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Effect of the interplay between protein and surface on the properties of adsorbed protein layers

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ABSTRACT

Although protein adsorption to surface is a common phenomenon, investigation of the process is challenging due to the complexity of the interplay between external factors, protein and surface properties. Therefore experimental approaches have to measure the properties of adsorbed protein layers with high accuracy in order to achieve a comprehensive description of the process. To this end, we used a combination of two biosensing techniques, dual polarization interferometry and quartz crystal microbalance with dissipation. From this, we are able to extract surface coverage values, layer structural parameters, water content and viscoelastic properties to examine the properties of protein layers formed at the liquid/solid interface. Layer parameters were examined upon adsorption of proteins of varying size and structural properties, on surfaces with opposite polarity. We show that "soft" proteins such as unfolded α-synuclein and high molecular weight albumin are highly influenced by the surface polarity, as they form a highly diffuse and hydrated layer on the hydrophilic silica surface as opposed to the denser, less hydrated layer formed on a hydrophobic methylated surface. These layer properties are a result of different orientations and packing of the proteins. By contrast, lysozyme is barely influenced by the surface polarity due to its intrinsic structural stability. Interestingly, we show that for a similar molecular weight, the unfolded α-synuclein forms a layer with the highest percentage of solvation not related to surface coverage but resulting from the highest water content trapped within the protein. Together, these data reveal a trend in layer properties highlighting the importance of the interplay between protein and surface for the design of biomaterials.

1. Introduction

Knowledge of protein adsorption has expanded rapidly in the past decades due to the development of techniques and methods providing more detailed experimental data [1]. The range of techniques used to characterize protein layers includes optical techniques such as ellipsometry [2,3], neutron reflectometry [4], surface plasmon resonance [5], waveguide lightmode spectroscopy [6], and dual polarisation interferometry (DPI) [7,8]; acoustic biosensing techniques such as quartz crystal microbalance with dissipation (QCM-D) [9,10]; surface imaging techniques such as atomic force microscopy [11,12] and finally techniques focusing on the secondary structure of the adsorbed protein such as attenuated total reflectance infrared spectroscopy [13]. These techniques measure the kinetics of protein adsorption, mass coverage and structure of the layer, thus achieving a description of the adsorption process. In addition to this, a combination of these techniques has been used to characterize specific protein layers in more details [14–16].

Apart from the external parameters, such as temperature [17], pH [18], and ionic strength [19], other critical fixed parameters influence the properties of the resulting layers; these include both protein and surface properties [1]. The properties of the adsorbed layer are highly dependent on the size, net charge and structure of constituent proteins as these factors influence surface affinity, protein packing and orientation and water content of the layer. Furthermore, the polarity [13,20] and roughness [21] of the surface influence the protein-surface interaction in terms of protein affinity, reversibility of the adsorption process and the extent of protein deformation. Therefore the complexity of the interplay of these parameters on the properties of protein layers requires a highly accurate experimental approach in order to achieve systematic model descriptions [1].

Our previous work described in details the properties of adsorbed lysozyme layers as function of surface coverage, using a combination of QCM-D and DPI [16]. The use of these two

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techniques has proved to be valuable in determining structure–property relationships between surface coverage and adsorbed lysozyme properties on a hydrophilic silica substrate. In particular, we show that solvent content, layer rigidity and protein orientation and packing are dependent of the surface coverage. These insights emerge from the complementary data extracted from the two techniques, such as 'dry' and 'wet' mass values, layer solvation, thickness, density and viscoelastic properties.

