Using Surface Plasmon Resonance Spectroscopy To Measure the Association of Detergents with Self-Assembled Monolayers of Hexadecanethiolate on Gold

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This paper describes the use of surface plasmon resonance (SPR) spectroscopy to measure the rates and extents of association of four detergents—sodium dodecyl sulfate (SDS), β-octyl glucoside, Triton X-100, and Tween 20—to self-assembled monolayers (SAMs) of alkanethiolates on gold. SAMs presenting hexaethylene glycol groups resisted the adsorption of all four detergents. These same detergents associated with hydrophobic SAMs presenting methyl groups; the concentration of detergent molecules on the surface was 120−280 pmol/cm^2. The associations of the detergents with the hydrophobic SAM were described well by the Langmuir adsorption isotherm. The dissociation constants K_d (M) for the desorption of the detergents from the surface correlated with the critical micelle concentration (cmc, M) of the detergents in solution, and followed the relationship cmc $\approx 7\ (\pm 2)K_d$. The efficacy of SDS in removing the protein fibrinogen adsorbed on a hydrophobic SAM depended strongly on the concentration of detergent. SDS at a concentration three times greater than the cmc removed (or displaced) the adsorbed layer of protein in seconds; SDS at a concentration three times smaller than the cmc did not desorb it even after several minutes. This paper shows that SPR is a useful analytical technique for characterizing the interactions of detergents—and other molecules having low molecular weight—with the well-defined surfaces of SAMs.

This report describes the use of surface plasmon resonance (SPR) spectroscopy to study the association of detergents with self-assembled monolayers (SAMs) of alkanethiolates on gold. Detergents are used in a wide range of applications. In biochemical research—the focus of this work—applications include cleaning surfaces,[1] reagents for the solubilization of membrane proteins,[2] aids for the crystallization of proteins that aggregate (such as membrane proteins),[3] blocking agents to prevent the nonspecific binding of proteins in solid-phase binding assays,[4] protein denaturants,[5] and antibacterial agents.[6] These applications all rely on the ability of detergents to associate with hydrophobic interfaces—including hydrophobic patches on the surface of a protein—in aqueous media.

It would be easier to study the adsorption of detergents at interfaces if there were a convenient model system that provided relevant information at structurally characterized surfaces under well-defined conditions (pH, ionic strength, presence of proteins or organic cosolvents, etc.). SAMs formed by the adsorption of terminally-functionalized alkanethiols on gold are particularly well-suited as substrates for such studies.[7] These model surfaces are structurally well-defined and allow the interfacial properties to be controlled through synthesis of the precursor alkanethiols. They are also compatible with a variety of analytical techniques that can measure the adsorption of molecules and proteins at the surface. Here, we have used SPR to measure four important characteristics of the association of four representative detergents—sodium dodecyl sulfate (SDS), β-octyl glucoside, Triton X-100, and Tween 20—to SAMs of hexadecanethiolate ($-S(CH_2)_{15}-CH_3$): (i) the affinity of the detergents for this hydrophobic surface; (ii) the density of detergent molecules in films formed on the SAM; (iii) the rate of desorption of detergents from the SAM; (iv) the efficacy of detergents in removing adsorbed protein. We also show empirically that the dissociation constant describing the interaction of the detergents with the hydrophobic surface correlated with the critical micelle concentration (cmc).

Surface Plasmon Resonance Spectroscopy. The adsorption of detergents at interfaces has been studied by a variety of techniques, including: *in situ* ellipsometry,[8−10] neutron and X-ray reflectivity,[11] surface tension measurements,[12] and radiolabeling.[12] We employed surface plasmon resonance (SPR) spectroscopy in this work for several reasons: it measures kinetic information about the adsorption of molecules at an interface; it has excellent sensitivity (down to ~500 pg/cm^2 of adsorbed analyte); it uses thin films of gold and is compatible with SAMs of alkanethiolates; it is experimentally convenient, since an instrument with good fluidic control is commercially available. SPR is an optical technique that measures changes in the refractive index of the medium near (within ~200 nm) a metal surface. The active sensing element is a thin (~40 nm) film of gold deposited on a glass substrate. Monochromatic, p-polarized light is reflected from the back side of the glass−gold interface. A plot of reflected intensity versus the angle of incidence (Θ) shows a minimum (Θ_m) corresponding to the excitation of surface plasmons at the gold−solution interface.[13] The value of Θ_m shifts with changes in the refractive index of the

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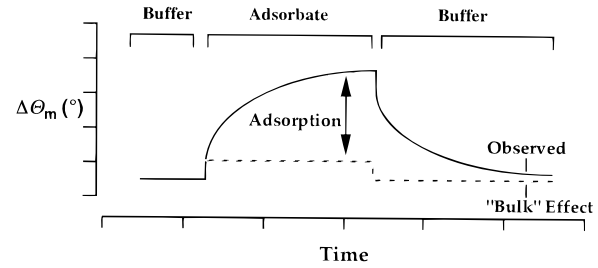


