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Review

The development and characterization of protein-based stationary phases for studying drug–protein and protein–protein interactions

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ABSTRACT

Protein-based liquid chromatography stationary phases are used in bioaffinity chromatography for studying drug–protein interactions, the determination of binding affinities, competitive and allosteric interactions, as well as for studying protein–protein interactions. This review addresses the development and characterization of protein-based stationary phase, and the application of these phases using frontal and zonal chromatography techniques. The approach will be illustrated using immobilized heat shock protein 90α and the immobilized estrogen related receptor stationary phases. In addition, the review discusses the use of the protein-coated magnetic beads for ligand and protein fishing as well as for the identification of unknown ligands from cellular or botanical extracts.

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1. Introduction

The measurement of protein–protein interactions has become a key factor in drug discovery. The methods currently used for these measurements include but are not limited to static light scattering (SLS) [1,2], ultracentrifugation [3], X-ray scattering [4], self-interaction chromatography (SIC) [5,6] and size-exclusion chromatography (SEC) [7,8]. The characterization of the protein–protein interactions using these methods is typically carried out by determining the second virial coefficient parameter. A positive coefficient implies that repulsion interactions dominate the protein–protein interaction, while a negative number is indicative of a net attraction for the protein–protein interactions [9]. Dumetz et al., for example, carried out SIC to measure the effect of salt concentration on the protein–protein interactions, [6] and determined that the protein interactions show very low salt dependence for sodium chloride solutions, with a very pronounced effect when ammonium sulfate is employed, for their proteins tested. A limitation of SIC is that it requires prior immobilization of protein, which can affect protein structure and thus protein–protein interaction. In addition, SIC uses the same protein as ligand and ligate and thus is not useful to study multiprotein complexes, and several other methods are limited to protein–protein interactions between two different proteins. Bloustine et al. used SEC technique to determine the solute distribution coefficient from the retention times measured by refractive index detector and diode array detector [7]. They also compared the result obtained by SEC technique to the results obtained by frontal chromatography [10,11] and from light-scattering measurements [12–14]. Therefore, SIC and SEC techniques have the advantage of a shorter duration time relative to the other techniques. Although, these methods are very useful, they still use larger amounts of protein, albeit, Garica et al. have recently shown that SIC technique can be miniaturized to a microchip, thus significantly reducing the amount of protein required for the determination of the second virial coefficient parameter [9]. SLS technique also requires large amounts of protein in order to determine the coefficient parameter and therefore is seldom used [1,2].

Protein–protein and protein-ligand interactions have also been explored through protein immobilization on solid supports of chromatographic and non-chromatographic experimental techniques such as microarrays [15,16], biosensors [17] and nanotechnology [18]. In protein microarrays, functionally active proteins were arrayed using microfabricated polyacrylamide gel pads to capture their samples [19], while more recently, Macbeath et al. immobilized the protein onto the surface of the plates in order to probe thousands of protein–protein and ligand–protein interactions [15,16]. This method was developed to take advantage of existing instrumentation. This was accomplished by immobilizing the protein onto a solid support while preserving its proper conformation. More specifically, the protein was immobilized covalently onto the surface of smooth flat surfaces of microscope slides. Although, several chemically derivatized slides were used the majority of the studies were carried out using the aldehyde-containing slides. This takes advantage of immobilizing the protein through primary amine containing residues, specifically the N-terminus lysine residue as they are a more reactive α-amine. For details on the fabrication of protein microarrays c.f. ref Macbeth et al. Briefly, nanoliter volumes of protein samples is delivered on the slides in PBS with 40% glycerol, which prevents evaporation of the nanodroplets, thus keeping the proteins hydrated. The unreactive functional groups are capped using BSA. These slides have been used to study protein–protein interactions as well as small molecule protein interactions. Protein interaction at specific sites can also be studied using protein-domain microarrays, Espejo et al., used a microarray approach to study signal transduction issues and interactions that are sensitive to arginine methylation. This was accomplished using a glutathione S-transferase (GST)-fusion protein that contained a peptide-specific binding motif, allowing for the immobilization onto a support to study the specific protein interaction at a specific site [20]. Several other methods have been reported for protein microarrays and have been previously reviewed [21]. For example, Zhu et al. constructed yeast proteome microarrays containing 5800 yeast proteins and screened it for various biochemical activities [16]. Many known kinases and calcineurins were identified along with 33 new calmodulin binding partners. Knezevic et al. showed the alteration in specific levels of more than ten cancer related proteins expression due to ionizing radiation treatment [22]. Miller et al. also successfully showed the antibody microarrays containing 184 antibodies to profile the serum of patient diagnosed with prostate cancer to identify potential biomarkers [23]. The further development and characterization of this technology can lead to its application in a personalized medicine, where a treatment can be tailored to specific individual for an increased efficacy.

An alternative approach is to use the immobilized protein as a stationary phase in bioaffinity chromatography. This technique is based on specific reversible interaction between the ligand and the immobilized protein. A widely used method for the synthesis of protein-based stationary phases is the immobilization of the protein on the solid support using adsorption or covalent immobilization. The resulting protein-based LC phases (SPs) can be used to determine and characterize ligand–protein interactions [24,25]. The theory and applicability of using immobilized proteins to explore the interactions between ligands/substrates and an immobilized cytosolic protein or enzyme were initially described by Chaiken [26] and Carr [27] and expanded to the study of transmembrane proteins by Lundahl et al. [24,25]. It was further expanded to the immobilization of cellular membrane fragments and their use in cellular membrane affinity chromatography (CMAC) [28]. Recent data using frontal and zonal chromatographic techniques have demonstrated that ligand binding affinities (K_d values) obtained using protein based stationary phases are comparable to affinities obtained using standard membrane binding techniques [29].

In addition, Belanger immobilized Mex67-Nep1 onto sepharose beads and determined protein–protein interactions by LC/MS and Western blotting techniques [30]. Magnetic beads have gained a significant amount of interest as an alternative method for 'fishing' experiments for both ligand and protein binders. It has been demonstrated and will be discussed in greater detail below that the formation of protein–protein complexes remain intact on the surface of the protein coated magnetic beads [31–34]. The protein coated magnetic beads, were successfully used to fish out binders from a mixture of binders and non-binders for HSA [35], and the identification of ligands that modulate protein–protein interactions [31]. Further, Jonker et al., have shown that the magnetic nano-particles can be used as a novel high throughput screening methodology, to determine whether a compound has an affinity for an immobilized target in a 'yes' or 'no' method [32]. The formation of a multiprotein complex was also carried out on the surface of the protein coated magnetic beads, and it has been demonstrated that the resulting protein coated magnetic bead was able to fish out a binding partner in a complex matrix, the KU-812 cellular matrix [34].

The development of protein-based stationary phase and their characterization using frontal and zonal chromatographic techniques will be demonstrated using Hsp90α and the estrogen related receptors. The use of HSA and Hsp90α-coated magnetic beads for ligand and protein fishing will also be reviewed.