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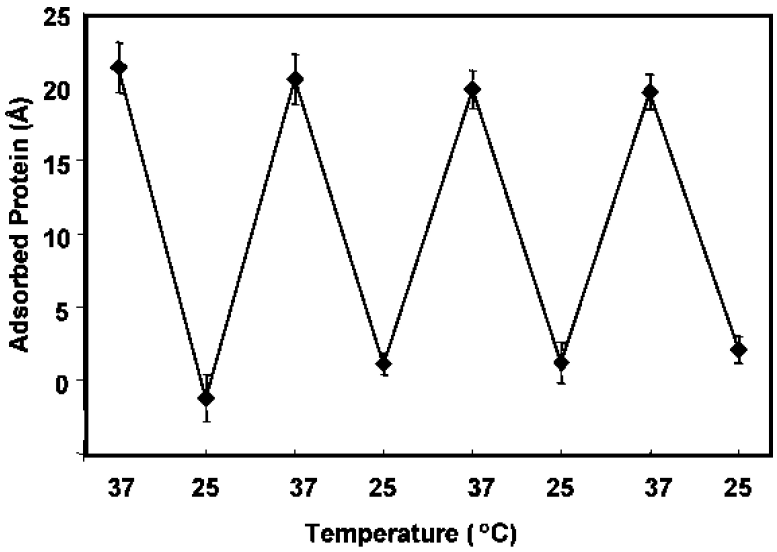

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Reversible Protein Adsorption and Bioadhesion on Monolayers Terminated with Mixtures of Oligo(ethylene glycol) and Methyl Groups

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Since the original work by Whitesides et al. that established self-assembled monolayers (SAMs) of oligo(ethylene glycol) (OEG)-terminated alkylthiolates as model surfaces that are resistant to protein adsorption,[1] there have been hundreds of reports on the investigation of their biointeractions. A handful of these studies have examined the temperature dependence of bioadhesion on single-component molecular assemblies of OEG or PEG and have demonstrated temperature transitions, none of which have been experimentally convenient for reversible bioadhesion.[2] In this communication, we investigate in detail and exploit a secondary finding of Prime and Whitesides: that certain compositions of mixed SAMs formed by the coadsorption of OEG- and methyl-terminated alkanethiols exhibit different tendencies for protein adsorption at room temperature and at 37 °C.[1a] Specifically, we demonstrate (i) that a sharp transition in protein adsorption and cellular adhesion as a function of temperature can occur on mixed SAMs, (ii) that this transition can be exploited to create model thermally responsive surfaces that exhibit reversible bioadhesion, and (iii) that the origin of the transition is consistent with a change in surface wettability and a change in the structure in mixed monolayers as detected by vibrational sum frequency generation (VSFG) spectroscopy.

The ability to rapidly and reversibly modulate protein adsorption,[3] and by extension, cellular attachment, at the liquid−solid interface is important in a variety of applications, including controlling protein adsorption and biofouling,[4] chromatography,[5] and cell culture.[6] Thermally responsive polymers, such as poly(*N*-isopropyl acrylamide), have been studied extensively for these and related applications. We measured protein adsorption and bacterial attachment to SAMs formed by immersing freshly deposited gold substrata into ethanolic solutions of $HS(CH_2)_{11}(OCH_2CH_2)_6OH$ (OEG−SAM), $HS(CH_2)_{11}CH_3$ (CH_3−SAM), or a 95:5 (v/v) mixture of the two (OEG/CH_3−SAM). The mole fraction of OEG in OEG/CH_3−SAMs was ∼0.5, which at room temperature is slightly above the concentration required for resistance to adsorption of pyruvate kinase and lysozyme, but near the minimum required for resistance to fibrinogen adsorption.[1a]

Figure 1 shows a sharp transition in adsorption at 32 °C for both pyruvate kinase and lysozyme; at T < 32 °C, neither protein adsorbed significantly on OEG/CH_3−SAMs, whereas at temperatures ≥32 °C, ellipsometric thicknesses indicated that a monolayer of protein had adsorbed (i.e., ∼170 ng cm^{-2} lysozyme and 500 ng cm^{-2} pyruvate kinase).[1a] No such temperature dependence was observed for CH_3−SAMs or pure OEG−SAMs. That the two different proteins showed sharp changes in their adsorption at the same temperature suggests that this change is not due to changes in protein properties, but rather to those of the solid−liquid interface.