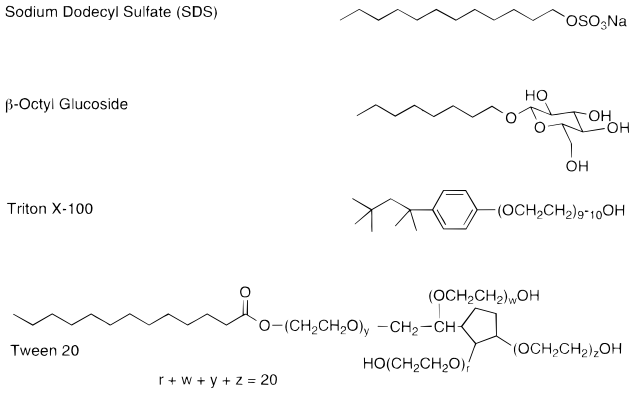
Figure 1. Hypothetical plot illustrating an SPR experiment for the reversible adsorption of an analyte to the sensing surface. SPR records the angle of minimum reflectivity of incident light versus time. In this example, buffer is allowed to flow through the cell, replaced by a solution containing the adsorbate, and then returned to buffer. Θ_m increases when the adsorbate is passed through the cell (and adsorbs to the surface) and then decreases when buffer is passed through the flow cell (due to dissociation of the adsorbate from the surface). The dashed curve represents the contribution to $\Delta\Theta_m$ of dissolved analyte that increases the refractive index of the buffer.

interfacial region near the surface of the gold (within approximately $^1/_4$ of a wavelength of the incident light). For thin (<50 nm) organic films and light with a wavelength of 760 nm, the shift in Θ_m is approximately proportional to the thickness of the film.[14]

Because SPR measures changes in the index of refraction of the medium within ~200 nm of the surface, it is sensitive to the adsorption of molecules at the interface, and to the presence of molecules dissolved in the medium. This later effect (the "bulk" effect) produces a displacement in Θ_m proportional to the concentration of the analyte in the solution. Figure 1 shows representative data for the reversible adsorption of an analyte to the sensing surface. The solid curve shows the change in Θ_m observed when buffer is allowed to flow through the cell, replaced with a solution of analyte, and then returned to buffer. The rise in Θ_m upon introduction of analyte in the cell is due principally to adsorption at the interface, and the fall in Θ_m when buffer is reintroduced into the flow cell is due to desorption. The dashed line shows the component of the response that is due to the presence of analyte dissolved in the buffer (due to an increased refractive index of the solution). The amount of analyte that *adsorbs* to the interface is proportional to the *difference* between the two curves.

Results

Preparation of Substrates. We employed a commercial instrument in this work.[15] Substrates were prepared by evaporating thin films of titanium (1.5 nm, for adhesion of gold) and gold (38 nm) onto glass cover slips. SAMs of hexadecanethiolate (HDT) or a hexaethylene glycol (EG_6OH) terminated alkanethiolate were allowed to assemble onto these substrates by immersing the substrates in solutions of the alkanethiols $HS(CH_2)_{15}-CH_3$ and $HS(CH_2)_{11}(OCH_2CH_2)_6OH$, respectively) in ethanol (2 mM) for 12 h. The substrates were cut and glued into cartridges for use in the BIACore instrument as described previously.[16]



SAMs Presenting EG_6OH Groups Resist the Adsorption of Detergent. The adsorption of each of the four detergents—SDS, β-octyl glucoside, Triton X-100, and Tween 20—on a SAM presenting hexaethylene glycol groups was measured using SPR. Our experiments began by allowing phosphate-buffered saline (PBS; 10 mM phosphate, 140 mM NaCl, pH 7.4) to flow through the cell. This flow was periodically replaced with solutions of the same buffer containing detergent at increasing concentrations, each for a period of 3 min. A constant rate of flow of solution over the surface was maintained during the experiment. The instrument recorded Θ_m as a function of time. The BIACore instrument reports Θ_m in resonance units (RU, 10 000 RU = 1°); we report values of $\Delta\Theta_m$ ($\Delta\Theta_m = \Theta_m - \Theta_m°$), which is the *change* in Θ_m during the experiment relative to the clean surface in PBS at the start of the experiment. In all cases, the SPR curves rapidly reached an equilibrium value during the 3-min injection of detergent (Figure 2A). Figure 2B shows plots relating the equilibrium displacement in the resonance angle to the concentration of detergent in the sample. For all four detergents, the change in Θ_m was related linearly to the concentration of detergent.

The linearity of the dependence over wide ranges of concentration both above and below the cmc suggests that the changes in Θ_m are due only to changes in the refractive index of the solution (n_s), and not to association of the detergent with the SAM presenting hexaethylene glycol groups. The best-fit lines to these data give ratios for the incremental change in Θ_m with the concentration of detergent (c). We call this parameter $R_s = \partial\Theta_m/\partial c$ (Figure 2B, in units of deg/mM). Under the conditions of the experiment, Θ_m is related linearly to n_s according to the relationship $\partial\Theta_m/\partial n_s = 108°$.[17] The incremental change in n_s with c ($R_n = \partial n_s/\partial c$, in units of mM^{-1}) is therefore related to R_s as described in eq 1.

$$R_n = R_s/108° \qquad (1)$$

Table 1 shows that the values of R_n determined from the SPR data agree well with values of R_n determined independently using an Abbe refractometer. These results also show that no detergent associated with the SAM terminated in hexaethylene glycol groups.

