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Review

Aptamer affinity ligands in protein chromatography

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ABSTRACT

The present review deals with the place of single chain oligonucleotide ligands (aptamers) in affinity chromatography applied to proteins. Aptamers are not the only affinity ligands available but they represent an emerging and highly promising route that advantageously competes with antibodies in immunopurification processes.

A historical background of affinity chromatography from the beginning of the discipline to the most recent outcomes is first presented. Then the focus is centered on aptamers which represent the last step so far to the long quest for affinity ligands associating very high specificity, availability and strong stability against most harsh cleaning agents required in chromatography. Then technologies of ligand selection from large libraries followed by the most appropriate chemical grafting approaches are described and supported by a number of bibliographic references.

Experimental results assembled from relevant published paper are reported; they are selected by their practical applicability and potential use at large scale.

The review concludes with specific remarks and future developments that are expected in the near future to turn this technology into a large acceptance for preparative applications.

Contents

1. Background on protein affinity chromatography and combinatorial ligands

Affinity chromatography is one of the most effective technologies for the purification of proteins from crude or semi-purified extracts. Since its introduction in the late '60 - early '70 [1,2] and thanks to the enormous merits of the technology pioneers, the method has made immense progress and is moreover used presently at preparative and industrial scale. Before the advent of this technology it was really challenging to isolate a single protein from complex mixtures. At that time fractionated precipitation was a common approach associated to ion exchange chromatography, gel filtration, hydrophobic chromatography or more empirically using hydroxyapatite chromatography.

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Abbreviations

SDS-PAGE	Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis
AMP	Adenosine Mono Phosphate
NAD	Nicotinamide Adenine Dinucleotide
PCR	Polymerase Chain Reaction
SELEX	Systematic Evolution of Ligands by EXponential enrichment
EDTA	Ethylene Diamine TertraAcetic acid
NHS	N-HydroxySuccinimide
NTA	NitriloTriacetic Acid
PBS	Phosphate Buffered Saline
IMAC	Immobilized Metal Affinity Chromatography
BSTE	*Bacillus stearothermophilus* Esterase
IgG	Immunoglobulin G
ConA	Concanavalin A
FVII	Coagulation Factor VII
FH	Factor H
FIX	Coagulation Factor IX
GTP	Guanosine TriPhosphate
FMN	Flavin MonoNucleotide
GMP	Good Manufacturing Practices
LNA	Locked Nucleic Acid

Affinity chromatography utilizes the ability of a protein to recognize and dock to natural or synthetic ligands. This property is exploited to the preparation of affinity sorbents where the ligand specific for the protein to purify is chemically attached directly or by means of a spacer arm. The interaction of the protein with the ligand is governed by the mass action law; its specificity is dependent on the affinity dissociation constant which is a probabilistic term indicating how fast the interaction between the molecular partners (the protein to separate and the ligand) occurs with the increase of the concentration of each partner [3]. In such a protein purification system the association rate (the inverse of the dissociation constant) must be high not only to rapidly obtain the complex protein-ligand during the loading phase, but also to maintain the complex stable during the washing step when the concentration of the target protein in the medium decreases close to zero. However, the complex must be dissociable to recover the captured protein; this is generally obtained by using chaotropic agents, deforming agents and various physicochemical conditions (pH, ionic strength, temperature,…) at which the interaction between the partners is annihilated. From these key imperatives it is clear that the most critical point is the selection of the appropriate ligand which is specific for each affinity chromatography case. Actually contrary to ion exchange chromatography or hydrophobic interaction chromatography, affinity systems are not of general applications except for group separation such as glycoproteins with lectin ligands [4], phosphoproteins or phosphopeptides with immobilized metal ion affinity ligands [5]. In many other situations dedicated ligands must be specifically selected.

When considering the specificity of affinity chromatography systems it is rapidly realized that a very large panel of ligand would have to be available to satisfy the separation of the huge number of different proteins. In practice the existence of specific ligands readily available is scarce. They are found among trivial collections of reactive dyes [6], amino acids [7], sugars [8] and other current natural molecules that most of them have only a limited applicability. Thus when wishing to isolate a single protein affinity ligands are only accessorily available.

With the advent of protein expression systems able to produce customized proteins for biochemical and therapeutic applications, efficient purification processes from complex media became crucial unavoidable operations. This is why affinity chromatography was and still is approached by immobilizing specific antibodies in systems named immunoaffinity chromatography techniques [9]. Unfortunately the very good selectivity of antibody ligands is counterbalanced by their poor stability when submitted to stringent chromatographic separations, on the one hand, and are of particularly high cost, on the other hand.

Alternatively in the last decade scientists tried to design collections of affinity ligands called libraries from where it could be possible to retrieve structures of choice. A number of affinity libraries comprising thousands if not millions of molecules are exploitable and may represent a source of affinity chromatography ligands. Among them it is interesting to mention chemical libraries, peptide libraries, polypeptide libraries and DNA libraries, the latter being the object of this review.

Although various synthetic chemical ligand libraries have been described (for example see reviews [10,11]), the ones dedicated to affinity chromatography are of limited number. The most known are triazine biomimetic structures [12], multicomponent UGI reaction libraries [13,14] and peptoids [15]. Combinatorial triazine biomimetic structures are obtained by reacting cyanuric chloride, a well known tripolar reactive symmetric compound, with three different chemical substituents, the reactions being modulated by temperature changes. The synthesis is simple as detailed by Roque et al. [16]; the key issue is to rationally select the substituents to react with the diversity points of triazine. This process has been successfully approached by the group of CR Lowe [17,18] for the purification of various proteins.

Multicomponent UGI reaction libraries, another category of chemical ligands, are produced by a single reaction involving a carboxylated compound, a primary amine, an aldehyde and an isonitrile. Considering the very large diversity of available primary amines and of carboxyl-containing compounds, the number of diversomers theoretically obtainable can reach several hundreds of millions. From this principle an erythropoietin specific ligand has been selected using a small library [19].

Peptoids are linear molecules integrating primary amines where the diversity is played on the side chains of the selected building blocks. Primary amines can be used under combinatorial mode thus generating diversomers in very large number. As an example from this technology a ligand for amyloid beta was retrieved using a library of 38,416 unique different hexamer structures [20].

Ligand libraries of peptides that are also produced synthetically are obtained by reacting sequentially single amino acids to elongate the chain. The synthesis is operated on solid phase and is easily organized to obtain combinations of structures [21,22]. The number of diversomers that can be obtained depends on the length of the peptide and the number of amino acids used. These libraries are presently used as mixed-mode mixed-bed affinity ligands in proteomics applications to reduce the dynamic range of protein extracts [23]. Many examples of affinity chromatography purifications involving hexapeptides from combinatorial libraries are described. The most representative examples are the purification of fibrinogen [24] and immunoglobulins G [25,26].

Recombinant technology contributes also to generate polypeptide ligands by means of phage display methodologies [27] and/or ribosome display [28]. Various strategies are taken depending on the initial polypeptide scaffold.

Affibodies are an example of polypeptides ligands for affinity chromatography originated from the Z domain of protein A, a well-known ligand for immunoglobulins G [29]. Thanks to their