Thirty years of affinity chromatography

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Abstract

Affinity chromatography was introduced 30 years ago and is the most powerful tool for purification of biologically active molecules. Affinity chromatography has also had a major impact and virtually revolutionized the entire field of modern biology, molecular biology, biochemistry, medicine and biotechnology. The development of affinity chromatography has led to many other studies and applications in which the concept of molecular recognition and biorecognition is used. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

It is difficult to believe that 30 years have already passed since Pedro Cuatricasas, Chris Anfinsen and one of us (MW) [1] introduced the concept of affinity chromatography. We therefore thank the organizers of the 98 POC Symposium for organizing a special session on this occasion.

Affinity chromatography is based on molecular recognition and is among the early systems in which the principles of molecular recognition were applied to solve important biological problems. The application of molecular recognition or biorecognition is now widespread in virtually all fields of biology, chemistry, molecular biology and biotechnology. In many cases, the entire field of research is based on this principle. Affinity chromatography by itself has also developed very nicely, and there are very few papers in the biological sciences in which affinity chromatography has not been applied.

2. Affinity chromatography and its application for purification

Affinity chromatography as demonstrated in Scheme 1 is based on the simple principle that every biomolecule usually recognizes another natural or artificial molecule, which can be denoted for this short review by the terms 'binder' and 'target'. If either the binder or target is immobilized on a polymeric carrier, it can be used to selectively capture its counterpart, by simply passing the cell extract through the column under favorable conditions. The desired target can then be eluted by changing the external conditions, e.g., ionic strength, pH,

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Consequently, a large number of macro-particle support materials for affinity chromatography separation are commercially available. By far, the most popular support used is agarose. Its success can be attributed not just to inherently good qualities for affinity chromatography, but also to its introduction at an early stage in the development of affinity chromatography technology and strong promotion in the technical and commercial literature. Indeed, a literature survey showed that agarose is used 90% of the time as a solid phase matrix for affinity chromatography.

A number of other supports now exist that can serve in affinity chromatographic application. Among these are cross-linked cellulose, trisacryl, Fractogel TSK and silica [5].

3.2. Activation and coupling

There are many methods for immobilization of ligands. In the following, we will describe a few of them and the most representative. The most frequently used method is the cyanogen bromide-activation of agarose (the activated resin is commercially available), which leads to a highly reactive cyanate ester. Subsequent coupling of ligands to the activated matrix results in an isourea linkage. Despite the popularity of this method, the isourea linkage of the ligand causes several problems during the purification procedure, including nonspecific binding due to charge and leakage of ligand due to instability of the isourea bond [6].

Since the early days of affinity chromatography, active esters, in particular N-hydroxysuccinimide (NHS) esters, have also been used for immobilizing ligands [7]. The preparation of active esters requires a matrix that contains carboxylic groups. Such matrices can be easily obtained from agarose by activation of the hydroxyl groups with different reagents, including cyanogen bromide, activated carbonates, etc. (Fig. 1), and successive reaction with ω-amino acids of different sizes depending on the length of the spacer arm required. The NHS ester is then prepared by mixing the carboxylic matrix

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The Principles of Affinity Chromatography and its applications

<table>
<thead>
<tr>
<th>Binder + Target</th>
<th>Purification</th>
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<tr>
<td>Antibodies and antigens</td>
<td>Lectins and glycoproteins</td>
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<td>Enzymes and inhibitors</td>
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<td>Regulatory enzymes</td>
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<tr>
<td>Hormone-binding proteins</td>
<td>Genetically engineered proteins</td>
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<tr>
<td>Vitamin-binding proteins</td>
<td>Others</td>
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Scheme 1.

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with dicyclohexylcarbodiimide and NHS. Due to a stability problem [8] we introduced a different method based on $N,N,N',N'$-Tetramethyl(succinimido) uronium tetrafluoroborate [9]. The covalent attachment of ligands to such activated carriers proceeds through the production of stable amide bonds.