Association of Detergents with Hydrophobic SAMs. Figure 3A shows the SPR response obtained for the association of SDS with a SAM of hexadecanethiolate. A series of samples containing increasing concentrations of the detergent were allowed to flow through the cell, interspersed with buffer. The increase in Θ_m on injection of the detergent solutions was greater than that observed when SAMs presenting EG_6OH groups were used (the data obtained using this SAM are also shown in Figure 3A); the *difference* between the two curves represents the amount of detergent that associated with the SAM of

(13) For a detailed physical description of SPR, see: Raether, H. *Phys. Thin Films* **1977**, *9*, 145−261.
(14) Stenberg, E.; Persson, B.; Roos, H. *J. Colloid Interface Sci.* **1991**, *143*, 513−526.
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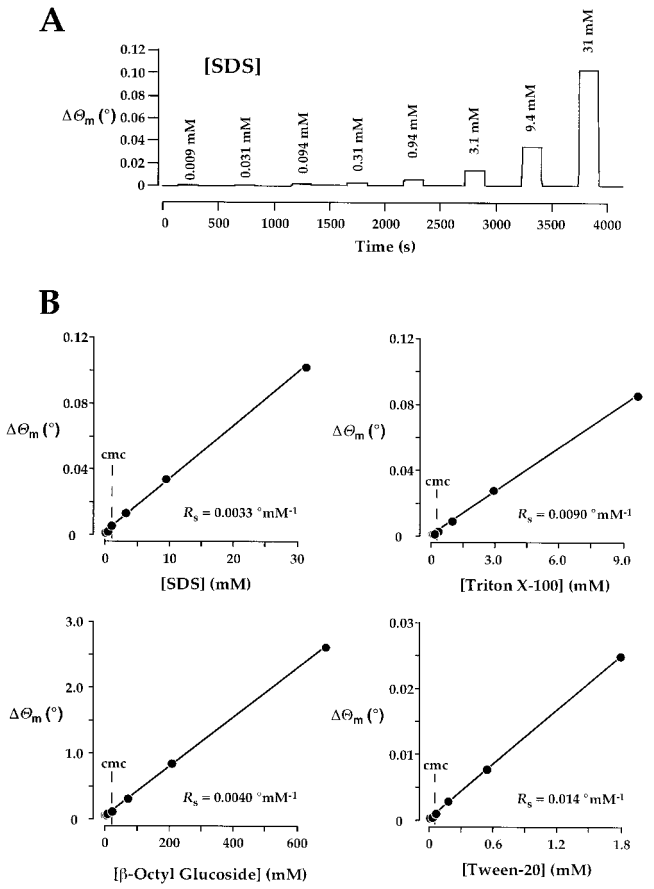

Figure 2. (A) Data from the SPR experiment for the treatment of SAMs presenting hexaethylene glycol groups with the detergent SDS. Increasing concentrations of SDS in PBS were allowed to flow through the cell for 3 min each, separated by flows of buffer for 2 min. The change in the resonance angle relative to the baseline value ($\Delta\Theta_m$) is plotted versus time. Tween 20, Triton X-100, and β-octyl glucoside behaved similarly (these data not shown). (B) The relationship between the equilibrium displacement of Θ_m and the concentration of detergent is linear for all four detergents. Error bars are not shown because the uncertainty in the data is smaller than the closed circles. These data show that changes in Θ_m arise only from changes in the refractive index of the aqueous buffer and that the detergents do not associate with the SAM presenting EG_6OH groups.

hexadecanethiolate. In all cases, the association of detergents with the surfaces was complete within the 3 min incubation period; the rate constants for association, however, were too large to be measured due to the slow response time of the instrument (mixing effects dominate the SPR response during the first 10 s of the incubation period). Assuming pseudo-first-order kinetics, the pseudo-first-order rate constant ($k'_{on} = k_{on}$[detergent]) can only be measured for values of $k'_{on} < \sim 0.1\ s^{-1}$).

Table 1. Dependence of Refractive Index on the Concentration of Detergent in Aqueous Solutions: A Comparison of Measurements by SPR and Refractometry

		R_n (mM^{-1})[b]	
detergent	R_s (deg/mM)[a]	SPR[c]	refractometry[d]
SDS	3.3×10^{-3}	3.1×10^{-5}	3.3×10^{-5}
octyl glucoside	4.0×10^{-3}	3.8×10^{-5}	4.0×10^{-5}
Triton X-100	9.0×10^{-3}	8.4×10^{-5}	9.0×10^{-5}
Tween 20	1.4×10^{-2}	1.3×10^{-4}	1.4×10^{-4}

[a] R_s is the ratio $\partial\Theta_m/\partial c$ determined (as described in the text) by passing solutions of detergents over a SAM presenting hexaethylene glycol groups (from Figure 2B). [b] R_n is the ratio $\partial n_s/\partial c$. [c] Values of R_n were determined from the values of R_s according to eq 1. [d] Values of R_n were determined by measuring the refractive index of solutions containing the detergents with an Abbe refractometer.