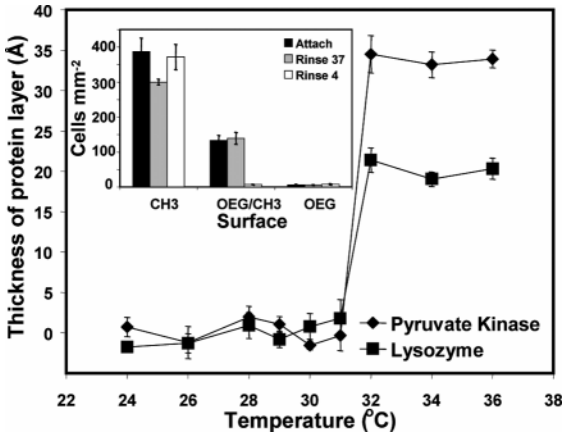

Figure 1. Adsorption of pyruvate kinase and lysozyme on OEG/CH_3−SAMs as a function of temperature, as measured by ellipsometry of dry samples. Inset: Number of bacterial cells attached to CH_3−, OEG/CH_3−, and OEG−SAMs after 2 h attachment at 37 °C and rinsing at either 37 or 4 °C.

Fibrinogen also showed an increase in adsorption with temperature; at 24 °C, a small amount of protein was adsorbed (∼8 Å; ∼40 ng cm^{-2}) and increased to ∼24 Å (∼150 ng cm^{-2}) at 36 °C. Both of these thicknesses were less than a monolayer of protein.

Protein adsorbed on OEG/CH_3−SAMs above 32 °C can be removed by rinsing at a lower temperature. Lysozyme was adsorbed at 36 °C for 2 h, and its thickness was measured by ellipsometry. The substrate was then briefly rinsed with distilled water at 24 °C and dried. A second measurement showed complete removal of the adsorbed protein. This adsorption/desorption cycle was repeated several times.

We also examined the temperature dependence attachment of the marine bacterium, *Cobetia marina*, to OEG/CH_3−SAMs. Whereas minimal attachment was observed at 22 °C for OEG-containing SAMs, at 37 °C, OEG/CH_3−SAMs accumulated significant numbers of cells; pure OEG−SAMs were still resistant to attachment (Figure 1 inset). CH_3−SAMs accumulated large numbers of cells at both temperatures. Moreover, most (97%) of the cells attached to mixed SAMs at 37 °C were released upon rinsing at 4 °C. The change in both protein adsorption and cellular attachment as a function of temperature was concomitant with a change in advancing water contact angle. While the water contact angle for the mixed SAMs did not change appreciably below 31 °C and above 32 °C, a sharp change of ∼9° was observed between 31 and 32 °C.

Figure 2 shows (i) the VSFG spectra (inset) taken in 20 °C water of a SAM formed by adsorption of $HS(CH_2)_{11}(OCH_2CH_2)_6OCH_3$ (OME−SAM) and a SAM formed from a 95:5 (v/v) mixture of the OME and $HS(CD_2)_{11}CD_3$ (OME/CD_3)−SAMs, and (ii) the temperature dependence of the methylene resonances of the ethylene

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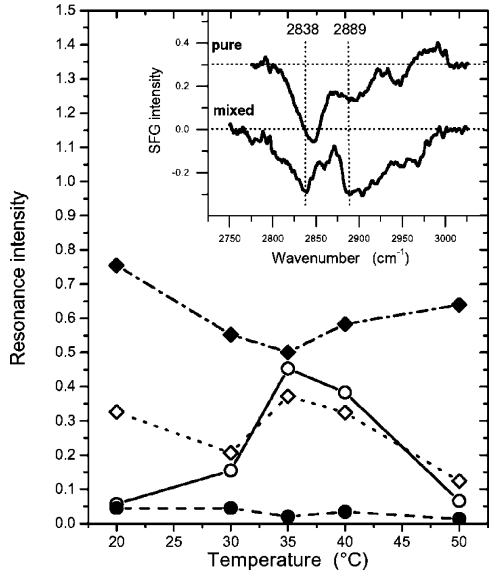

