Journal of Chromatography B, 968 (2014) 49–63

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

Analysis of biomolecular interactions using affinity microcolumns: A review

Xiwei Zheng, Zhao Li, Sandya Beeram, Maria Podariu, Ryan Matsuda, Erika L. Pfaunmiller, Christopher J. White II, NaTasha Carter, David S. Hage*

Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304, USA

ARTICLE INFO

Article history:
Received 30 September 2013
Accepted 19 January 2014
Available online 27 January 2014

Keywords:
Affinity microcolumns
High-performance affinity chromatography
Biointeraction analysis
Zonal elution
Frontal affinity chromatography
Ultrafast affinity extraction

ABSTRACT

Affinity chromatography has become an important tool for characterizing biomolecular interactions. The use of affinity microcolumns, which contain immobilized binding agents and have volumes in the mid-to-low microliter range, has received particular attention in recent years. Potential advantages of affinity microcolumns include the many analysis and detection formats that can be used with these columns, as well as the need for only small amounts of supports and immobilized binding agents. This review examines how affinity microcolumns have been used to examine biomolecular interactions. Both capillary-based microcolumns and short microcolumns are considered. The use of affinity microcolumns with zonal elution and frontal analysis methods are discussed. The techniques of peak decay analysis, ultrafast affinity extraction, split-peak analysis, and band-broadening studies are also explored. The principles of these methods are examined and various applications are provided to illustrate the use of these methods with affinity microcolumns. It is shown how these techniques can be utilized to provide information on the binding strength and kinetics of an interaction, as well as on the number and types of binding sites. It is further demonstrated how information on competition or displacement effects can be obtained by these methods.

Contents

* Corresponding author. Tel.: +1 402 472 2744; fax: +1 402 472 9402.
E-mail address: dhage1@unl.edu (D.S. Hage).

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http://dx.doi.org/10.1016/j.jchromb.2014.01.026

1. Introduction

Biomolecular interactions make up an important component of the many pathways and responses that are present in living systems. These interactions include the binding of substrates and co-factors to enzymes, antigens to antibodies, proteins to proteins, and sugars to lectins, as well as the binding of small molecules such as hormones and drugs with transport proteins and receptors [1–4]. These interactions can determine the eventual activity, distribution, excretion, metabolism, and effects of a solute or biomolecule in the body. In addition, the binding of small molecules with proteins can determine the solubility of hydrophobic compounds and can be an important source of direct or indirect competition between different solutes with the same binding protein (e.g., drug–drug interactions) [5–12].

Various techniques can be employed for examining biomolecular interactions. These methods include X-ray crystallography, fluorescence spectroscopy, absorption spectroscopy, ultrafiltration, equilibrium dialysis, capillary electrophoresis, surface plasmon resonance (SPR), and nuclear magnetic resonance spectroscopy (NMR) [5,6,10,13–27]. Two other, related methods that have seen increasing use in the study of biomolecular interactions are affinity chromatography and high-performance affinity chromatography (HPAC, also known as high-performance liquid affinity chromatography or HPLAC) [1–4,9,11,28–30]. These methods use a chromatographic column and support that contain an immobilized biologically related agent (e.g., a protein or receptor) as the stationary phase. This immobilized agent can then be used to study the binding of injected compounds to the column or as a probe to examine the interaction of an injected compound with another binding agent in the mobile phase [2,3]. Various types of columns and formats can be used in these experiments [1–3,9,11,31–33]. However, one topic that has been of growing interest in the analysis of biomolecular interactions is the use of affinity microcolumns for such work (i.e., columns containing affinity ligands and with volumes in the mid-to-low microliter range) [1,2,34–39].

This review examines the developments and applications that have appeared in the use of affinity microcolumns as related to the characterization of biomolecular interactions. First, the basic principles behind affinity chromatography and HPAC are described, especially as related to the use of these methods in investigating biomolecular interactions. The general types of affinity microcolumns that have been reported for binding studies are next considered. These microcolumns range from open-tubular capillaries and packed capillaries to small columns based on monoliths or particulate supports [2,4,40–55]. The various approaches that have been used with affinity microcolumns for binding studies are then discussed. This discussion includes various formats based on zonal elution and frontal analysis [1,2,11,31,34,50–59]. In addition, techniques such as peak decay analysis, split-peak analysis, ultrafast affinity extraction, and band-broadening measurements are discussed [2,11,31,34,55]. The principles behind each method are described, along with the advantages and possible limitations of these methods. Various examples are also provided to illustrate the use of these techniques with affinity microcolumns. These examples range from drug–protein interactions (including those involving chiral drugs), to antibody–antigen interactions, the binding of enzymes with inhibitors, and the interactions of lectins with sugars, among others.

1.1. Basic principles of affinity chromatography and HPAC

Affinity chromatography is a liquid chromatographic technique that uses a biologically related binding agent, or “affinity ligand”, as the stationary phase to separate or analyze sample components [3,60–64]. This stationary phase can be created by covalently attaching, entrapping, absorbing or in some other way immobilizing the affinity ligand to a chromatographic support [3,60,61]. This solid support and the stationary phase are placed within a column or capillary that can then be used for the purification, separation or analysis of targets capable of binding to the affinity ligand [28,61–70]. The retention and separation of a target from other sample components is based on the specific and reversible interactions that characterize many biological interactions, such as the binding of an antibody with an antigen or a hormone with a receptor [1–3,61–64]. If the interaction is strong (i.e., with an association equilibrium constant greater than 10^5–10^6 M^{-1}), an elution buffer and a change in the pH, temperature, or mobile phase composition may be required to remove the target from the column [34,71,72]. If weaker binding is present (i.e., an association equilibrium constant of 10^5–10^6 M^{-1} or less), it may be possible to elute the target under isocratic conditions. This latter method is sometimes referred to as weak affinity chromatography (WAC) [29,30]. The variety of elution formats, immobilized ligands, and columns that can be used in affinity chromatography has made this method a valuable tool for the study of biomolecular interactions [1–3,31,51,57], as will be discussed in this review.

In any type of affinity chromatography, the support that is used for the immobilized affinity ligand should have low non-specific binding to sample components and yet be easy to modify for ligand attachment [60–64,73–76]. Traditional affinity chromatography typically employs relatively inexpensive supports and non-rigid materials with low-to-moderate efficiencies, such as agarose gels or other carbohydrate-based materials [3,62,63,73]. In the method of HPAC, which is the type of affinity chromatography utilized with most affinity microcolumns, the support is a material that has sufficient mechanical stability and efficiency for use in HPLC [3,9,61,64,73,74]. This type of support, in turn, tends to provide HPAC with better speed and precision than traditional affinity chromatography, along with greater ease of automation through the use of HPLC systems [1,4,59,61,73–76]. Possible supports for HPAC include particulate materials based on modified silica or glass, azalactone beads, and hydroxylated polystyrene media [1,3,61,73,74]. Various types of monolithic supports have also been considered for use in HPAC and affinity chromatography, such as those based on organic polymers, silica monoliths, cryogels and modified forms of agarose [4,73,75–82]. The recent interest in monoliths for these affinity-based separations is due to several useful features of these supports, including their rapid mass transfer properties, low back pressures, and ability to be made in a variety of shapes and sizes [4,73,75,76,79,82].

The use of affinity chromatography and HPAC for the study of biomolecular interactions is sometimes referred to as analytical affinity chromatography, quantitative affinity chromatography, or biointeraction chromatography [2,3,11,31,51–59,61,83]. This type