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Hydration–dehydration of adsorbed protein films studied by AFM and QCM-D

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Abstract

The hydration–dehydration process of an adsorbed human serum albumin film has been studied using atomic force microscopy (AFM) and a quartz crystal microbalance (QCM). All measurements were performed with identically prepared protein films deposited on highly hydrophilic substrates. Both techniques are shown to be suitable for following in situ the kinetics of protein hydration, and for providing quantitative values of the adsorbed adlayer mass. The results obtained by the two methods have been compared and combined to study changes of physical properties of the films in terms of viscosity, shear, Young's modulus, density and film thickness. These properties were found to be reversible during hydration–dehydration cycles.

Keywords: Protein films; Resonance frequency; Cantilever sensor; AFM; QCM-D

1. Introduction

Surface modification, by the adsorption or deposition of thin films of natural or synthetic polymers is a firmly established and widely used technique in materials processing technology. Typical examples include the deposition of protein films and hydrophilic coatings on biomedical devices in order to increase biocompatibility or cell adhesion and, via lithographic printing techniques, for spatially controlling these processes. Other important applications include the deposition of polymer coatings and diffusion membranes in fluid separation systems; polymer coatings applied to isolate or protect components in electronic devices including remote sensors; protective layers applied to materials in corrosive or other aggressive environments and cosmetic polymer films applied in a huge range of finishing and packaging industries. In all of these, and many other, situations it is extremely important to understand, and in some cases be able to predict, the behaviour of the surface film in a variety of environments, which relate to its technological use. Experimental and modelling methods which aid our understanding of the changes, which can occur in surface films as a function of environment are therefore of great practical use.

The elastic properties of proteins have been explored by different techniques such as ultrasound velocimetry (Kharakoz, 2000; Sarvazyan, 1991; Speziale et al., 2003) and mechanical rheometry (Valentine et al., 2005). Bending of the sample in a periodic magnetic field has also been investigated (Zenchenko et al., 1996). More recently development of the quartz crystal microbalance (QCM) technique has offered new opportunities to study the processes of thin polymer film formation as well as the modification of their physical properties under varying environmental conditions (Höök et al., 2002, 2001). This method has shown to be an especially fruitful and reliable when combined with others techniques for the characterisation of surface coatings (Bailey et al., 2002; Vikinge et al., 2000; Vörös, 2004).

In the work presented here we investigate the novel combination of atomic force microscopy (AFM) and the quartz crystal microbalance (QCM) to study sorption and swelling phenomena in protein layers. The combined data from these two techniques was used to demonstrate the potential of the approach for deriving additional information about diffusion rates, viscosity, shear and Young's modulus. We also further extend the previously published investigations on the formation and the structure of

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the adsorbed protein layer (Browne et al., 2004; Lubarsky et al., 2005).

2. Materials and methods

2.1. Preparation of the protein-coated samples

Cantilevers used throughout this work were uncoated probes made out of single crystalline silicon, with the resonance frequency <300 kHz and a force constant <40 N m^{-1} (Nanoscience Instruments Inc., Phoenix, AZ, USA). This kind of cantilever is widely used in oscillation modes of AFM. Following nominal dimensions describe the geometry of the cantilever: the length of the cantilever is 125 μm, the thickness is 4 μm and the width is 30 μm. Polished 5 MHz quartz crystals from Maxtek Inc. (Torrance, CA, USA) were used as QCM-D sensors.

Cleaning and hydrophilisation of the surfaces were done according to the procedure described by Ito et al. (1997). Samples surfaces were exposed to UVO for 15 min using a UVO reactor. The UVO treatment is known to effectively remove contaminants such as hydrocarbons from surface (Vig, 1985). After the UVO treatment, the samples were first immersed in 0.5 M NaOH for 20 min, then in 0.1 M HCl for 10 min and finally in 0.5 M NaOH for 10 min. Subsequently, they were rinsed in Milli-Q deionised water (with resistivity <18.3 MΩ cm^{-1}) and dried in nitrogen for 10 min.