Figure 2. VSFG spectra of a pure OME−SAM and OME/CD_3−SAM recorded in water at 20 °C (inset). Spectra were recorded at different temperatures and deconvoluted as described previously.[8] The figure displays the evolution of the intensities of the symmetric ether methylene resonances of the ethylene glycol chain at 2889 cm^{-1} (circles) and methoxy terminus at 2838 cm^{-1} (diamonds) indicated in the inset, respectively. Dark symbols represent intensities for the OME−SAM and open symbols the OME/CD_3−SAM.

glycol chain at 2889 cm^{-1} and methoxy terminus at 2838 cm^{-1}. OME was used instead of OEG since, in an aqueous environment, the methoxy groups can be detected much easier than the hydroxyl terminus of OEG and, thus, can serve as an indicator for the orientation of the chain terminus. A perdeuterated alkanethiol was chosen as a diluent to allow a background-free analysis of the OME. Resonance intensities were calculated according to

$$I_q = \int_{-\infty}^{\infty} \left|\frac{A_q}{\omega_{IR} - \omega_q + i\Gamma_q}\right|^2 d\omega_{IR} = \frac{A_q^2}{\Gamma_q}$$

where A_q is the amplitude, Γ_q the bandwidth, and ω_q the position of mode q. The intensities are normalized to the nonresonant signal of the gold substrate and are directly comparable.[7] The methylene resonances are constant with temperature, while the resonances of the terminal methoxy group show a slight minimum at ∼35 °C. This indicates that the OEG units and the terminal methoxy groups in these SAMs are sufficiently constrained so they cannot undergo substantial conformational changes. In contrast, for the mixed SAMs, both the methylene and methoxy resonances increase between 30 and 35 °C and then decrease toward zero at about 50 °C. Similar changes in the methylene resonances for OEG/CD_3−SAMs were observed.

Because of the dependence of VSFG intensity on local symmetry, an increase in intensity indicates partial ordering of the molecular chains and preferential orientation of the methoxy groups.[8] Zero intensity of the methylene resonances corresponds to either a complete disorder of chain conformation or a (unlikely) perfectly helical or all-trans conformation of the (oxy)methylene chain, with the methylene groups in an centro-symmetric environment. Hence, the temperature-induced changes are most likely due to a disorder-to-partial order transition of the hydrated chains, followed by further randomization of the molecular conformations. Effects of changes in the order and hydration of chains on protein resistance have been demonstrated in studies on the effects of density of OEG of protein resistance. For example, a change from a helical or amorphous structure to the all-trans state resulted in loss of protein resistance,[9] and the penetration of water into the OEG− and OME−SAMs is a necessary condition to render them protein resistant.[10] Hydration of OEG requires strongly bonded water,[11] and pure OEG− and OME−SAMs are protein resistant up to 80 °C.[2a] Dehydration would be reflected by drastic changes in the relative and total intensities with observable frequency shifts, which are both absent up to 50 °C in the pure and mixed SAMs studied here, further suggesting that changes in resistance to bioadhesion are due to conformational changes of the OEG chains. This could be due to a conformation-dependent (negative) surface potential of the mixed SAMs and concomitant changes in electrostatic repulsion, which have been postulated to be important in the protein resistance of OEG−SAMs.[10] Alternatively, conformational changes above 30 °C could expose hydrophobic moieties and, thereby, induce density changes in the vicinal water (hydration) layer, which would also change the hydration forces between the SAM and an approaching protein or bacterium. Additional theoretical work will be required to derive a precise structural model for the observed temperature-induced changes in the mixed hydrated SAMs.

We have demonstrated that thermally induced changes in the structure of mixed SAMs containing OEG groups coincide with changes in their resistance to protein adsorption, bacterial cell attachment, and wettability. In addition, temperature cycling can be used to almost completely remove adsorbed proteins and cells. Such switchable SAMs, being of precisely defined chemistry and architecture, lend themselves to use in correlating results of studies of interfacial biointeractions with models generated by molecular simulations. Because the temperature required for transition from the resistant to the nonresistant state is one which is compatible with most biomolecules and cells, OEG/CH_3−SAMs could serve as useful platforms for a variety of biotechnological applications.

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Supporting Information Available: Cyclic adsorption of lysozyme (Figures S-1), contact angle analysis (Figure S-2), surface analysis, calculation of protein concentration, and experimental details for protein and bacterial adsorption and VSFG. This material is available free of charge via the Internet at http://pubs.acs.org.

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