Another method for activating polysaccharides is the use of $N/N$-disuccinimidyl carbonate (DSC), which forms highly reactive carbonate derivatives with polymers containing hydroxyl groups [10]. These derivatives react with nucleophiles under mild, physiological conditions (pH 7.4), and the procedure results in a stable carbamate linkage of the ligand coupled to the carrier. The immobilization of different ligands (e.g., enzymes, enzyme inhibitors, antigens and antibodies) to activated carbonate carriers has been achieved, together with excellent maintenance of biological activity of the proteins [9,10].

Since we introduce this method it has become the method of choice in our own and in other laboratories [11].

4. Derivative affinity techniques

The broad scope of the various applications of affinity has generated the development of
subspecialty techniques, many of which are now recognized by their own nomenclature. Scheme 2 summarizes some of these techniques, and we will only discuss the most useful and recent examples.

As can be seen from Scheme 2, some of these subcategories have become accepted useful techniques [3].

4.1. Immunoaffinity chromatography [4,12]

Among the most popular of these affinity-derived technologies is immunoaffinity chromatography on antibody columns to purify antigens. There are scarcely any biologically oriented papers in which immunoaffinity chromatography is not used for isolating of receptors, enzymes, DNA fragments, etc. One of the reasons for the rapid growth of immunoaffinity chromatography is the rapid advancement in the field of molecular biology and biotechnology, and the need for purification of pharmacologically active proteins for which ligands are not always available. On the other hand, antibodies can be produced against any compound, and these can be used upon immobilization to purify the parent compounds. Immobilized antibodies have also been used for removal of toxic components from blood by hemoperfusion and for various applications in solid phase immunoassay. It seems that the application of immunoaffinity chromatography
will continue to grow, at least for laboratory scale applications. For biotechnological applications in industry, we will need more efficient methods for large-scale preparations of monoclonal antibodies and columns thereof, which will exhibit favorable properties for adsorption and elution.

4.2. More recent affinity methods

Most protein sequence determinations have recently been accomplished through its DNA, and isolation of a protein from its native source, if done at all, is usually performed on a very small scale. Today, most proteins are produced by recombinant technology and genetic engineering. Therefore during protein expression, the means for purification are usually pre-engineered by introducing a suitable biologically active element into the gene itself [13,14]. These include various temporary affinity tags or affinity tails such as His-Tag which can be purified on metal-chelate affinity chromatography column (particularly a nickel column). Flag™-peptide which is an eight amino acid sequence, includes both an antigenic site and an enterokinase cleavage site. The affinity tag can therefore be used to purify a fusion protein on an immunoaffinity column, and the native protein can be recovered by enterokinase cleavage.

In addition, fusion proteins have also been produced with large proteins such as glutathione transferase, protein A, maltose-binding proteins, cellulose binding domains and biotinylated sequences, all of which have specific ligands through which the fused protein can be purified. In many cases, however, a cleavage site is not introduced and therefore these large contaminating components can adversely affect the designed proteins.

As mentioned above, immunoaffinity chromatography has mainly been developed due to the unavailability of specific ligands which would otherwise be suitable for immobilization and affinity chromatography for most of proteins of interest. Due to the establishment of recombinant technology and immunoaffinity chromatography, many purified proteins are available, and the time is now ripe for a protein to look for a ligand, rather than vice versa. This can be achieved using a phage display library or a combinatorial peptide library to screen the peptides which bind to a specific protein [15]. Such peptides can then be used as affinity ligands for economic and efficient protein purification.

5. Conclusions

It is interesting to note that affinity chromatography started with a single ligand, looking for a specific protein in a God-given 'combinatorial library' of proteins, i.e., from a cell. We have now completed the cycle by reaching a stage whereby a single protein is looking for a specific ligand in a man-made combinatorial library of ligands.

In this short review we have described some of the developments of affinity chromatography. Looking back, has made a significant contribution to the rapid progress which we have witnessed in biological sciences over the past 30 years. Due to its interdisciplinary nature, affinity chromatography has also been the means by which many scientists from different disciplines have been introduced to the exciting fields of modern biology. In the final analysis, it will be scientists from many disciplines who will together elucidate the molecular mechanisms of interactions among different molecules which underly their biological activities and the secrets of life.

References