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Surface enrichment of proteins at quartz/water interfaces: A neutron reflectivity study

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Abstract

Neutron reflectivity (NR) was used to study the adsorption of human serum albumin and human fibrinogen on quartz. The proteins were individually and sequentially adsorbed from heavy water and heavy water/methanol mixtures at pH 4 and 7.0. The technique allows for the subnanometer resolution of the adsorbed layer thickness and gross morphology. Under the conditions of our measurements we found that fibrinogen formed a distinct layer that we interpret as a mat of the protein three layers thick whereas albumin formed only diffuse layers. The adsorption pattern of the two proteins changed radically when one protein was adsorbed on top of the other (previously adsorbed). In general our measurements indicate that the adsorbed protein layers on quartz are rather loosely bound and that these layers, incorporating as much as 80% water, extend further into the bulk fluid than might have been expected.

Keywords: Protein adsorption; Neutron reflectivity; Fibrinogen; Albumin; Sequential adsorption

1. Introduction

Protein adsorption on surfaces is important in the performance of a wide range of biotechnological devices including implants, biosensors, and bioseparation units. Adsorbed proteins "catalyze" cell adhesion on implants inducing rejection of the foreign body by the immunosystem. They clog artificial arteries, hemodialysis and hemofiltration membranes and foul membrane biosensors, membranes in protein purification devices and heat exchangers in food processing industries, significantly reducing their operational efficiency and lifetimes.

The importance of understanding and as desired either preventing or promoting protein adsorption on solid surfaces has led to extensive study (for reviews see [1–7]). Most of the published work has been focused on the determination of adsorption isotherms on different substrates, on the characterization of the adsorbed layer, and on the phenomenological modeling of the adsorption process. The adsorbed layer has been characterized by ellipsometry, transmission electron microscopy, and total internal reflection IR spectroscopy. Nevertheless, a number of experimental limitations have given rise to controversy about the mechanisms by which proteins adsorb on a solid surface and about the structure of the adsorbed protein layer. Even in the simplest scenario that we can conceive, the adsorption of a single protein from solution on a well-defined surface, many questions arise about the nature of the adsorption process. Among the most obvious: the orientation of the proteins at the surface, the deformation of flexible molecules as they interact with the surface, and whether or not multilayers are formed. One of the difficulties that one encounters in characterizing the structure of adsorbed protein layers is that some techniques require a dry sample. We can expect that drying a protein layer adsorbed in equilibrium from solution will create artifacts and thus preclude the appropriate interpretation of the results. Also, most techniques do not have the capability of revealing structural features without which it is practically

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impossible to fully understand the adsorption process. For example, a common approach is to estimate the number of layers from surface concentration measurements alone assuming dense packing and overlooking the possibility of having a dilute or "patchy" multilayer with defects—cases which, as we shall show, are distinctly different when examined with the neutron reflectivity (NR) technique employed here.

Recently the specular reflection of neutrons has been used to characterize the adsorbed layer of albumin on lipid bilayers prepared on a silicon substrate [8] on a poly(acrylic acid) brush on silicon substrate [9], on self-assembled monolayers [10–12], on quartz [13], on silicon/water interfaces [14–16], on a hydrogel polymer [17], at air/water interfaces [18,19] the adsorption of lysozyme on silicon oxide and octadecyltrichlorosilane [20–24], and the adsorption of fibrinogen on silicon [25]. The use of neutron reflectivity in protein adsorption studies is very attractive because it can potentially give information about adsorbed layer profiles at subnanometer resolution. The objective of this study is to use this technique to determine the thickness and gross morphology of the adsorbed protein layers of different flexibility deposited on a solid substrate. Because of their importance, their differences in flexibility and shape, and their abundance in plasma we decided to investigate the adsorption of fibrinogen and human serum albumin (HSA) on quartz. Our choice of substrate is based on the numerous devices designed to handle plasma that are partially glass. Because of this early interest on glass/protein interactions, there is an abundant collection of experimental data in which various aspects of plasma proteins adsorption on glass have been studied. This data is invaluable for critical comparison of our own experimental data.

