Reversibly Controlling the Rigidity of Adsorbed Polycations

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Changes in the properties of an adsorbed layer at a solid−liquid interface are of considerable interest for their use as controllable surfaces.[1] Of particular interest are the polyelectrolyte adsorbed layers or brushes which can change their structures due to external stimuli such as pH, salt, solvent, etc.[2] Collapse of polyelectrolyte brushes due to addition of salt solutions is generally explained by screening of the charges. In addition to the screening of charges, the presence of ions can result in specific interactions which can dramatically influence the mechanochemical properties like lubrication and hydrophobicity of a polyelectrolyte brush.[3] These kinds of smart adsorbed layer and brushes can be used for many applications especially in drug delivery and sensors in fluidic devices.

An important property of smart surfaces is the rigidity of the adsorbed layer. Farhan et al.[4] have studied the effect of anions on the rigidity of poly[2-(methacryloyloxy)ethyl trimethylammonium chloride] (PMETAC) brushes using atomic force microscopy. Here, we have studied the changes in rigidity of an adsorbed poly(L-lysine) (PLL) layer due to addition of different anions using quartz crystal microbalance with dissipation monitoring (QCM-D), a high-resolution mass sensor, and attenuated total reflection Fourier transform infrared spectroscopy (ATR/FTIR). In a QCM-D, a gold-plated AT-cut piezoelectric quartz crystal is oscillated in shear mode at its fundamental resonant frequency (5 MHz) by passing an alternating current through the crystal. The normalized changes in frequency ($\Delta f/n$) and dissipation (ΔD) of the crystal are recorded in real time. The adsorbed layer with higher rigidity will have lower $|\Delta D/(\Delta f/n)|$ ratio. Detail discussion about the working principles and use of QCM-D has been reported elsewhere.[5,6]

A gold coated quartz crystal (QSX301, Q-Sense, Sweden) was oxidized in an UV/O_3 chamber for 15 min to clean the surface before conducting the QCM-D experiment. An amount of 250 μL of 300 μg/mL PLL hydro bromide (molecular weight >300 kDa, Sigma) in 100 mM NaBr solution at pH 7.4 was adsorbed onto a gold coated quartz crystal using a QCM-D (D300, Q-Sense, Sweden) for ∼30 min. Then, the crystal was washed by injecting 250 μL of deionized (DI) water, and this was repeated several times to remove the excess PLL and NaBr from the system. Following this, 250 μL of Na_2SO_4 (pH 7.4) was injected twice to induce changes in structure of the adsorbed layer and washed with DI water several times to remove excess Na_2SO_4 from the system. The concentration of Na_2SO_4 was 33.3 mM so as to keep the ionic strength of the solution constant. This cycle of steps was repeated twice. The normalized changes in frequency ($\Delta f/n$) and dissipation (ΔD) were monitored and are shown in parts a and b of Figure 1. For simplicity we have shown only the data for the 7th overtone and normalized the change in frequency with overtone number ($n = 7$). The adsorption of PLL in the presence of NaBr onto the gold surface resulted in a change of $\Delta f/n$ and ΔD to ∼−19.7 Hz and $\sim 3 \times 10^{-6}$, respectively. Washing with DI water increased the $\Delta f/n$ and ΔD to ∼−10.5 Hz and $\sim 6.9 \times 10^{-6}$, respectively (*1 in Figure 1). Injection of Na_2SO_4 solution decreased the frequency and dissipation to ∼−17.9 Hz and $\sim 1.9 \times 10^{-6}$, respectively. Washing with DI increased the $\Delta f/n$ to ∼−11.1 Hz and kept ΔD constant (*2 in Figure 1). Injection of NaBr solution followed by washing with DI water changed the $\Delta f/n$ and ΔD to ∼−14.3 Hz and 9.0×10^{-6}, respectively (*3 in Figure 1). The same cycles were repeated again (*4−*5 in Figure 1). Similarly, Br^- was also exchanged with ClO_4^- using 100 mM $NaClO_4$ (pH 7.4) solution (Figure 2). The ratio of $|\Delta D/(\Delta f/n)|$ is a measure of the rigidity of an adsorbed film.[7] Such values are obtained from Figures 1 and 2 and plotted in Figure 3a for exchanging anions from Br^- to SO_4^{-2} and to ClO_4^-. The adsorption of PLL in NaBr solution and subsequent washing with DI water resulted in $|\Delta D/(\Delta f/n)|$ ratio of $0.56 \pm 0.10 \times 10^{-6}$ Hz^{-1} (cycle 1). The change in frequency and dissipation due to addition of Na_2SO_4 is due to a combination of the change in rigidity of the adsorbed layer as well as a change in viscosity and density of the bulk liquid. Exchanging the Br^- with SO_4^{-2} resulted in $|\Delta D/(\Delta f/n)|$ ratio of $0.10 \pm 0.07 \times 10^{-6}$ Hz^{-1} (cycle 2). Injection of 100 mM NaBr and subsequent washing with DI water increased the $|\Delta D/(\Delta f/n)|$ ratio for the adsorbed layer back to $0.59 \pm 0.04 \times 10^{-6}$ Hz^{-1} (cycle 3). For the next cycle, a reversible change in $|\Delta D/(\Delta f/n)|$ ratio back to $0.12 \pm 0.08 \times 10^{-6}$ was observed due to the addition of Na_2SO_4 (cycle 4). Finally, exchanging the SO_4^{-2} with Br^- resulted in a $|\Delta D/(\Delta f/n)|$ ratio of $0.59 \pm 0.05 \times 10^{-6}$ (cycle 5). An adsorbed layer with a lower value of the $|\Delta D/(\Delta f/n)|$ ratio indicates its greater rigidity.[7] Thus, exchanging Br^- with SO_4^{-2} made the adsorbed layer more rigid. This change was also reversible. The divalent SO_4^{-2} ion presumably cross-linked the adsorbed polycationic chain via electrostatic interactions and resulted in a more rigid layer. This approach provides a platform to reversibly control the rigidity of an adsorbed layer for various applications like nanoactuators and drug delivery.

