. Most proteins have unique recognition sites that can be used to select the appropriate affinity ligand. The binding between the protein of interest and the selected ligand must be specific and reversible. Typical affinity purification includes several steps. First, apply the sample under conditions that favour maximum binding to the affinity ligand. After applying the sample, a wash step is performed to remove unbound substances, but the target (bound) molecule remains attached to the affinity support. To release and elute bound molecules, they are usually done non-specifically by using competing ligands specifically in changing the atmosphere of the medium (changes in ionic strength, pH, polarity, etc.). Perform the desorption step (Zachariou, 2008) [2]. Elution allows the purified protein to be collected in a concentrated form.

Biomolecules purified by affinity chromatography: Affinity chromatography is often chosen to purify biomolecules due to its excellent specificity, ease of operation, yield, and throughput [3]. In addition, affinity chromatography has the ability to remove pathogens, which is necessary if the purified biomolecules are to be used in clinical applications. The purity and recovery of target biomolecules is controlled by the specificity and binding constant of the affinity ligand. In general, the association constants of affinity ligands used for biomolecule purification range from 103 to 108 M1. A common affinity ligand used in these purifications is an antibody, but other affinity ligands such as biomimetic dyeligands, DNA, proteins, and small peptides have been used as well[4].

Purification from blood. byaffinity chromatography: Substances such as bilirubin that bind tightly to plasma proteins cannot be easily removed from the blood. This section describes the use of affinity chromatography as a new approach to removing protein-bound metabolites and toxins from the blood [5]. Agarose beads were bound to human serum albumin cyanogen bromide and contained 3050 mg of albumin/g wet weight. When such beads are exposed to plasma from patients with bilirubin-labeled congenital non-hemolytic jaundice, they bind more than 150 g

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bilirubin/g beads. Binding was saturable, concentration-dependent, relatively flow-independent, and reversible by elution with plasma, albumin, or 50% (v/v) ethanol. Beads can be reused for repeated ethanol elution in a cold long reservoir without impairing efficiency. Cortisol, cortisol and taurocollate binding were delayed by beads, but eluting with neutral buffer. Theroxin, taurolitoplate, Shenodoxicolatat, and dicexin were firmly bound but eluted with 50% ethanol. Digoxin does not bind at all. Toward agarosebumin beads, bilirubin was removed and calcium and magnesium were slightly reduced, but red blood cells, leukocytes, platelets, coagulation factors, and various electrolytes and proteins did not change much. Agarose albumin beads may help remove protein-binding substances from the blood of patients with liver failure, protein-binding drug poisoning, or certain metabolic disorders. In addition, it may be possible to make useful adsorbents by attaching other proteins to agarose or other polymer beads.

CONCLUSION

Affinity separation, especially affinity chromatography, has become a popular tool for the selective purification and separation of biological compounds, recombinant proteins, and biopharmacy. These methods are also important techniques for measuring or pretreating samples prior to chemical analysis and can be used to study the binding strength, kinetics, and stoichiometry of biological interactions. All of these methods are based on the selective and reversible interactions that occur in nature with different binding partners. This article provided an overview of the support, immobilisation methods, types of affinity ligands that can be used, and other general principles of affinity separation and affinity chromatography. Various biological and non-biological binders that can be used which have also been contemplated. These binders include antibodies, lectins, protein A/protein G, biomimetic dyes, immobilised metal ion chelates, and boronates. This list includes both naturally occurring binders and binders created synthetically or by rational design. Affinity separation applications are also being considered, including both preparative and analytical applications.

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