Two further advantages of the specular neutron reflection technique are that it is nondestructive, in that thermal neutrons at the fluxes used in these measurements do not damage protein structure even over long time scales, allowing slowly progressing adsorption processes to be tracked. Since the sample is measured in situ, it does not need to be dried as is necessary to obtain TEM/SEM images. It is difficult to believe that drying does not give rise to artifacts in adsorbed layer thickness and structure that do not accurately reflect the situation when an adsorbed biological molecule is exposed to water. One slight disadvantage of neutron scattering that should be mentioned in the present case is the somewhat reduced solubility of proteins in D_2O, the necessary solvent to obtain good neutron contrast against a hydrogenated protein. This reduced solubility of proteins in heavy water is a consequence of changes in hydrogen bonding near the protein's surface [26] and necessitates careful verification of the protein concentration in our sample solutions.

Adsorbed proteins are commonly visualized as forming a well-defined layer built upon specific interactions between the protein and the surface. Is that simple picture correct or is protein adsorption from solution in some cases more appropriately described as a diffuse protein layer close to a surface—equivalent to surface enrichment? Whether or not multilayers are formed, the orientation of the protein on the surface and the homogeneity of the deposits depend, in part, upon the flexibility of the protein. It is accepted that structurally stable proteins adsorb on hydrophobic surfaces under all charge conditions and on hydrophilic surfaces only if they are electrostatically attracted; while, in contrast, structurally unstable proteins adsorb on all surfaces because the increase in entropy due to conformational rearrangements outweighs the unfavorable enthalpy of adsorption [7]. The effect of the differences in the stability between HSA and fibrinogen on their adsorption on solid surfaces is explored here.

The adsorption of more than one protein on a surface is of practical interest and its interpretation a scientific challenge. In sequential protein adsorption, one protein is first adsorbed onto a surface, left there for a period of time after which a second protein is added to this protein–adsorbent complex. Two distinct scenarios are normally encountered: (1) the adsorption of one protein first "induces" the adsorption of other proteins in the mixture, and (2) after the first protein is adsorbed, it may be displaced by other proteins or may be followed by the adsorption of other proteins [27,28]. For example, fibrinogen in plasma is adsorbed first and then is displaced, probably by kininogen [29,30]. This displacement is known as the Vroman effect [31]. In this work we apply neutron reflectometry to the sequential absorption of HSA and fibrinogen.

Fibrinogen is an elongated molecule whose complete microstructure is as yet unknown. It is present at a concentration of $\sim$4.5 mg/cm^3 in normal plasma and is a "sticky" protein that plays a major role in the blood coagulation cascade by forming fibrin upon interaction with platelets. As might therefore be expected, fibrinogen adsorbs readily on a variety of surfaces and is known to form multiple layers [1,32–34], which in some cases initiate thrombogenesis. It has a molecular weight of 340,000 and consists of three pairs of nonidentical polypeptide chains and two pairs of oligosaccharides with a total of 19 galactose residues, 22 mannoses, 19 glucosamines and 6 sialic acid groups. It has molecular volume of $3.9–4.1 \times 10^5$ Å^3 and a partial specific volume of 0.71–0.72 cm^3/g. The molecule of fibrinogen can be represented by three nearly globular domains joined by two helical domains to which the carbohydrates are attached, and a pair of α–C domains that extend from the distal globular domains (D) towards the central globular domain (E). These C domains can bind the central domain or can bind together two fibrinogen molecules. The C and D domains are flexible and they may deform in contact with a surface. The total length of the fibrinogen molecule is approximately 470 Å (D: 60 Å + helical: 150 Å + E: 50 Å + helical: 150 Å + D: 60 Å). The globular domains are negatively charged, whereas the C domains are positively charged at physiological pH, and are more hydrophobic than the C domains. The isoelectric point of fibrinogen is 5.5; so, it is negatively charged at physiological pH. Typically, fibrinogen is adsorbed from solutions containing a few mg/cm^3.