The $|\Delta D/(\Delta f/n)|$ ratio has widely been used in literature to study the conformational changes of proteins[7a] and synthetic polymers.[7b,c] Liu et al.[7b] have studied the collapse and swelling of thermally responsive poly(*N*-isopropylacrylamide) (PNIPAM) brushes using QCM-D. They showed that the $|\Delta D/(\Delta f/n)|$ ratio for PNIPAM decreased due to heating the polymer from 20 °C to above its lower critical solution temperature (32 °C) due to collapse of the brush layer. The $|\Delta D/(\Delta f/n)|$ ratio increased when the collapsed polymer brush was cooled back to 20 °C.

The rigidity of an adsorbed polyelectrolyte layer can also be controlled by changing the pH of a solution and will depend on the pK_a of the functional group of the polymer. For PLL, the change should occur above pH 10.5 which is physiologically not relevant. The pH response of PLL has been used for drug delivery by synthesizing its copolymer with isophthalamide with different degrees of PEGylation.[8] Researchers have combined the pH responsiveness of PLL with the thermal responsiveness of PNIPAM to create a pH- and thermoresponsive polymer.[9] However, salt induced changes in rigidity of an adsorbed layer allows one to tune the physicochemical property of the adsorbed

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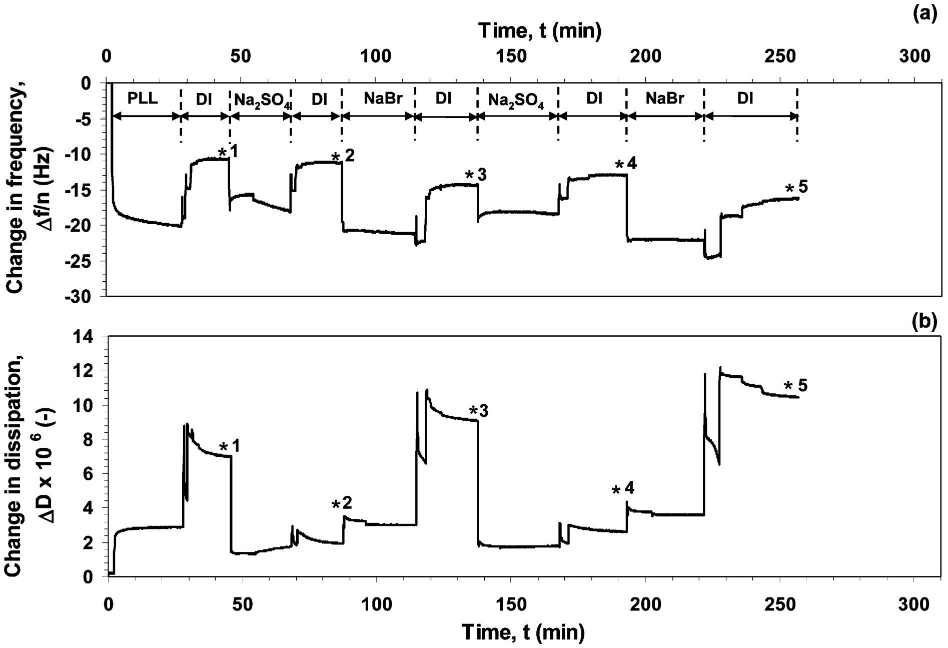

Figure 1. Effect of exchanging Br^- with SO_4^{-2} ions on the rigidity of an adsorbed poly(L-lysine) layer. (a) The normalized frequency ($\Delta f/n$) decreased as a function of time for the seventh overtones ($n = 7$; $f_0 = 5$ MHz). (b) Change in dissipation, ΔD with time. The $*$ indicates the $\Delta f/n$ and ΔD values after washing with DI water and are used to calculate the $|\Delta D/(\Delta f/n)|$ ratio.

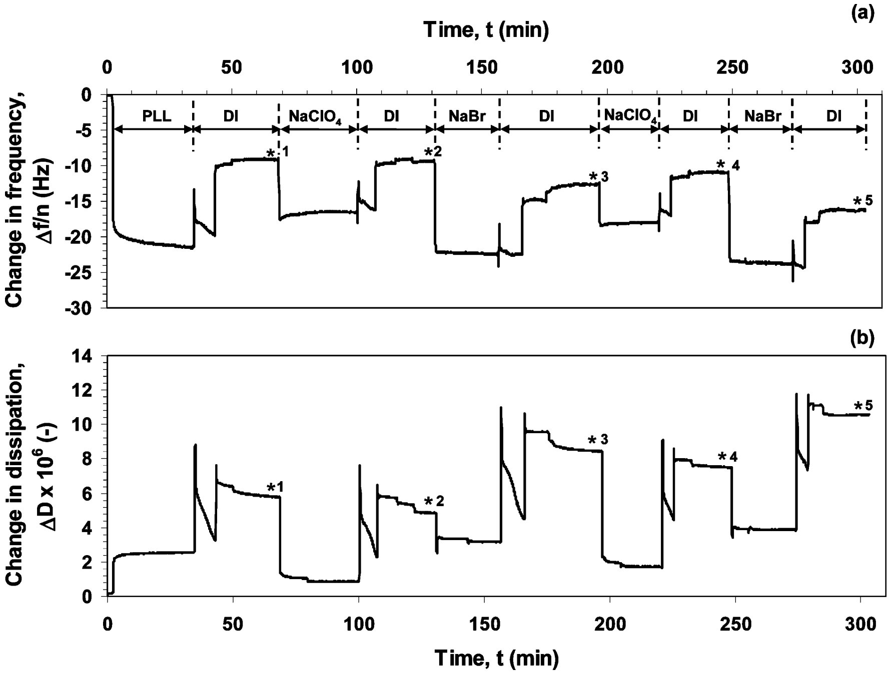

Figure 2. Effect of exchanging Br^- with ClO_4^- ions on the rigidity of adsorbed poly(L-lysine) layer. (a) The normalized frequency ($\Delta f/n$) decreased as a function of time for the seventh overtones ($n = 7$; $f_0 = 5$ MHz). (b) Change in dissipation, ΔD with time. The $*$ indicates the $\Delta f/n$ and ΔD values after washing with DI water and are used to calculate the $|\Delta D/(\Delta f/n)|$ ratio.