In the present study, we use the same approach to investigate the layer properties of three proteins of different size and structure, adsorbed onto two surfaces of opposite polarity. A comparison of the layer properties is made between alpha-synuclein (α-Syn) and lysozyme, which are of similar molecular weight (~14 kDa) but contrasting structure: highly ordered in the case of lysozyme and unfolded in the case of α-Syn. Further comparison is made with bovine serum albumin (BSA), a globular protein with a higher molecular weight (~66 kDa). These proteins are adsorbed onto a hydrophilic, negatively charged silica substrate and onto a hydrophobic methylated substrate. The effects of surface polarity on the proteins' affinity and their resulting packing and orientation upon adsorption are investigated. Overall, this study aims to draw some conclusions about the influence of the interplay between the protein and surface on the properties of the resulting adsorbed protein layers.

2. Materials and methods

2.1. Materials

Human milk lysozyme (14.4 kDa), bovine serum albumin (BSA, 66.4 kDa), HPLC Grade water (resistivity >18 MΩ cm), monobasic and dibasic phosphate, sodium deodecyl sulphate (SDS), trichloro(methyl)silane, and toluene (anhydrous, 99.8%) were purchased from Sigma (Sigma & Aldrich, UK) and used as received. α-synuclein (α-Syn, 14.46 kDa) was prepared as previously described [22]. Millex syringe filter (pore size = 0.22 μm) was obtained from Fisher (Fisher Scientific, UK). Diluted Hellmanex® III (Hellma Analytics, Germany) solution (2%, in deionised water) was used to clean the DPI injection loops.

Phosphate solution (10 mM, pH 7.4) used as buffer solution was prepared using monobasic and dibasic phosphate and HPLC grade water. Filtered protein stock solutions were each prepared in the same buffer solution and then diluted for injection at the desired concentrations. The concentrations of diluted protein solutions were checked by UV–Vis spectroscopy (Varian Cary® 300 UV–Vis Spectrophotometer, Agilent Technologies, UK) at 280 nm. Untreated silicon oxynitride (Farfield–Biolin Scientific AB, Sweden) and silicon dioxide (Q-Sense-Biolin Scientific AB, Sweden) sensor chips used for the DPI and QCM-D experiments respectively, were cleaned prior to the experiments and were referred to as the 'hydrophilic surface' and 'Silicon oxide' surface in later sections (for detailed cleaning procedure, please refer to reference [16]). Silanization of the cleaned sensor chips was performed in the following way: toluene solution immersion with agitation for 30 s, followed by immersion of toluene with 4% trichloro(methyl) silane solution for 1 h. The chips were then blown dried with nitrogen gas. The silane-modified chips are referred to as the 'hydrophobic surface' and 'Methyl' surface in later sections. Contact angle measurements were performed to check the polarity of the hydrophilic and hydrophobic surfaces. The values were determined with a goniometer (CAM200, KSV NIMA-Biolin Scientific, Finland) and a detailed method can be found in Ref. [16]. Contact angles of 8.7° ± 0.8 and 8.5° ± 0.7 were obtained for the hydrophilic DPI chips and QCM-D chips, respectively. Contact angles of 88.1° ± 2.5 and 88.9° ± 5.5 were obtained for the hydrophobic DPI and QCM-D chips, respectively. The surfaces are therefore considered as 'super-hydrophilic' and 'hydrophobic' as the contact angle of one type is less than 10° and of another type is approximately 90° [23].

2.2. DPI

An *Ana*light® dual polarization interferometer (*Ana*light® 4D, Farfield–Biolin Scientific AB, Sweden) was used to optically characterize adsorption of the three proteins on both hydrophilic and hydrophobic sensor chips. Details of the instrumentation can be found elsewhere [16,24]. The instrument alternately generates two orthogonal polarizations of light that excite waveguide modes supported by the DPI sensor chip. These two polarization waveguide modes are known as the transverse electric (TE) and transverse magnetic (TM) modes. The parameters employed in the experiments such as the operating temperature, flow rate, protein solution injection volume and the bulk solution exchange rate, together with the standard calibration procedure prior to the experiments, the cleaning procedure have been described in our previous work [16]. Successive protein solutions were injected until surface saturation was reached (as indicated by the TM and TE signals where further injections would not lead to further signal phase increment), then followed by a 30 min buffer rinsing. Data were analysed using *Ana*light explorer (Farfield–Biolin Scientific AB, Sweden) to calculate layer refractive index (RI), thickness, mass and density. During protein incubation, correction of the protein solution RI was performed, in order to obtain accurate values of the protein layer density and mass. Details of the new bulk RI calculations can be found in our previous work [16].