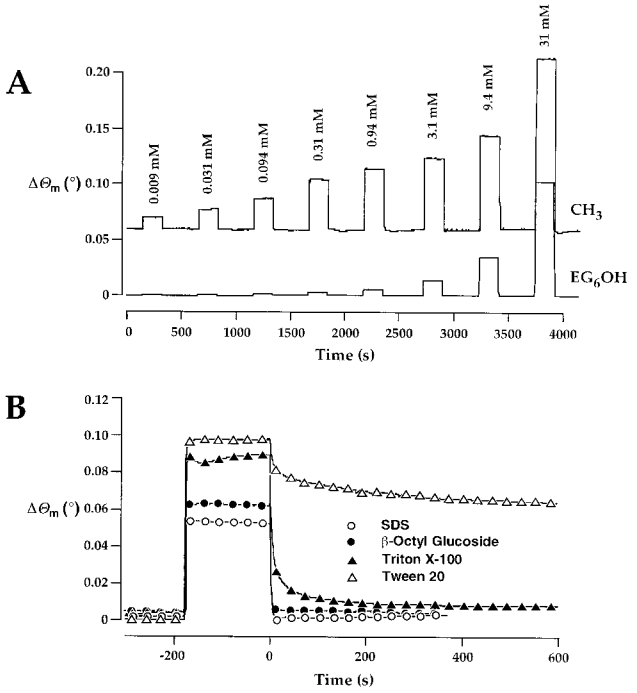

Figure 3. (A) Data from SPR involving treatment of SAMs presenting either methyl groups or hexaethylene glycol groups with SDS. Increasing concentrations of detergent in PBS were passed through the cell for 3 min each, separated by flows of buffer for 5 min. The change in the resonance angle relative to the baseline value ($\Delta\Theta_m$) is plotted versus time for each surface. The vertical scale indicates relative changes in $\Delta\Theta_m$: the curves are offset vertically for clarity. (B) Dissociation of detergent from the hydrophobic surface. SAMs of HDT were treated for 3 min with solutions of SDS, β-octyl glucoside, Triton X-100, or Tween 20 at concentrations equal to three times their cmc (3, 75, 0.9, and 0.18 mM, respectively). The detergent was then allowed to dissociate under a flow of buffer for 10 min. The experiments were repeated on a SAM presenting EG_6OH groups to measure the shifts in Θ_m due to the changes in refractive index in the bulk solution. The plot shows the difference between the data obtained on the two surfaces. Selected data points are represented with symbols to help distinguish the curves from each other.

Dissociation of Detergents from Hydrophobic SAMs. Figure 3B shows the time course for the dissociation of the detergents from SAMs of hexadecanethiolate. The kinetics for the dissociation of the detergents were measured by treating the SAMs with solutions containing the detergents (at concentrations three times greater than the cmc) and then allowing buffer to flow through the cell for several minutes to observe the dissociation curve. In order to measure the contribution of the bulk refractive index on the shifts in Θ_m, we repeated the dissociation experiments over SAMs presenting EG_6-

(17) The theoretical SPR response to changes in the index of refraction of the bulk liquid and to deposition of thin detergent films was determined by calculating the reflection of p-polarized light from a stratified, planar, isotropic structure, as described by: Azzam, R. M. A.; Bashara, N. M. *Ellipsometry and Polarized Light*; North-Holland: New York, 1977. The model used two layers with finite thicknesses (gold and detergent) between two semi-infinite media (glass and solution). The indices of refraction for the gold ($0.17 + 4.93i$), and glass (1.511) were taken from ref 14. The index of refraction of the buffer (1.335) was taken from the *CRC Handbook of Chemistry and Physics*; Weast, R. C., Lide, D. R., Astle, M. J., Beyer, W. H., Eds.; CRC Press: Boca Raton, FL, 1989. We modeled the adsorbed detergent as a liquid film of varying thickness and an index of refraction of 1.45. The introduction of the SAM of alkanethiolates as an additional layer in the calculations had negligible effects on the magnitude of the calculated changes in Θ_m and was therefore omitted for simplicity. We note that the calculated dependence of Θ_m on refractive index (108° shift in Θ_m per unit of refractive index) compares favorably with the value given by an experimental measurement (111° per unit of refractive index, ref 14). The calculated shift in Θ_m due to an adsorbed film with a refractive index of 1.45 is 0.071°/nm of film thickness.

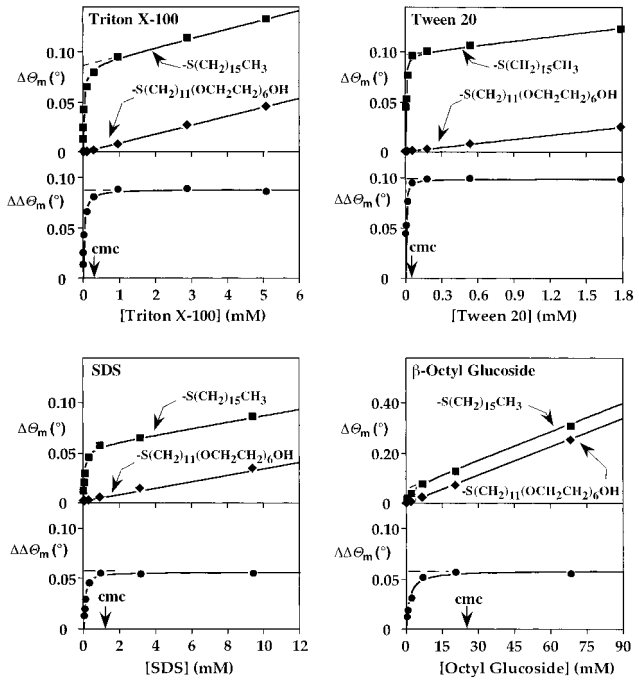

Figure 4. Curves at the top of each graph show the equilibrium displacement in Θ_m when buffer containing Triton X-100, SDS, β-octyl glucoside, or Tween 20 at increasing concentration was passed through the cell for SAMs presenting either methyl groups or hexaethylene glycol groups. The data for SAMs presenting EG_6OH groups were fit to a line intersecting the origin. The data for the HDT surface were fit to the sum of a line intersecting the origin and a Langmuir binding isotherm. The curve at the bottom of each graph plots the difference in the equilibrium values of $\Delta\Delta\Theta_m$ for the two SAMs; the difference represents that amount of detergent that is *associated* with the SAM. These data were fit to a Langmuir binding isotherm (eq 3).