layer without drastically changing the pH of the system. When Br^- was exchanged with ClO_4^- using a 100 mM $NaClO_4$ solution (Figure 2), we did not observe any significant changes in the $|\Delta D/(\Delta f/n)|$ ratio (rigidity) of the adsorbed layer as shown in Figure 3a. Azzaroni et al.[10] have shown the hydrophobic collapse of a grafted PMETAC brush due to exchanging the Cl^- with scarcely hydrated ClO_4^- due to ion-pair formation between a tertiary amine group of the brush molecule and the ClO_4^-. We conjecture that this kind of ion-pair does not form between the primary amine group of the PLL molecule and the ClO_4^- anion. Ma et al.[11] have shown that ion-pair formation occurs between ammonium groups of PLL and ClO_4^- in bulk solution. They also showed that the effect of ClO_4^- at a low concentration (0.1 M) of ClO_4^- is very small. In fact, they state that "in 0.1 M $NaClO_4$ the α-helix-like conformations are almost fully melted by 20 °C".[11] In our case, we probed the behavior of PLL at the solid−liquid interface and at a low concentration of ClO_4^- (0.1 M) at 24 ± 0.1 °C. Hence, we did not observe any significant ion-pair formation between the ammonium groups of PLL and ClO_4^- at the solid−liquid interface. Because

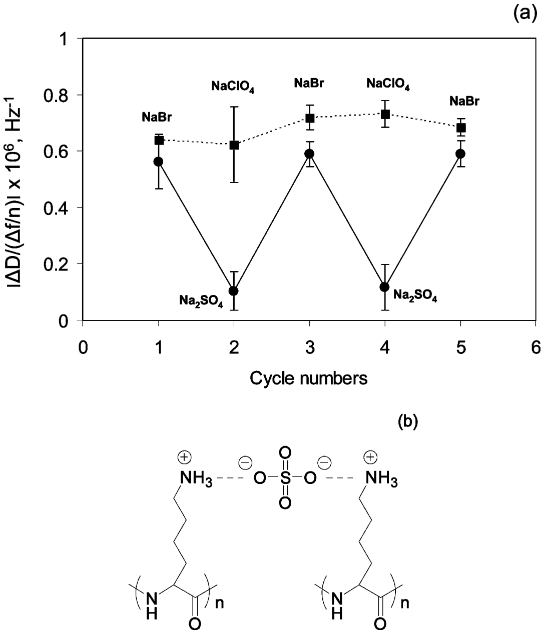

Figure 3. (a) The $|\Delta D/(\Delta f/n)|$ ratio of an adsorbed layer obtained after washing with DI water. Change in frequency was normalized with the overtone number ($n = 7$). Exchange of Br^- with SO_4^{-2} ions resulted in a decrease in the $|\Delta D/(\Delta f/n)|$ ratio while the reverse increased the $|\Delta D/(\Delta f/n)|$ ratio almost back to its initial value. The decrease in $|\Delta D/(\Delta f/n)|$ ratio due to exchanging the Br^- with SO_4^{-2} ions indicates an increase in rigidity of the adsorbed layer (● and —). Exchanging the Br^- with ClO_4^- ions did not cause a significant change in the rigidity of the adsorbed PLL layer (■ and · · ·). (b) Binding of SO_4^{-2} anions with the ammonium groups of PLL.

the $|\Delta D/(\Delta f/n)|$ ratio values of the adsorbed layer after treating with DI water were compared, it is less likely that the change in rigidity of the polycationic layer was due to electrostatic collapse via screening of charges because the salt in the bulk solution was replaced by DI water. It is more likely, however, that the SO_4^{-2} anions strongly bind with the primary amines and cross-link the polycationic layer (Figure 3b).

