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Real time, high resolution studies of protein adsorption and structure at the solid–liquid interface using dual polarization interferometry

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Abstract

A novel method for the analysis of thin biological films, called dual polarization interferometry (DPI), is described. This high resolution (<1 Å), laboratory-based technique allows the thickness and refractive index (density) of biological molecules adsorbing or reacting at the solid–liquid interface to be measured in real time (up to 10 measurements per second). Results from the adsorption of bovine serum albumin (BSA) on to a silicon oxynitride chip surface are presented to demonstrate how time dependent molecular behaviour can be examined using DPI. Mechanistic and structural information relating to the adsorption process is obtained as a function of the solution pH.

1. Introduction

Many biological processes involve proteins changing shape as they function. A key aspect of understanding these processes is to be able to detect and quantify the structural changes of such proteins and determine the conditions under which the changes occur.

Significant advances in this area have been achieved through neutron reflectivity experiments, where highly detailed structural analysis has been undertaken of proteins at interfaces [1]. However, in addition to necessitating large scale remote facilities, these experiments are limited in providing real time analysis of protein behaviour without a drastic loss in resolution.

In this work a new laboratory-based analytical tool called dual polarization interferometry (DPI) has been used to study the adsorption of the globular protein bovine serum

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albumin (BSA) at the solid–liquid interface as a model system. By using two polarizations of the light source, DPI allows unambiguous determination of the thickness and refractive index for a given layer. With a measurement capability of 10 data points per second at a resolution of 1 Å, detailed complementary real time information on protein adsorption behaviour can be extracted.

The characteristics of BSA in bulk solution are well documented [2]. Essentially, BSA is a prolate ellipsoid with approximate dimensions of 140×40 Å. It has three principal domains with different net charges that are pH dependent and an isoelectric point of about pH 5. The different conformational isomers of BSA that form reversibly in bulk solution with changing pH are already known and classified [3]. These are referred to as the N form, the F (fast) form and the E (expanded) form occurring at pH 5–8, pH 3–4 and below pH 3, respectively.

Neutron reflectivity experiments to study the surface adsorption of BSA have already demonstrated the reduced adsorption of BSA at pH 3 and pH 7 compared to that at pH 5 [4]. It is therefore anticipated that the real time structural information from the DPI experiments should provide key information regarding the mechanism of BSA adsorption. For example, given that the bulk solution structure of BSA at pH 3 is quite different to that at pH 7 it is quite likely that the mechanism of adsorption would be different.

2. Technique

The DPI instrument (Farfield *Ana*Light® BIO200) used for the experiments consists of a dual slab waveguide sensor chip illuminated with alternately polarized light [5]. Each polarized light mode travels down the waveguides and diffracts at the output to give Young's interference fringes. Molecular changes occurring on the surface of the chip will interact with the upper (sensing) waveguide evanescent field and cause a change in the phase of the light exiting the sensing waveguide with respect to the reference waveguide. This results in a phase shift in the camera fringe image which can be electronically tracked. The two polarizations give two independent phase shift responses. These responses can be resolved to give unique solutions for refractive index and thickness (at a resolution of 0.1 nm (1 Å)) of the molecular layer at a given point in time for up to 10 measurements per second.

3. Experimental details

The surface adsorption behaviour of BSA, (0.1 mg l^{-1} in phosphate buffer solution) at the hydrophilic solid–liquid interface was studied as the solution pH was varied between pH 3, pH 5 and pH 7.

An unmodified dual channel sensor chip was loaded into the instrument and phosphate buffer solution (PBS), 2 mM, pH 7 was flowed over each channel at 50 μl min^{-1}. With the temperature stabilized at $20 \pm 0.002\,^{\circ}$C, Decon (5% v/v dilution) was injected for 2 min to wash the chip surface, and then EtOH in water (80% v/v) followed by ultrapure (UHQ) water for 2 min each for calibration purposes. The flow rate was reduced to 30 μl min^{-1} and once the temperature had stabilized, 0.1 mg ml^{-1} BSA in PBS (pH 7) was injected for 11 min then incubated for 20 min. Without returning the flow to the running buffer, 0.1 mg ml^{-1} BSA in PBS (pH 5) was injected at 30 μl min^{-1} for 11 min and then incubated for 20 min. This step was repeated using the first BSA solution (pH 7) before the flow was returned to the running buffer (pH 7) at 30 μl min^{-1}. The adsorption was reversed by washing the chip surface with Decon (5% v/v) for 2 min.

The procedure was repeated using PBS (2 mM, pH 5) as the running buffer and switching the pH of the BSA solution from 5 to 7 then back to 5 and then with PBS (2 mM, pH 3) as the running buffer and switching the pH of the BSA solution from 3 to 5 then back to 3.

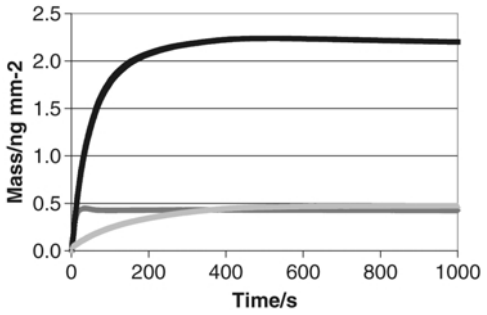

Figure 1. Change in mass of BSA adsorbed with time at pH 3 (dark grey curve), pH 5 (black curve) and pH 7 (light grey curve).