OH groups. We subtracted the contribution due to the refractive index of the bulk solution by calculating the difference between the curves measured over the two surfaces. The corrected association and dissociation curves for the four detergents show that SDS and octyl glucoside dissociated completely from the hydrophobic surface in less than 10 s. The dissociation of Triton X-100 and Tween 20, however, was slow enough to observe on the time scale of the experiment. We note that these dissociation rate constants probably underestimate the true dissociation rate constants, because detergent molecules that have desorbed can again adsorb to the surface before being swept outside of the flow cell with the buffer.[18]

Binding Isotherms for the Adsorption of Detergents on Hydrophobic SAMs. Figure 4 shows the dependence of the equilibrium value of $\Delta\Theta_m$ for association of the detergents with SAMs of HDT and EG_6OH on the concentration of detergent. The SPR response obtained by treating the SAM of HDT with solutions of the detergents had two independent components due to the superposition of the association of detergent with the SAM and the "bulk" effect; at low concentrations of detergent, the signal is dominated by the adsorption of detergent, and at high concentrations of detergent the signal is dominated by the refractive index of the solutions of detergent (as in Figure 1). Subtraction of the data obtained with the EG_6OH surface from that with the HDT surface gives a binding isotherm that is due only to association of the detergent with the SAM; the isotherm approaches a saturating value for adsorbed detergent at high concentrations of detergent (Figure 4).

Table 2. Parameters That Characterize the Association of Detergents with SAMs of HDT[a]

detergent	cmc (mM)[b]	K_d (mM)[c]	cmc/K_d	Γ_{sat} (pmol/cm2)[d]	mol area (Å^2)[e]
SDS	1.0	0.13	7.7	280	59
octyl glucoside	25	2.6	9.6	260	64
Triton X-100	0.30	0.058	5.1	170	97
Tween 20	0.059	0.0068	8.7	120	140

[a] The association of these four detergents with a SAM of hexadecanethiolate was studied as described in the text. [b] The values of cmc are taken from ref 2. [c] Values of K_d were obtained by fitting the experimental data to eq 2 (after correcting for the refractive index in the bulk solution). [d] Values were obtained using eq 6. [e] The molecular area refers to the average surface area per detergent molecule at saturation and was calculated from Γ_{sat}.

We found that the binding isotherm was described well by the Langmuir equation:

$$\Delta\Theta_m = \Delta\Theta_m{}^{sat}\frac{c}{K_d + c} \qquad (2)$$

where c is the concentration of detergent in solution, K_d is the effective dissociation constant for the detergent from the surface, and $\Delta\Theta_m{}^{sat}$ is the limiting value of $\Delta\Theta_m$ due only to adsorbed detergent on the surface (and not the "bulk" effect). We note that the adsorption of detergent to the surface is not a Langmuir process (there is most likely cooperativity in the adsorption of detergent molecules), but this model describes the data well, and it provides a useful empirical method for comparing data obtained for different detergents and under different conditions. Table 2 gives the values of Γ_{sat} and K_d measured for each detergent.

In practice, it was not necessary to collect data over the EG_6OH surface in order to correct for the bulk refractive index. R_s could be determined directly from the data for the association of detergent with the SAM of HDT by a least-squares fit of the data in the linear portion of the plots (i.e., [detergent] > cmc). The values of R_s determined in this fashion differed from the values of R_s determned over the SAM of EG_6OH by < 5%. Once R_s was known, we could apply a linear correction to the SPR data as shown in eq 3.

$$\Theta_m(\text{adsorption}) = \Theta_m(\text{measured}) - cR_s \qquad (3)$$

Density of Detergent Molecules Associated with the SAM. The number of adsorbed detergent molecules per unit of surface area under saturating conditions (Γ_{sat}, in units of pmol/cm^2) can be calculated according to eq 4[19]

$$\Gamma_{sat} = 0.1\frac{d_{sat}(n_f - n_s)}{R_n} \qquad (4)$$

where n_f is the refractive index of the close-packed film (with an assumed value of 1.45), n_s is the refractive index of the buffer solution in the absence of detergent (1.335), d_{sat} is the thickness (nm) of the film, and R_n is the incremental change in the refractive index of the solution with detergent concentration (mM^{-1}).

We calculated the dependence of Θ_m on d for films with refractive index of $n_f = 1.45$ in a solution with refractive index of $n_s = 1.335$.[17] For thin (<50 nm) films Θ_m is proportional to d. The proportionality constant, which we name R_d, has a value of 0.071 deg/nm (eq 5).[17]

(18) We also tested the stability of films of Tween 20 by *ex situ* ellipsometry. SAMs of HDT were treated for 3 min with a solution containing Tween 20 at a concentration of 2 mM in PBS. The films were then washed briefly with distilled water (~5 s). Ellipsometry showed no detergent remaining on the surface.

