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Analysing protein competition on self-assembled mono-layers studied with quartz crystal microbalance

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ABSTRACT

The mechanisms by which proteins adsorb to surfaces of biomaterials have long been of interest. The present work started with the premise that small/hard and large/soft proteins will yield different sets of normalized frequency shift and dissipation signals when studied with a quartz crystal microbalance. The aim was to evaluate the usefulness of these raw data to study protein competition using protein incubations in sequence and from mixtures of albumin (BSA) and gamma-globulin (BGG) at various ratios. Increasing the concentration of BSA decreases the adsorption of subsequently incubated BGG. For BSA/BGG mixtures the dissipation is similar for all logarithmic molar ratios BGG/BSA below 1 but soon decreases when the molar ratio of BSA/BGG (and opposite for the normalized frequency shift) is above 1, indicating preferential binding of BGG. Modelling indicated that differences in the film shear modulus and viscosity depend more on the properties of the self-assembling mono-layers (SAMs) than on the proteins. Films high in BSA tentatively differ in film shear modulus and viscosity from that of films high in BGG but only on the hydrophobic surfaces. The results were encouraging as the raw data were deemed to be able to point at protein adsorption competition.

1. Introduction

Key aspects of protein adsorption onto the surface of biomaterials are not only related to the adsorption kinetics and total amount but also to various aspects of competitive behaviour of blood proteins. The latter is probably more important when looking into the behaviour of complex protein solutions, especially with regard to activation of the humoral systems and exposure of cell receptor ligands. The present work is focused on competitive protein binding as a phenomenon on its own right. This is not a new field, going back to the pioneer works by researchers such as Vroman, whose early experiments with ellipsometry indicated that there are differences in which proteins can be detected with antibodies after different serum incubation times [1], and Brash, whose lab performed (as far as the authors know) the first experiment to show protein exchange [2,3], thus indicating that protein competition is important for biomaterials. They have been followed by many others using a large variety of techniques to study protein competition [4–18]. In the background to this work is also previous studies indicating that competitive protein binding can affect the interaction between cells (at least in vitro) with the surfaces of biomaterials [19–21], again highlighting the interest in competitive protein adsorption in relation to biomaterials.

The starting point for this work is the premise that proteins with different size and overall characteristics (e.g. small/hard and large/soft) will yield a different response pair (frequency shift and dissipation) in a quartz crystal microbalance with dissipation (QCM-D), when incubated at similar concentrations (Fig. 1). Such a response most likely will also depend on the surface properties of the biomaterial. QCM-D is a unique technique that relies on two aspects of adsorbed films: (i) the adsorbed mass is a function of the changes in resonance frequency of the measurement crystals upon protein adsorption, and (ii) the decay of the resonance amplitude is related to viscosity of the film. One of the main drawbacks is that the obtained signal also is sensitive to the mass of the water captured in films and the viscosity of solutions, which makes the subsequent necessary modelling the largest hurdle in analysing the results.

The focal point of this work is thus to evaluate to what extent the analysis of the raw data from QCM-D will indicate about protein competition in sequence and mixtures, using albumin and gamma-globulin as the model system since there is a marked difference in their size and mass. Also, the competitive behaviour of adsorption of albumin and gamma-globulin or IgG has been studied by other techniques [5,7,8] which is important when discussing the approach.

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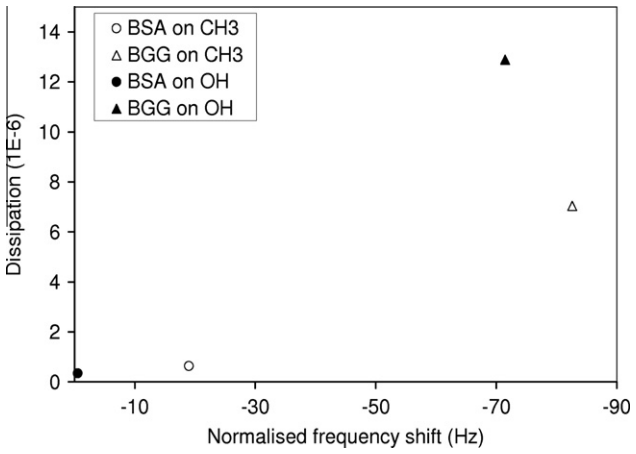

Fig. 1. Dissipation vs. frequency shift of BSA and BGG adsorbed alone at OH- and CH_3-terminated SAMs for 15 min followed by 15 min rinse in 50 mM Tris 145 mM NaCl pH 7.4.