The properties of PLL at a solid−liquid interface were also studied using ATR/FTIR. 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. Spectra were collected at a gain of 8 and resolution of 2 cm^{-1} with 256 scans for each sample. To analyze the pure PLL spectra, baseline corrected buffer and water vapor spectra were subtracted from the PLL spectra in aqueous solutions.[12] Two major peaks are observed in the range 4000−1200 cm^{-1}: the peak at ∼3375 cm^{-1} corresponds to the amine group and the peak at ∼1639 cm^{-1} corresponds to the amide I band (1700−1600 cm^{-1}) of the polypeptide (Figure 4). The intensities of the peak at ∼3375 cm^{-1} in the presence of Br^-, SO_4^{-2}, and ClO_4^- anions were 0.318 ± 0.013, 0.389 ± 0.008, and 0.310 ± 0.008, respectively. The intensities of the peak at ∼1639 cm^{-1} in the presence of Br^-, SO_4^{-2}, and ClO_4^- anions were estimated to be 0.146 ± 0.006, 0.172 ± 0.003, and 0.147 ± 0.002, respectively. Thus, a ∼20% increase in peak intensity occurred in exchanging bromide or perchlorate to sulfate for both peaks. This increase is also obtained when the area under the amide I band for the polypeptide in the presence of the three different anions is measured. Areas in the presence of Br^-, SO_4^{-2}, and ClO_4^- were estimated as 4.6 ± 0.2, 5.6 ± 0.1, and 4.5 ± 0.3, respectively. The three anions used in this study are present in the Hoffmeister series in the order $SO_4^{-2} > Br^- > ClO_4^-$. Sulfate anions interact with the polypeptide and water molecules resulting in salting out of the polypeptide. This increases the effective concentration of PLL on the ATR crystal which is evident from the increase of peak intensity or area for sulfate ions as compared with bromide or perchlorate ions. These observations are in agreement with our QCM-D data.

In conclusion, one of the important aspects in changing the rigidity by addition of SO_4^{-2} is that it is reversible and can be reverted to its original state by adding Br^- ions. This reversible cross-linking and uncross-linking of the adsorbed polycationic layer could be useful for drug delivery and nanoactuator applications.

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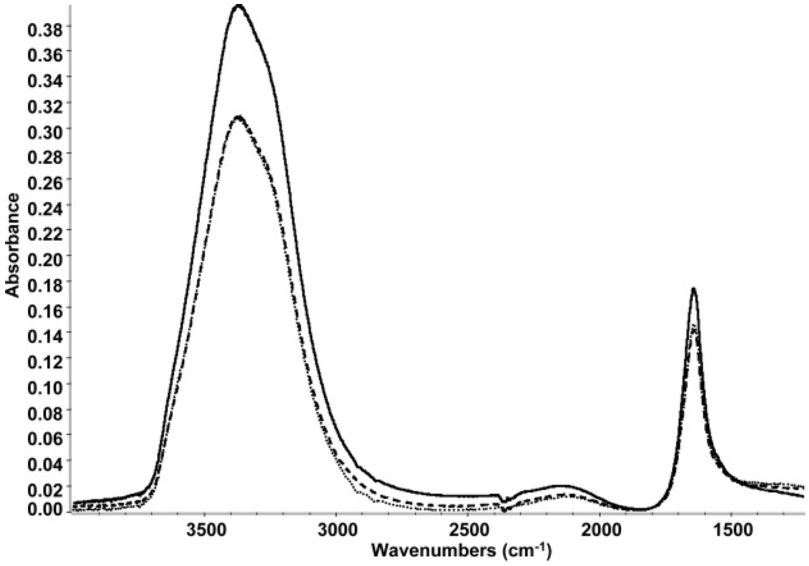

Figure 4. ATR/FTIR spectra of PLL in the presence of SO_4^{-2} (—), Br^- (— — —), and ClO_4^- (···). Spectra were collected at a gain of 8 and resolution of 2 cm^{-1} with 256 scans for each sample. The peaks at ∼3375 cm^{-1} and ∼1639 cm^{-1} are due to the amine group and amide I band, respectively.

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