(19) de Feijter, J. A.; Benjamins, J.; Veer, F. A. *Biopolymers* **1978**, *17*, 1759.

$$\Delta\Theta_m = R_d d = (0.071°/\text{nm})\ \text{d}, \quad \text{for } (n_f - n_s) = 0.1154 \tag{5}$$

Combining eqs 1 and 5 with eq 4 gives Γ_{sat} (in units of pmol/cm^2) as a function of the values of $\Delta\Theta_m^{sat}$ and R_s that were determined experimentally (eq 6).

$$\Gamma_{sat} = 0.1 \frac{\Delta\Theta_m^{sat}}{R_d}(n_f - n_s)\frac{108°}{R_s} \tag{6}$$

Table 2 gives the experimentally determined values of Γ_{sat}, as well as literature values for the cmc, for each detergent. The cmc values of the detergents investigated in Table 2 were approximately 7 times greater than the measured values of K_d (±30%).

Effect of Ionic Strength on the Adsorption of SDS. We measured the binding isotherms for adsorption of SDS to SAMs of HDT using phosphate buffers (5 mM) containing different concentrations of sodium chloride (from 0 to 150 mM). For each buffer, samples containing increasing concentrations of SDS—from 0 to 3.6 mM—were allowed to flow through the cell, separated by periods of buffer. The experiment was repeated over a SAM presenting EG_6-OH groups to correct for changes in the refractive index of the bulk solution. The corrected equilibrium displacement in Θ_m is plotted against the concentration of SDS in Figure 5. The data were fit to eq 2 as described earlier to afford equilibrium dissociation constants for SDS in each buffer. The apparent dissociation constant of SDS for the SAM decreased with the ionic strength of the solution. This correlation has been observed previously for the localization of SDS at the air−water interface and has been ascribed to shielding of the charged head groups by salts in the solution.[20,21] The quantity $7K_d$ was plotted against the concentration of sodium ion; comparison of these data to values of cmc for SDS obtained from the literature[22] show that the empirical relationship cmc ≈ $7K_d$ provides a good estimate for the cmc of the detergent (Figure 5b). The molecular area of detergent molecules on the surface at saturation (55−58 Å^2), however, is not strongly affected by ionic strength for solutions containing sodium ions at concentrations greater than 5 mM. This result contrasts with radiotracer studies of the adsorption of tritiated SDS at the air−water interface that show a difference in the molecular area at high and low concentrations of salt (40 Å^2 at [Na^+] = 0 mM and 52 Å^2 at [Na^+] = 115 mM); the molecular areas at intermediate concentrations of sodium ion were not determined by the radiotracer study.[20]

Desorption of Adsorbed Protein with Detergents. We determined the ability of SDS to remove fibrinogen adsorbed to a SAM of HDT. A layer of fibrinogen was adsorbed to the SAM by allowing a solution of the protein (1 mg/mL in PBS; 2.5 μM) to flow through the cell for 7 min; when the protein-containing solution was replaced with buffer, the protein did not dissociate from the SAM (Figure 6A). When a solution containing SDS at the cmc (1 mM) in PBS was subsequently passed through the cell, the protein dissociated slowly from the SAM. Subtraction of the component of the signal due to the bulk solution (obtained by repeating the experiment on a SAM presenting EG_6OH groups) from these data shows the change in $\Delta\Theta_m$ due only to desorption of protein from the surface (Figure 6B). SDS at a concentration equal to the cmc led to a slow ($t_{1/2}$ ~ 100 s, assuming first-order kinetics) and incomplete (~50% completion) desorption of adsorbed fibrinogen. By contrast, a solution containing SDS at a concentration of only three times greater than the cmc almost completely (>90%) desorbed the protein layer in <20 s; a solution containing SDS at a concentration three times less than the cmc had no effect on the protein layer.