Protein solutions of 0.1 and 0.05 mg ml^{-1} were prepared with human serum albumin, essentially fatty acid and globulin free lyophilised powder (Cat. no. A3782, Sigma–Aldrich Company, Dorset, UK) in Dulbecco's phosphate buffered saline at pH 7.2 (PBS, Cat. no. D8537, Sigma–Aldrich). A 50 μl drop of solution was placed on each sample and allowed to incubate at 37 °C. After 1 h samples were removed from the incubator and rinsed twice in Milli-Q water. From kinetic studies an adsorption time of an hour was found to be sufficient to reach equilibrium without significant effects of concentration of the protein solution due to water evaporation. Samples were then re-immersed in Milli-Q and placed on a shaker for 4 h, rinsed twice again in Milli-Q and then allowed to dry overnight at room temperature. There were two groups of both cantilevers and quartz crystals prepared by coating in solutions with concentrations of 0.1 and 0.05 mg ml^{-1}, respectively. Each group contained of five cantilever chips and two quartz crystals.

2.2. Cantilever sensor method

The oscillating cantilever sensor allows measurement of the resonant frequency shift of the cantilever $\Delta\nu$ with the high accuracy. In the work described, the system has been modified to include a cantilever which is surface coated with a material under study. In response to the environment, the coating is modified in terms of its physical properties, which in turn generates a mechanical signal that changes the resonance frequency of the cantilever. In present work cantilevers coated with the protein layer are exposed in a nitrogen environment with the variable level of relative humidity. The humidity-dependent resonant frequency shift of the cantilever in this system $\Delta\nu^{RH} = \nu_u - \nu_c^{RH}$, where ν_u is the resonant frequency of the uncoated cantilever and ν_c^{RH} is the resonant frequency of the coated cantilever in the humid environment.

The apparatus used was based around a Digital Instruments Multimode SPM system (Fig. 1). This system uses a high-resolution, laser-based detection scheme that enables measurement of cantilever deflections down to the nanometer-scale and measures sub-hertz resonant frequency shift. The apparatus was upgraded with a system for humidity control in the AFM head chamber. An atmospheric hood, equipped with a humidity sensor HIH-3610 (Honeywell, Freeport, IL) and nitrogen and water vapour sources, was used to enclose the cantilever allowing close control over its environment throughout the experiment. The gas inlets were equipped with a porous diffuser to allow uniform gas introduction without affecting the AFM operation. The gas flow through the atmospheric hood was kept at a constant rate of 150 ml min^{-1} or approximately two cell volumes per minute.

The resonant frequencies (ν_u) of all uncoated (as received) cantilevers were measured immediately after the cleaning procedure and prior to the protein coating producing. These oscillating experiments were performed in a dry nitrogen atmosphere. The cantilevers were then coated with a protein film, as described above. Next, the resonance frequency of each cantilever was

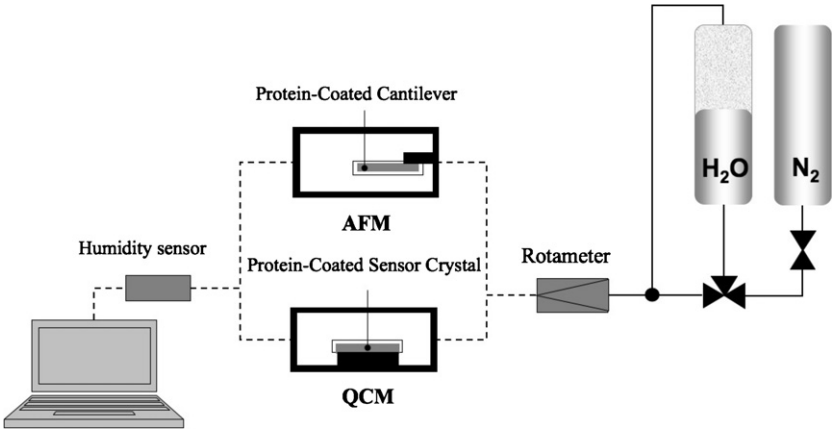

Fig. 1. Experimental set-ups used to study physical properties of HSA films in varying humidity.