2. Materials and methods

Bovine serum albumin (BSA, pI 5, M_W 66 kDa, Sigma) and bovine gamma-globulin (BGG, pI 6.5–9.5, M_W 150–900 kDa, Sigma) were dissolved in 50 mM Tris (Merck), 145 mM NaCl, 0.05% NaN_3 at pH 7.4 at room temperature (RT). Solutions were stored at 8 °C for less than a week. These proteins were incubated for 15–30 min at 25 °C at total 1 mg ml^{-1} in a quartz crystal microbalance (E4, Qsense) onto gold coated crystals (Tangidyne and Qsense) that were previously coated with alkane thiols. The gold surfaces were cleaned by sonication for 3 min each in acetone and ethanol followed by 1 h UVO/ozone (Pro, Bioforce nanosciences) on each side, then more 3 min sonication in ethanol to remove loose organic remnants. The cleaned crystals were incubated in 20 μM ethanol solution of C16-SH (Fluka) or HS-C11-OH (Sigma) for at least two nights to ensure well formed mono-layers [22]. The alkane thiol coatings were analysed with contact angle goniometry (OCA15+, Dataphysics) using the circle fit algorithm with sessile drop of water (2 μl, HPLC quality). The crystals were dried with flowing nitrogen prior to UVO treatment and measurements. The QCM responses in frequency and dissipation were used to study the feasibility of multilayer formation and potential preferential binding for BSA and BGG. The pumping speed was set at 50 μl min^{-1}, giving laminar flow as indicated by the manufacturer. The crystals were run in buffer (Tris) for at least 15 min to assure a stable baseline. Except for the repeats of BSA and BGG the data points were only taken from Tris rinsing after the protein incubations to rule out influence of the protein solution itself on the QCM responses, although 1 mg ml^{-1} solutions of these proteins usually only impose small changes (apart from the adsorbed proteins). The frequency and dissipation values at the end of each experiment were used as the basis for most of the analysis. The full experimental runs were in some cases modelled using the QTools software (QSense) with the Voigt model which uses the assumption that the viscoelastic properties of a material can be described by a parallel spring and viscous damper. In some of the graphs the logarithm of the molar ratio of BSA and BGG is used, log(mol(BSA/BGG)), under the assumption that BGG is only IgG. Since it is difficult to find good experimental fits by using a as wide as possible range for the fitting parameters, initially a smaller range was chosen based on published result [6,23] as follows: fundamental tone 5 MHz, fluid viscosity 1 mPas, fluid density 1000 kg m^{-3}, film density 1150 kg m^{-3}, layer viscosity 0.001–0.1 mPas, layer shear modulus 100–2000 kPa and layer thickness 0.01–100 nm. Choosing to fix the fluid viscosity and density to that of water [6] was deemed reasonable for two reasons: firstly, only low protein concentrations were used which was also noted in the very low change of dissipation going from incubation to rinse; secondly, the current study was more concerned with the values after rinse, not of those during the actual incubation. Notably the density of the protein film can actually depend on the molecular mass of the studied proteins [24]. In modelling density and thickness are reciprocal, implying that variations in either will to a large extent cancel each other when calculating surface mass density. Since this study used protein mixtures it was deemed more practical to assume a fixed value for the protein film density. Statistical evaluation of the triplicate or more experiments (except Fig. 1) was done with Student's *t*-test, only reporting differences if they were found to be equal or better than 5% confidence level. The values in the graphs are mean ± standard error of mean.

3. Results and discussion

The water contact angles were found to be 113°(6) for CH_3-SAMs and 17°(6) for OH-SAMs, thus with distinctly and statistically different levels of surface energy. In this section, the obtained QCM data will be discussed in the light of some published approaches to assess the QCM raw data vs. QCM modelling using the Voigt model and published results on protein competition and adsorption, with QCM and other methods.

In sequential incubation of proteins, albumin (BSA) seems to adsorb at a very limited extent on BSA from previous incubations under the used experimental setup (Fig. 2). In contrast, gamma-

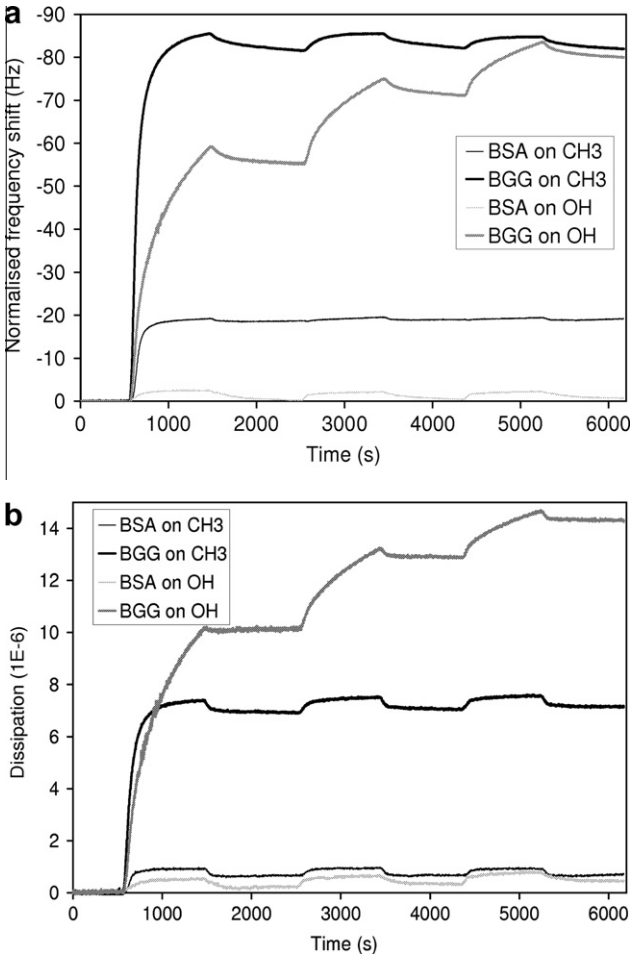

Fig. 2. (a) Normalized frequency shift of third overtone of 3 repeated incubations of 1 mg ml^{-1} BSA and BGG incubated on OH and CH_3-terminated SAMs followed by rinse in Tris. (b) Dissipation of same experiments as in (a).

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