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# Viscoelastic properties of adsorbed and cross-linked polypeptide and protein layers at a solid–liquid interface

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ABSTRACT

The real-time changes in viscoelasticity of adsorbed poly(L-lysine) (PLL) and adsorbed histone (lysine rich fraction) due to cross-linking by glutaraldehyde and corresponding release of associated water were investigated using a quartz crystal microbalance with dissipation monitoring (QCM-D) and attenuated total reflection Fourier transform infrared spectroscopy (ATR/FTIR). The kinetics of PLL and histone adsorption were measured through changes in mass adsorbed onto a gold-coated quartz surface from changes in frequency and dissipation and using the Voigt viscoelastic model. Prior to cross-linking, the shear viscosity and shear modulus of the adsorbed PLL layer were $\sim 3.0 \times 10^{-3}$ Pa s and $\sim 2.5 \times 10^{5}$ Pa, respectively, while after cross-linking, they increased to $\sim 17.5 \times 10^{-3}$ Pa s and $\sim 2.5 \times 10^{6}$ Pa, respectively. For the adsorbed histone layer, shear viscosity and shear modulus increased modestly from $\sim 1.3 \times 10^{-3}$ to $\sim 2.0 \times 10^{-3}$ Pa s and from $\sim 1.2 \times 10^{4}$ to $\sim 1.6 \times 10^{4}$ Pa, respectively. The adsorbed mass estimated from the Sauerbrey equation (perfectly elastic) and the Voigt viscoelastic model differ appreciably prior to cross-linking whereas after cross-linking they converged. This is because trapped water molecules were released during cross-linking. This was confirmed experimentally via ATR/FTIR measurements. The variation in viscoelastic properties increased substantially after cross-linking presumably due to fluctuation of the randomly cross-linked network structure. An increase in fluctuation of the viscoelastic properties and the loss of imbibed water could be used as a signature of the formation of a cross-linked network and the amount of cross-linking, respectively.



## 1. Introduction

There is increasing interest in cross-linking polypeptides and proteins to increase protein stability [1,2]. Recent evidence has shown that proteins, either from cross-linking [3] or from poly(ethylene glycol) [4] addition, have longer in vivo half-lives and hence are likely be more efficacious provided they retain their activity. Similarly, stabilizing proteins at interfaces are of relevance to bio-fouling, [5] nano-fluidics and protein-based sensor technology [6]. In all these cases, the rheological properties of aggregates or films are important [7]. Protein cross-linking also occurs in biological systems during post-translational modification [8]. Transglutaminases (Tgases) cross-link protein via $\varepsilon$-($\gamma$-glutamyl)lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. These cross-linked proteins have high mechanical strength, are resistant to proteolytic degradation, and they accumulate in a number of tissues and processes where such properties are important such as skin, hair, and blood clotting [8]. Many researchers have investigated the rheological properties of adsorbed protein layers [9–11]. Though cross-linking proteins in bulk solution to increase their stability has been widely studied, similar studies at solid–liquid interfaces are limited. To monitor the changes in rheological properties during cross-linking in real time at an interface, we have chosen to study a model polypeptide, poly(L-lysine), and a model protein, histone (lysine rich fraction) adsorbed onto a flat gold surface using a quartz crystal microbalance with dissipation monitoring (QCM-D).

QCM-D is a simple and highly sensitive mass sensor by which a wide range of interfacial adsorption reactions can be monitored, on a variety of supports, in real time. In a QCM-D, a gold-plated AT-cut piezoelectric quartz crystal is oscillated in shear mode at its fundamental resonant frequency by passing an alternating current through the crystal. The dissipation and resonant frequency of the crystal can be recorded in real time. The principles behind this technique have been reported elsewhere [12]. The measurement of changes in frequency and dissipation using QCM-D allows one to estimate the viscoelasticity of the non-elastic adsorbed layer by using Voigt viscoelastic model [13–15], which has been used to probe the rheological properties of proteins [16,17] and synthetic polymers [18–20] at the solid–liquid interface. Höök et al. [16]

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used this model to study the viscoelasticity of an adsorbed *Mytilus edulis* foot protein (Mefp-1) film both before and after cross-linking of the adsorbed layer by $NaIO_4$. Adsorption and viscoelastic properties of type I collagen, in native and heat-denatured forms, on polystyrene was studied by Gurdak et al. [17]. Adsorption kinetics and cross-linking of ethyl(hydroxyethyl) cellulose (EHEC) and hydrophobically modified EHEC at hydrophilic and hydrophobic modified surfaces have been investigated in detail by Hedin et al. [18].

Here, we have used the same technique to study the changes in viscoelasticity of an adsorbed PLL and a histone layer due to cross-linking by glutaraldehyde (GTA). We choose histone as it is a well-studied protein rich in lysine residues for effective cross-linking (MW 21.5 kDa, 24–32% lysine). Core histones of which the histone chosen for this study is one component, assemble to form one octameric nucleosome core particle by wrapping base-pairs of DNA around a protein spool [21]. They also play a role in gene regulation. Our work shows that structural differences between PLL and histone result in differences in viscoelastic properties of the adsorbed layer. Cross-linking of PLL and histone with GTA resulted in an increase in rigidity of the adsorbed polypeptide and protein layers at the solid–liquid interface and an increase in the fluctuation of their constitutive viscoelastic properties.

