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Review

Protein behavior at surfaces: Orientation, conformational transitions and transport

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ABSTRACT

Chromatography is the key technology in protein purification as well as in protein refolding. Taking the scientific development and technological innovation of protein chromatography as the objective, this article is devoted to an overview of protein behavior at chromatographic surfaces, including protein orientation, conformational transitions (unfolding and refolding), and protein transport. Recent advances achieved by using molecular simulations as well as theoretical and experimental investigations are elaborated and discussed with emphasis on their implications to the rational design of novel chromatographic surfaces or materials and mobile phase conditions for the development of high-performance protein chromatography.

Contents

1. Introduction

Adsorption chromatography based on complex protein binding to and dissociation from surfaces is a key technology in

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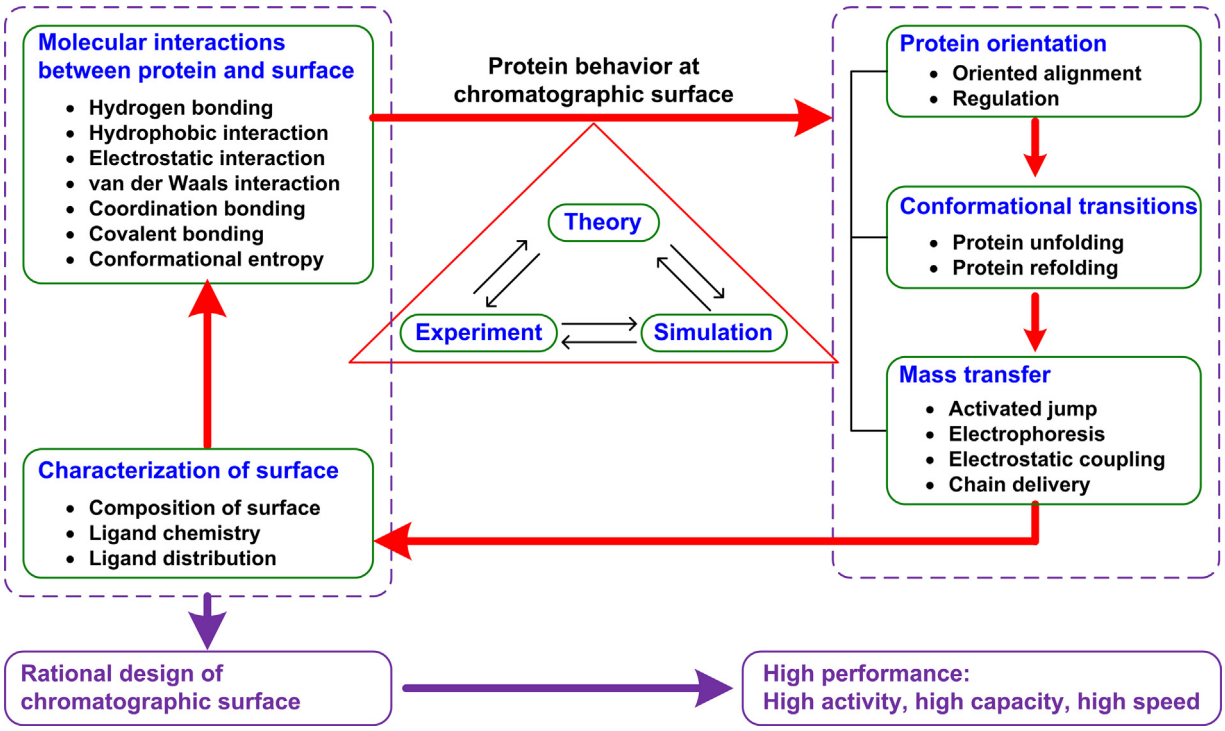

Fig. 1. Protein behavior at chromatographic surface.

protein purification [1–3]. Protein adsorption at chromatographic surfaces involves various interactions between the protein and surface or the ligands attached to the surface. The interactions that contribute to protein adsorption include hydrogen bonding, hydrophobic interaction, electrostatic interaction, van der Waals interaction, coordination bonding, covalent bonding, and conformational entropy [1,2,4]. Examinations of these interactions facilitate the knowledge development and findings on protein behavior at chromatographic surfaces, which have provided some understanding of the fundamentals of protein chromatography, such as protein orientation, conformational transitions (unfolding and refolding) and mass transfer, as shown in Fig. 1. Still many problems exist at present that need to be solved or clarified, such as the difficulties in experimental examination and regulation of protein orientation or conformational transitions at surfaces, and the micro-scale elucidation of surface transport mechanisms. Some of the technological problems are being studied by using molecular simulations [5–8] combined with theoretical [1,9] and experimental investigations [10,11].

The objective of the present review is to summarize recent advances in the investigation of protein behavior at chromatographic surfaces, and to provide more insight into the rational design of novel and more efficient surfaces by optimizing the composition, ligand chemistry, ligand density and ligand distribution (Fig. 1). Protein adsorption phenomena at solid surfaces have been comprehensively discussed in recent reviews [4,10], focusing on both individual and ensemble behaviors of protein molecules driven by their attraction to solid surfaces. Relevant experimental and computational strategies to practically approach the field of protein adsorption mechanisms have also been summarized. Thus, as shown in Fig. 1, the present review will focus on the surfaces of protein chromatography, including both attractive and repulsive surfaces studied in recent years. Molecular interactions between protein and surfaces are briefly described at first. It is the dominant factor for protein orientation at a surface, which is described in the following part by focusing on the oriented alignment of protein molecules and its regulation. Thereafter, an introduction of protein conformational transitions (unfolding and refolding) at surfaces is provided to highlight recent research progresses such as the greatly enhanced refolding yield by like-charged surfaces. Then, emphases are placed on the recent advances in protein transport at surfaces, in which the possible mechanisms of surface transport reported recently are detailed, including activated jump, electrophoresis, electrostatic coupling, and chain delivery. The knowledge and findings reviewed are discussed for the rational design of novel chromatographic surfaces for high-performance protein chromatography. These surfaces should provide well-tuned molecular interactions for proteins and thus offer good conservation of protein activities and high-performance mass transport, leading to high-performance protein chromatography characterized by high activity and high capacity at high speed (high productivity) (Fig. 1).

2. Protein orientation

2.1. Introduction

Proteins are complex biopolymers composed of 20 naturally occurring amino acids as monomeric units [10]. The diversity of amino acids, e.g., hydrophobicity/hydrophilicity or charged/neutral features, results in an extraordinary structural and functional complexity of protein molecules. Chicken egg white lysozyme (CEWL), for example, is positively charged at pH 7 because it is contributed by 17 positively charged amino acid residues (R and K) and 9 negatively charged residues (E and D) at the protein surface. That is, a protein molecule commonly has both positive and negative charge patches. Things become more complex when considering the folding of protein molecules into their secondary and tertiary structures [10]. Protein folding leads to a heterogeneous surface that can be decomposed into individual patches exhibiting specific properties such as hydrophobic/hydrophilic, or charged/neutral, as shown in Fig. 2. Thus, protein behavior at chromatographic surfaces is complex, often a result of interplay of attraction and repulsion of individual patches on the heterogeneous surface of protein that