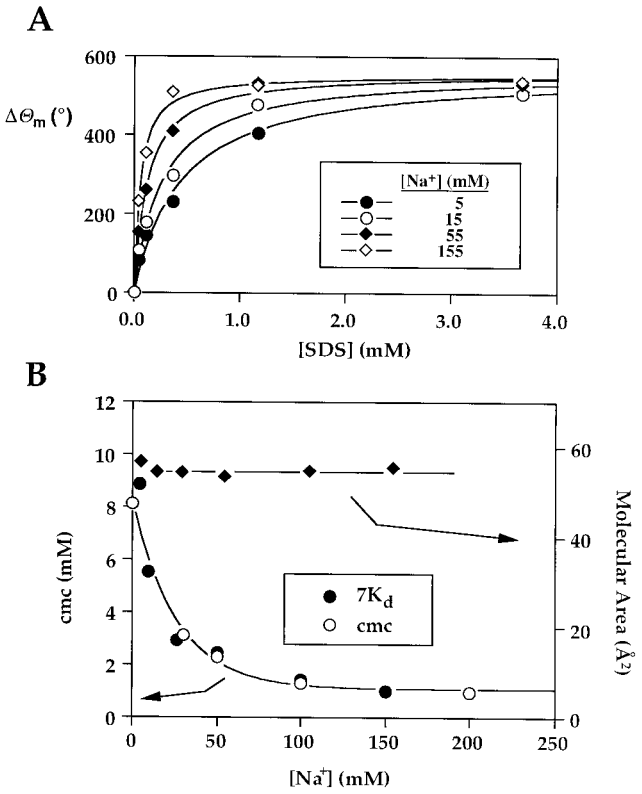

Figure 5. (A) The effect of ionic strength on the adsorption of SDS to SAMs of HDT. The ionic strength of a phosphate buffer (5 mM NaH_2PO_4, pH 7.2) was increased by adding sodium chloride (0−150 mM). For each buffer, samples containing increasing concentrations of SDS in the same buffer were allowed to flow through the cell; the equilibrium displacement in Θ_m is plotted versus the concentration of SDS in the solution. The curves were obtained by fitting these data to eq 3 after correcting for changes in the refractive index by subtracting the displacements measured for identical experiments on SAMs presenting EG_6OH groups. (B) The values of K_d obtained from fitting the data were used to plot the quantity $7K_d$ versus the concentration of sodium ion in the buffer (closed circles); values obtained from the literature for the cmc of SDS in the presence of NaCl are represented by the open circles (ref 23). Values for the molecular area (diamonds) at the limiting density of SDS on the surface show that the final density of detergent molecules is independent of ionic strength.

Discussion

We have previously described the combination of SPR and SAMs as an experimental system with which to study the adsorption of proteins on surfaces.[16] The present work shows that this system is also well suited for studies of the interactions of small molecules (MW ~ 300) with surfaces. The commercial instrument used in this work can measure changes in Θ_m down to 0.0005°; for the association of SDS with a SAM of HDT, this value corresponds to a sensitivity of ~700 pg/cm^2, that is, ~15 000 molecules/μm^2, or less than 1% of a monolayer. SPR has several other advantages as an analytical technique: it is a nondestructive technique that provides information about association under a variety of solution conditions; it provides both thermodynamic and kinetic information; it uses thin films of gold and is compatible with the extensive body of information concerning SAMs of alkanethiolates on gold.

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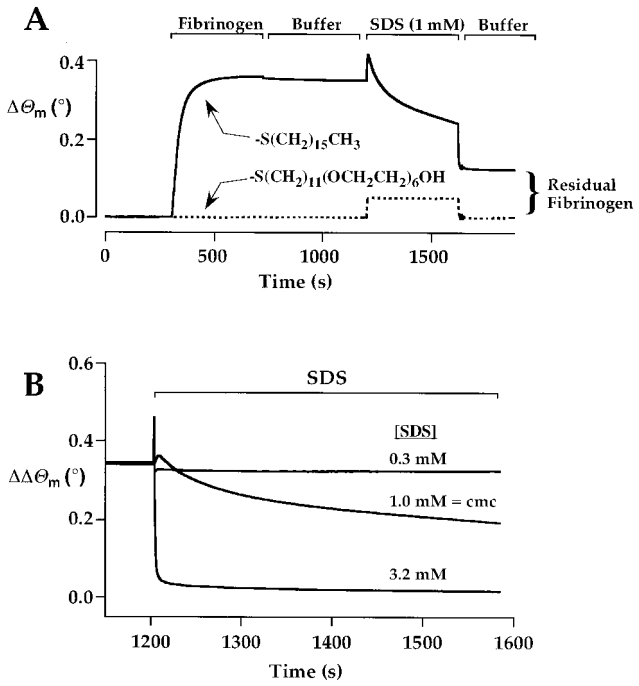

Figure 6. Dissociation of fibrinogen adsorbed to a SAM of HDT in the presence of SDS. (A) PBS was allowed to flow over the SAM for 5 min, replaced with a solution of fibrinogen (1 mg/mL) in PBS for 7 min, and then replaced with PBS for 5 min. A solution of SDS (1.0 mM) in PBS was then allowed to flow over the surface with its adsorbed film of fibrinogen for 7 min. The dashed line shows the data obtained using a SAM presenting EG_6OH groups. The vertical scale provides a relative comparison: the curves are offset vertically for clarity. (B) The difference in $\Delta\Theta_m$ for the two SAMs is shown for the region of the response curve during which SDS was present in the buffer and fibrinogen dissociated from the SAM.

Table 2 gives the measured values of K_d, Γ_{sat}, and the molecular area of adsorbed detergent for four commonly used detergents on the SAM of HDT. The behavior of all the detergents on this SAM was described well by eq 2. The molecular surface area of SDS groups at saturation in the presence of 150 mM NaCl (~59 Å^2) is considerably less dense than would be expected for a tightly packed monolayer (~28 Å^2, based on the cross-sectional area of a hydrated sulfate ion;[21] ~21 Å^2 based on the cross-sectional area of HDT in the SAM);[23] this result, however, is consistent with the values determined for other hydrophobic surfaces using radiolabeling and *in situ* ellipsometry (40 and 68 Å^2 for SDS at the air−water interface and on the surface of methylated silica, respectively).[20,24] The large molecular surface area at saturation suggests that the detergent is present on the surface in a fluid-like phase, rather than as a highly ordered crystalline phase; we presume that the short length of the alkyl chain (C_{12}) and the charge−charge repulsion of sulfate groups make a closer packing unfavorable. Similarly, the size and flexibility of the head groups on the nonionic detergents that were tested make the formation of tightly packed monolayers of those detergents unfavorable.

The data show that detergent molecules associate with the surface of a monolayer of HDT at concentrations significantly below the cmc; since the SAM presents a large, hydrophobic surface, it is intuitively reasonable that aggregation should occur at lower concentrations of SDS in solution than that required to nucleate formation of a micelle. Association of SDS with the SAM of HDT follows a Langmuir adsorption isotherm; this behavior is in contrast to the highly cooperative formation of micelles. The Langmuir model may not be completely correct, since it completely discounts the interactions between detergent molecules on the surface; nonetheless, the data are described well by this model, and the apparent association constants are useful for comparing the association of different detergents with surfaces under different conditions.