## 2. Materials and methods

### 2.1. QCM-D

Aqueous solutions at 300 μg/ml of poly(L-lysine) hydro bromide (molecular weight > 300 kDa, 81356, Sigma, Saint Louis, MO) and 21.5 μg/ml of histone (21.5 kDa, H5505, Sigma) and 100 μg/ml glutaraldehyde (GTA, 340855, Sigma) were prepared in phosphate buffered saline (PBS) at pH 7.4. Concentration of PLL, histone and glutaraldehyde was kept very low so that they do not affect the viscosity of the solution. Polished gold coated AT-cut quartz crystals with fundamental frequencies of ∼5 MHz (QSX301, Q-Sense AB, Göteborg, Sweden) were cleaned by immersion in a 1:1:5 mixture of $H_2O_2$ (30%), $NH_3$ (25%), and distilled water at 60 °C for 20 min followed by exposure to UV/$O_3$ for 10 min. 250 μl of the polypeptide/protein solution were injected through the sensor loop and allowed to adsorb for 40 min. The adsorbed protein on the crystal was then washed with buffer for 20 min and then cross-linked by injecting 250 μl of glutaraldehyde solution for ∼20 min. Approximately 100% of the reactive lysine present in the protein/polypeptide layer was cross-linked due to addition of GTA [22]. Mass and dissipation measurements were performed using a QCM-D (D300, Q-Sense AB, Göteborg, Sweden) in batch mode at 24 ± 0.1 °C. More details about the experimental procedure have been reported elsewhere [23].

### 2.2. ATR/FTIR

All polypeptide/protein solution spectra were recorded in their respective aqueous buffer solution. A horizontal ATR (Magna-IR 550 Series II, Thermo Nicolet Instruments Corp., Madison, WI) accessory with a trapezoidal germanium crystal having ends cut to 45° generated 12 internal reflections. The spectrometer was equipped with a liquid nitrogen-cooled mercury cadmium telluride detector. To reduce the contributions of water vapor and carbon dioxide, the IR system was continuously purged with air from an FTIR Purge gas generator (Model 74-45, Balston, Haverhill, MA). Spectra were collected at a gain of 8 and an aperture of 40 with spectral resolution of 2 $cm^{-1}$. All spectra were collected in the 4000–1000 $cm^{-1}$ range as sets of 256 time-averaged interferograms. Omnic (v 7.0) software (Thermo Nicolet Instruments Corp., Madison, WI) was used to analyze and measure peak heights for different sample spectra.

### 2.3. Viscoelastic modeling

The Voigt element is a parallel combination of a spring and a dashpot to represent the elastic (storage) and inelastic (damping) behavior of a material, respectively. Using the Voigt model, Voinova et al. [13] developed the following equations to describe the viscoelasticity of an adsorbed layer:

$$\Delta f = \frac{\mathrm{Im}(\beta)}{2\pi d_q \rho_q}, \tag{1}$$

$$\Delta D = -\frac{\mathrm{Re}(\beta)}{\pi f d_q \rho_q}, \tag{2}$$

where

$$\beta = \frac{(2\pi f\eta\xi_1 - i\mu\xi_1)(1-\alpha\exp(2\xi_1 d))}{2\pi f(1+\alpha\exp(2\xi_1 d))}, \tag{3}$$

$$\alpha = \frac{(2\pi f\eta\xi_1 - i\mu\xi_1 + 2\pi f\eta_l\xi_2)}{(2\pi f\eta\xi_1 - i\mu\xi_1 - 2\pi f\eta_l\xi_2)}, \tag{4}$$

$$\xi_1 = \sqrt{-\frac{(2\pi f)^2\rho}{\mu + i2\pi f\eta}}, \tag{5}$$

$$\xi_2 = \sqrt{\frac{i2\pi f\rho_l}{\eta_l}}. \tag{6}$$

$d_q$ and $\rho_q$ are the quartz thickness and density, respectively. $f_0$ is the fundamental resonant frequency, and $f = nf_0$ (with $n = 3, 5,$ and 7 are the overtone numbers). $\rho_l$ and $\eta_l$ are the bulk liquid density and viscosity, respectively. Changes in frequency ($\Delta f$) and dissipation ($\Delta D$) for three overtones (3, 5, 7) were used to estimate the thickness ($d$) (or mass adsorbed) and two viscoelastic parameters, shear modulus ($\mu$) and shear viscosity ($\eta$) of the adsorbed layer using QTools software (Q-Sense). More details on the model have been reported elsewhere [13,14]. This model does not take into account the frequency dependence of viscoelasticity. However, Höök et al. [16] and Larsson et al. [24] have shown that effect of frequency on viscoelasticity is very small.

## 3. Results and discussion

The adsorption of PLL and histone from PBS at pH 7.4 and 24 ± 0.1 °C onto a gold coated quartz crystal was measured with QCM-D by recording changes in frequency and dissipation with time. In order to compare the data for the three overtones, the decrease in frequency was normalized by their overtone number. The adsorption of PLL and histone resulted in a decrease in the normalized frequency and an increase in the dissipation for the three overtones as shown in Figs. 1a and 1b, respectively. The normalized change in frequency ($\Delta f/n$) and dissipation ($\Delta D$) for the three overtones did not overlap. This indicates that the adsorbed layer is not perfectly elastic [16]. If a layer is not perfectly elastic, it dissipates energy and the adsorbed mass is not proportional to change in normalized frequency. Hence, $\Delta f/n$ for three overtones does not overlap for a viscoelastic adsorbed layer such as ours. For a perfectly elastic and thin adsorbed layer, researchers have successfully used the Sauerbrey equation [25]. According to this equation, the adsorbed mass, $\Delta m$ ($ng/cm^2$) is proportional to a normalized decrease in the frequency, $\Delta f/n$ (Hz). If the Sauerbrey equation holds, then,

$$\Delta m = C\frac{\Delta f}{n}, \tag{7}$$

where $C$ is the mass sensitivity constant ($C = 17.7\ \mathrm{ng\,cm^{-2}\,Hz^{-1}}$). Due to the viscoelastic nature of the adsorbed layer, we then fit the Voigt viscoelastic model to the data using QTools (Q-Sense)