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Study of the conformational change of adsorbed proteins on biomaterial surfaces using hydrogen-deuterium exchange with mass spectroscopy

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ABSTRACT

There is no doubt that protein adsorption plays a crucial role in determining biocompatibility of biomaterials. Despite the information of the identity and composition of blood plasma/serum proteins adsorbed on surfaces of biomaterials to understand which proteins are involved in blood/biomaterial interactions, it still does not provide information about the conformations and orientations of adsorbed protein, which are very important in determining biological responses to biomaterials. Therefore, our laboratory has developed an experimental technology to probe protein conformations on materials that is applicable to mixtures of proteins. Herein, the new application of hydrogen/deuterium (H/D) exchange combined with mass spectrometry was applied to determine conformational changes of adsorbed proteins at biomaterial surfaces. The results suggest that there may be a significant conformational change in adsorbed proteins at 'low' bulk concentrations that leads to a large change in the kinetics of H/D exchange as compared to 'high' bulk concentrations. This technique may eventually be useful for the study of the kinetics of protein conformational changes.

1. Introduction

The adsorption of protein molecules at liquid-solid interfaces is an important phenomenon that affects the response of cells to biomaterial [1–5]. Protein adsorption is especially important in the development and design of biomaterials, particularly blood-contacting devices [6–11]. Therefore, a fundamental understanding of the mechanism of protein adsorption onto biomaterials is of central importance in the ultimate development of protein-repellent materials for use as implanted biomaterials [6,8,9]. Competitive adsorption of proteins is important in the failure of biomaterials that occurs in the presence of blood proteins. For example, when blood comes into contact with artificial biomaterials, blood proteins quickly adsorb to the material surfaces, followed by complex changes in conformation of the proteins at the interface [9]. However, the first proteins to arrive at the surface (e.g. albumin, alpha-1-antitrypsin) do not promote a biological response to the material [12,13]. A biological response is triggered by the displacement of these proteins by other proteins, which have a high affinity for the surface and which become activated upon adsorption [14–16]. The displacement of adsorbed proteins by proteins with higher affinities for the surface is known as the "Vroman effect" [17,18]. Many investigations have focused on studying this process, to gain an understanding of the dynamics of protein adsorption using theoretical approaches [19–21] and experimental studies [20–22]. However, due to the complexity of the adsorption process and experimental limitations, the dynamics of protein adsorption on the surface is still not comprehensively described. Among the modeling-oriented approaches, some recent efforts to understand the Vroman effect have been devoted to understanding the correlation between protein transport rate and adsorption kinetics on the surface [21,23]. However, these investigations have some limitations, such as modeling only one protein [23] or neglecting mass transport [21]. While Chittur's group suggested a more comprehensive model of multi-protein adsorption and showed the Vroman effect with their models [19,24], they assumed that there is no spreading of the protein molecules on the surface.

When proteins interact with a surface, multiple chemical groups on the protein can interact with the surface. Over time, adsorbed protein molecules tend to undergo a molecular relaxation or spreading process on the surface, producing a higher affinity interaction with the surface [21,25]. It has been demonstrated that the reversibility of protein adsorption depends on residence time [18]. Thus, the dynamics of the Vroman effect will be altered by spreading effects. In some cases, the Vroman effect may be eliminated if