2.3. QCM-D

In parallel to DPI, QCM-D was also performed to record real time change of frequency and dissipation value during protein adsorption (QCM-D E4, Q-Sense-Biolin Scientific AB, Sweden). A detailed description of the instrument and the experiment design can be found in our previous work [16]. Alternating protein and buffer solutions of fixed volume were passed over the substrate until the surface was saturated. This was followed by 30 min of buffer rinsing.

For the adsorbed layer mass calculation, the Sauerbrey equation is employed in lysozyme adsorption on both surfaces as the average dissipation value is less than 1×10^{-6} (Eq. (1)). The Voigt model is used to calculate BSA and α-Syn adsorbed mass due to greater dissipation values.

$$\Delta M = -C\Delta f/n \tag{1}$$

Where ΔM, C, and n represent the adsorbed mass per unit area, mass sensitivity constant (17.7 ng cm^{-2} Hz^{-1}), and the overtone number, respectively. The fifth overtone was used for analysis.

The Voigt model was also used to obtain the viscoelastic properties of all three adsorbed protein layers on both surfaces. The fixed parameters were bulk solution density and bulk solution viscosity, which were assumed as 1000 kg/m^3 and 0.001 kg/ms, respectively. The parameters available to fit were the layer viscosity, layer shear modulus and layer thickness, which were set in the range of 0.0001–0.1 kg/ms, 1×10^4 and 1×10^8 Pa, and 1×10^{-10} and 1×10^{-6} m, respectively. Overtones $n = 3$, 5, 7, 9, 11, and 13 were employed for the modelling [16].

For the percentage layer solvation calculation, the 'dry' mass obtained from the DPI (ΔM_{ads}) was subtracted from the QCM-D calculated 'wet' mass (ΔM_{qcmd}), then divided by ΔM_{qcmd} (Eq. (2)).

$$\text{wt\% solvation} = \left(\Delta M_{qcmd} - \Delta M_{ads}\right) \Big/ \Delta M_{qcmd} \tag{2}$$

3. Results

3.1. Quantification of protein layer solvation

One important property obtained from combining the adsorbed mass values from both DPI and QCM-D is the protein layer solvation (wt% solvation). Quantification of the entrapped solvent and its evolution throughout the adsorption process are important factors that directly link to the performance of artificial materials [15,25,26]. The change of wt% solvation during the adsorption and desorption processes of lysozyme, BSA and α-Syn on both hydrophilic and hydrophobic surfaces are calculated using Eq. (2) and presented in Fig. 1A and Fig. 1B, respectively. As noted in Fig. 1, the wt% solvation decreases as the surface coverage increases for all the proteins adsorbed on both surfaces until surface saturation (indicated by the *). However, this is most significant for lysozyme, with starting values of 70% (Fig. 1A) or above (Fig. 1B), that drops to approximately 45%. In comparison, the changes of wt% solvation for BSA and α-Syn through the processes are much less significant.

Another interesting finding is related to the wt% layer hydration of different proteins at the same surface coverage, which follows a trend of α-Syn> BSA > lysozyme. Moreover, comparing the effect of the surface polarity on the layer hydration for a given protein, a higher hydration level is observed on the hydrophilic surface at surface saturation coverage (as indicated by the *) for BSA and α-Syn (91% on 'Silicon oxide' substrate compared to 83% on 'Methyl' substrate for α-Syn; 88% on 'Silicon oxide' substrate compared to 77% on Methyl substrate for BSA) whereas this effect is not significant for lysozyme (both adsorbed layers have approximately 50% solvation).

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