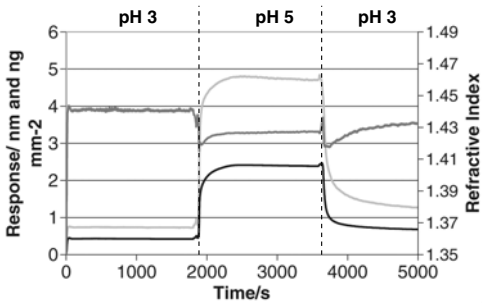

Figure 2. Change in layer thickness (light grey curve), refractive index (dark grey curve) and mass (black curve) of BSA adsorbed when the pH was cycled from 3 to 5 and back to 3.

Table 1. Comparison of the mass of BSA adsorbed at equilibrium with analogous neutron reflectivity data.

Method	[BSA] (mg l^{-1})	Mass adsorbed at pH 3 (ng mm^{-2})	Mass adsorbed at pH 5 (ng mm^{-2})	Mass adsorbed at pH 7 (ng mm^{-2})
Neutron reflectivity	0.15	0.50	2.50	0.50
DPI	0.10	0.48	2.11	0.42

4. Results and discussion

It was found that the mass of BSA adsorbed reached a maximum at pH 5, close to the isoelectric point of BSA (figure 1). For pH 3 compared with pH 7, the amounts of BSA adsorbed at equilibrium were similar, although reduced with respect to the result at pH 5. Comparison of the adsorption rates at these extreme pHs showed an initial rapid adsorption of BSA at pH 3 but a more gradual adsorption at pH 7, implying different adsorption mechanisms. The mass data at equilibrium were compared with analogous neutron reflectivity data [4] and were found to correlate well (table 1).

When the pH was cycled, measurements of the variation in protein structure showed large conformational changes that could be reversed (figure 2). This indicated that the protein did not denature on the surface. It has previously been observed that proteins retaining their globular frameworks tend to adsorb reversibly with respect to pH cycling [6] whilst denatured proteins show irreversible adsorption [7].

From the information gained, likely surface structures and adsorption mechanisms for BSA can be proposed. Given the dimensions of the crystal structure provided above, it can be calculated for a saturated monolayer of BSA that the area per molecule will be approximately 5600 $Å^2$ and the layer thickness will be approximately 40 Å if the molecules adsorb parallel to the surface, whereas if the molecules adsorb perpendicularly, the area per molecule will be approximately 1600 $Å^2$ and the layer thickness will be approximately 140 Å.

At pH 3, the mass adsorbed at equilibrium was 0.48 mg m^{-2}. This is equivalent to an area per molecule of 26 367 $Å^2$. The adsorbed layer thickness was less than 1 nm, but the refractive index of the layer (1.440) was relatively high and equates to approximately 80% of the layer being occupied by protein, the remainder being filled by buffer solution. These results suggest that the BSA was spread thinly on the surface (figure 3(a)). At pH 5, the area per molecule was 5248 $Å^2$ and the layer thickness was 39 Å at equilibrium, with 76% of the layer occupied

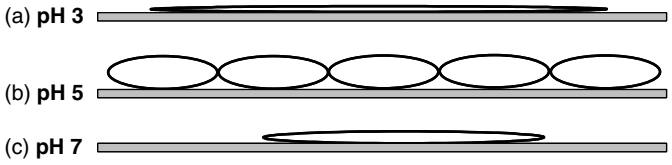

Figure 3. Schematic representations of the likely surface structures of adsorbed BSA at pH 3, pH 5 and pH 7.

by protein, suggesting a structure close to that of a saturated monolayer with the molecules orientated parallel to the surface (figure 3(b)). At pH 7, the BSA layer was 11 Å thick and had an area per molecule of 23 071 $Å^2$ at equilibrium, with a protein fraction of 59%, which suggests that the BSA molecules had again spread on the surface but not to the same extent as at pH 3 (figure 3(c)).

By bringing together the information on the surface adsorbed structures described above, the measured adsorption rates (figure 1) and the known bulk solution structures [2], it is possible to gain an understanding of the mechanisms involved in the surface adsorption of BSA. At pH 3 it seems that the adsorption process for the already unfolded protein is predominantly driven by electrostatic interactions between the positively charged protein molecule and the negatively charged silica waveguide surface, and that this occurs relatively rapidly. At pH 5, close to the isoelectric point for the protein, the adsorption process is still rapid, but slower than at pH 3 as the process is presumably driven by forces such as van der Waals, induced dipole and dispersion forces. Also, at this pH the molecules can pack closely together without repelling one another, resulting in high surface mass. At pH 7, the molecules are negatively charged and the rate at which they interact with the surface is much attenuated as significant electrostatic repulsion must be overcome before adsorption can proceed. It is likely that this effect is further exacerbated as a consequence of the molecule unfolding as it adsorbs on the surface (it has been reported that BSA adopts a prolate ellipsoid structure in solution at pH 7 [2]).

5. Summary

The pH-dependent surface adsorption of BSA has been examined in real time using DPI. A significant level of structural and mechanistic detail has been unravelled for the first time. The amounts adsorbed at equilibrium correlate strongly with similar neutron reflectivity data. This demonstrates DPI as an invaluable laboratory-based tool for studying molecular structure and function at interfaces.

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