Estimation of Critical Micellar Concentration. We found an empirical relationship between the cmc of the detergents and the measured values of K_d for dissociation from the hydrophobic SAM (cmc $\approx 7K_d$). The applicability of the empirical relationship to variety of detergents in solutions of different ionic strengths indicates that the shape of the binding isotherm is relatively constant under the range of conditions that were tested. The relationship cmc $\approx 7K_d$ may be useful to predict the cmc of the detergents within a factor of 2, which is sufficient for many applications. The range in the ratios of cmc/K_d could also be due to imprecision in the reported values of the cmc, because measured values of the cmc can be strongly influenced by the technique used to measure the cmc, by the conditions under which the measurement was made (ionic strength, temperature, etc.), and by the presence of impurities in the detergent solutions.[2]

SAMs Presenting Hexaethylene Glycol Groups Resist Association of Detergents. The observation that SAMs terminated in EG_6OH groups resist association with detergents agrees with a previous study that used *in situ* ellipsometric measurements on wettability gradient surfaces to show that uncharged hydrophilic surfaces do not adsorb detergent molecules.[8] SAMs presenting oligoethylene glycol groups also resist the adsorption of protein;[25] the mechanism for this resistance is not presently known. We have not explored the basis for these same SAMs to resist association with detergents. Further studies that investigate the association of detergents with SAMs that present other hydrophilic groups (hydroxyl, carboxy, amido, etc.) will help elucidate the factors that cause surfaces to resist association with detergents.

Desorption of Proteins from SAMs in the Presence of SDS. Figure 6B shows that the ability of SDS to remove fibrinogen adsorbed to the SAM depends sensitively on the concentration of the detergent. SDS at a concentration three times greater than the cmc removes the adsorbed protein in less than 5 s, whereas a concentration of SDS at one-third that of the cmc does not remove any fibrinogen even after several minutes. These results suggest that the removal or displacement of protein from the surface requires the formation of aggregates of detergent or aggregates of protein and detergent. Both the activity of SDS molecules and the ability of SDS to form a monolayer on a SAM of HDT are already near (or at) their maximum values for concentrations of detergent at the cmc; it is therefore unlikely that the sharp increase in rates of desorption for concentrations of detergent above the cmc can be explained by a mechanism that involves only the competition of single molecules of SDS with protein for association with the hydrophobic surface. These results do not, however, distinguish between the kinetic involvement of complete micelles, smaller aggregates, or individual molecules of detergent in the displacement.

An interesting feature of the desorption curve in the presence of 1 mM SDS is the initial increase in Θ_m on introduction of the solution of detergent. This change must represent the association of detergent with the adsorbed protein molecules *prior* to desorption from the

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surface. This effect suggests that SPR may be useful in studying protein−detergent interactions (using proteins that are covalently immobilized at the surface of the SAM, for example). We note that the results reported here are similar to those observed on a different model system: proteins adsorbed on a methylated silica surface, with desorption studied by ellipsometry.[26]

Experimental Section

Materials. All materials and reagents were used as received. Phosphate-buffered saline (P3813) and fibrinogen (F4883; 94% clottability) were purchased from Sigma. Electrophoresis grade detergents—sodium dodecyl sulfate (BioRad), β-octyl glucoside (Sigma), Triton X-100 (Fisher) and Tween 20 (Sigma)—were used in all the studies. Hexadecanethiol was purchased from Aldrich and purified by silica gel column chromatography using 19:1 hexanes/ethyl acetate as the eluent; the hexa ethylene glycol terminated alkanethiol was synthesized as described previously.[27] All buffers and solutions of detergents and fibrinogen were filtered through 0.45 μm filters immediately before use.

Surface Plasmon Resonance Spectroscopy. We used the BIACore instrument (Pharmacia) for all studies described here. We modified the manufacture's cassettes to accept our substrates as described previously.[16,28] Briefly, substrates were prepared by evaporation of titanium (1.5 nm) and gold (39 nm Au) onto glass cover slips (0.20 mm, No. 2, Corning, refractive index = 1.52). The metalized substrates were cut into squares 1 cm^2 in size, immersed in solutions of hexadecanethiol or $HS(CH_2)_{11}-(OCH_2CH_2)_6OH$ in ethanol (2 mM thiol) for 1 h, rinsed with ethanol, and dried with nitrogen. The substrates were glued into BIACore cassettes with a two-part epoxy (Devcon). Special care was taken to prevent artifacts due to accumulation of air bubbles or hydrophobic impurities at the hydrophobic SAMs. Prior to each set of experiments, the fluidics of the SPR instrument were cleaned with a solution of SDS according to the manufacturers instruction. All buffers and samples were degassed under vacuum.

Refractometry. We used an Abbe refractometer (Bausch and Lomb) to measure the refractive index of solutions containing varying concentrations of the detergents—up to 10% (w/v)—in PBS. R_n was determined from a plot of the refractive index versus the concentration of detergent by calculating the slope of the best fit line through the data.

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(28) The manufacturer of the SPR instrument used in this study (Pharmacia Biosensor) now provides substrates that are modified with a SAM of octadecanethiolate; these substrates are functionally identical to the SAMs of HDT used here.