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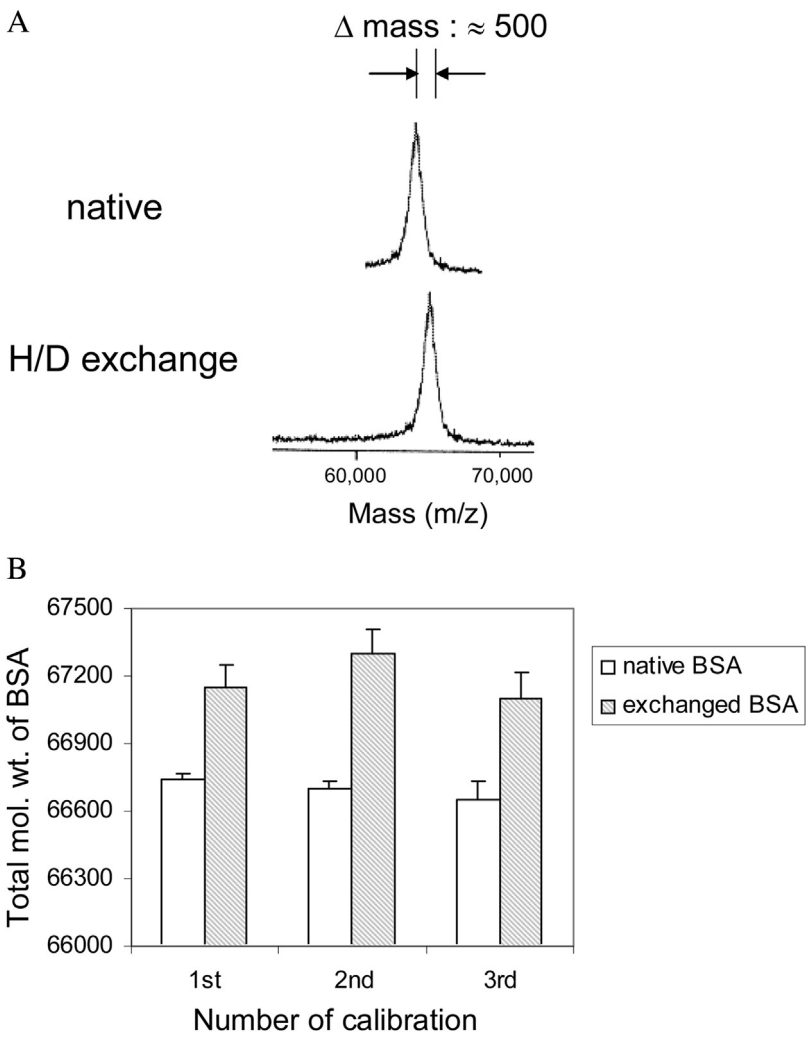

Fig. 1. Hydrogen/Deuterium exchange of bovine serum albumin analyzed by MALDI-TOF mass spectrometer. (A) The MALDI-TOF mass spectra of a non-exchanged albumin (native) and exchanged-in-solution albumin (exchanged BSA). (B) The Calibration of MALDI-TOF mass spectrometer using bovine serum albumin. Error bars represent standard deviations between the measurements (n = 3).

protein spreading is very fast (i.e., if proteins become irreversibly bound before competing proteins can displace the adsorbed proteins).

Changes in conformation of adsorbed proteins must be considered in the design of biomaterials, with respect to the blood compatibility of the material. For instance, a conformational change within fibrinogen adsorbed on biomaterials allows interaction with the platelet receptor, glycoprotein complex IIb/IIIa, that does not occur in solution [9]. A better understanding of this phenomenon is important for improving the design of biomaterials with enhanced biocompatibility.

It is known that the extent of conformational change in adsorbed protein is determined by the amount of unoccupied surface area and the amount of time the adsorbed protein resides on the surface. For example, Cheng et al. have shown that the fibronectin undergoes a conformational change more significantly at low solution concentration than at high concentration by using Fourier Transform Infrared/Attenuated Total Reflectance (FTIR/ATR) techniques [26]. They suggested that the available free surface area for the protein is greater at low solution concentration, compared with high solution concentration, resulting in more significant conformational changes, while at high concentration the protein-protein interactions due to rapid filling of vacant sites on the surface hinders conformational change. Therefore, the solution concentration plays a crucial role in determining the extent of conformational change in adsorbed proteins.

Hydrogen/Deuterium (H/D) exchange methods have been used for the study of protein structure, dynamics and protein stability [27–32]. In principle, some hydrogen atoms in proteins are easily exchangeable when they are exposed to surrounding deuterated solvents, such as D_2O.

However, under certain circumstances such as protein-ligand binding, some protons are no longer easily exchangeable, due to ligand binding-induced stability and H/D exchange has been used for the quantification of protein-ligand interactions by mass spectrometry [31,32]. Powell et al. extracted the values of denaturant concentration at the transition midpoint, $C^{1/2}_{SUPREX}$ from their SUPREX (Stability of Unpurified PRoteins from rates of H/D EXchange) curves using H/D experiments [31]. They found that $C^{1/2}_{SUPREX}$ values are higher in the presence of ligands, due to binding-induced stabilization. From those values, they obtained thermodynamic information about protein-ligand interactions, such as folding free energies and ligand dissociation constants. In a similar way, it is expected that adsorbed proteins that undergo more significant conformational changes are more resistant to H/D exchange due to changes in the local solvent environment, steric exclusion, surface-induced stability, or exposure of hydrophobic residues at