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Abstract: This report describes the use of surface plasmon spectroscopy to study the effect of surface wettability on the nonspecific adsorption of proteins and detergents to self-assembled monolayers (SAMs) of alkanethiolates on gold. The adsorption of both proteins and detergents to uncharged SAMs showed a general dependence on the wettability of the surface as determined by the contact angle of water on the SAM under cyclooctane (θ_{co}). The effect of the wettability of the SAMs on the adsorption of sodium dodecyl sulfate (SDS) was dependent on whether micelles were present. Above the critical micelle concentration (cmc), SDS adsorbed only on surfaces that gave contact angles with values of $\cos \theta_{co} < 0$ (i.e., the transfer of the surface from water to cyclooctane has a favorable free energy). Below the cmc, the requirement for adsorption was much more stringent: SDS adsorbed only on the surfaces that gave values of $\cos \theta_{co} < -0.9$. Similarly, the effect of the wettability of the SAMs on the adsorption of proteins showed a dependence on the size of the proteins. The smaller proteins tested (ribonuclease A and lysozyme) adsorbed only on the least wettable surfaces tested ($\cos \theta_{co} < -0.83$). The larger proteins tested (pyruvate kinase, fibrinogen, and γ-globulin) also adsorbed best to the least wettable surfaces, but adsorbed to some extent on almost all the surfaces; the single exception was a SAM presenting hexa(ethylene glycol) groups at the surface, to which no protein adsorbed. Films of adsorbed proteins were desorbed from the SAMs by treatment with detergent.

Introduction

This report describes the use of surface plasmon resonance spectroscopy (SPR) to study the effect of surface wettability on the nonspecific adsorption of proteins and detergents to self-assembled monolayers (SAMs) of alkanethiolates on gold. Control over the nonspecific adsorption of proteins to surfaces is fundamentally important in technologies that involve the contact of synthetic surfaces with biological fluids. Examples include (i) sensitive solid-phase immunoassays that retain selectivity even in the presence of high concentrations of serum proteins,[1] (ii) biochemical separations using media that are resistant to biofouling,[2] (iii) surgically implanted prostheses that are biocompatible,[3] and (iv) solid-phase supports for the growth of adherent cells.[4]

Although nonspecific protein adsorption is complex and not well understood, it can be discussed in terms of two limiting mechanisms: adsorption by charge−charge interaction and adsorption by hydrophobic interaction (a combination of these two effects may, of course, occur). The properties of a surface required for the hydrophobic adsorption of proteins have been the subject of many studies, as well as periodic reviews.[5−9] Perhaps the most careful of the studies has been the work of Elwing et al.[10,11] These studies used gradients of wettability generated on glass slides by the diffusion of dichlorodimethylsilane vapor along the length of the slide. Proteins and detergents were then adsorbed onto the slides, and the amount of adsorbed protein (as determined by ellipsometry) was plotted against the wettability of the surface. For negatively charged proteins, the amount of adsorbed protein increased rapidly with the increasing contact angle of water; nonionic and negatively charged detergents did not adsorb to the more hydrophilic surfaces.

One limitation of these studies was that an unmodified glass surface is negatively charged at physiological pH. It was, therefore, difficult to separate the binding caused by hydrophobic interactions of proteins with the surface from that caused by electrostatic interactions; positively charged proteins adsorbed over the entire wettability gradient. Other studies of protein adsorption as a function of the wettability of the surface have relied on even less well defined surfaces (for example, treated polymer surfaces).[12,13]

Detailed studies of protein adsorption require a system of molecularly well-defined surfaces. SAMs of alkanethiolates on gold are the best model surfaces now available for these types of studies:[14−16] (i) SAMs of alkanethiols on gold are well

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(2) Kessler, G. H.; Lund, D. B. In Kessler, G. H., Lund, D. B., Eds.; Prien Chiemsee: 1989.
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(4) Schakenraad, J. M.; Busscher, H. J. *J. Colloids Surf.* **1989**, *42*, 331−339.
(5) Andrade, J. D.; Hlady, V. *Adv. Polym. Sci.* **1986**, *79*, 1−63.
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(8) Sadana, A. *Chem. Rev.* **1992**, *92*, 1799−1818.
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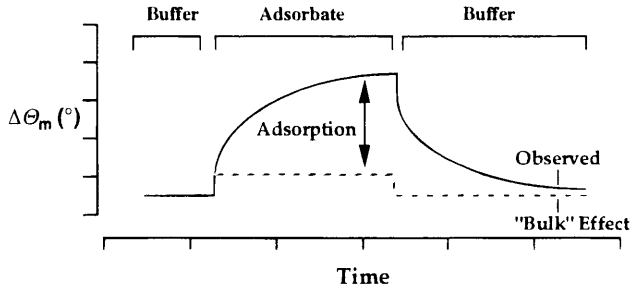

Figure 1. Hypothetical plot illustrating an SPR experiment for the reversible adsorption of an analyte to the sensing surface. SPR records the angle of minimum reflectivity of incident light versus time. In this example, buffer is allowed to flow through the cell, replaced by a solution containing the 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 Θ_m due to the "bulk" effect of increasing the refractive index of the buffer by dissolving analyte. The difference between the observed signal and the signal due to the bulk effect is the signal due to the adsorption of the analyte on the surface.

characterized,[17-19] (ii) the character of the surface can be controlled by using linear alkanethiols terminated with functional groups, and (iii) thin gold films as substrates allow SPR to be used as an analytical tool for measuring protein adsorption.[15] In this paper, we describe the preparation of uncharged model surfaces by the formation of SAMs consisting of alkanethiolates presenting functional groups varying in polarity. We demonstrate that the propensity of these surfaces to adsorb proteins and detergents is related (with important exceptions) to the interfacial free energy of these surfaces *under water*.

Surface Plasmon Resonance Spectroscopy. We employed SPR to measure the adsorption of proteins and detergents to SAMs. SPR is an optical technique that measures changes in the refractive index of the medium near 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 backside 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.[20] The value of Θ_m shifts with changes in the refractive index of the interfacial region near the surface of the gold (within approximately one wavelength of the incident light). For thin (<100 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.[21]

Because SPR measures changes in the index of refraction of the medium within ~200 nm of the surface, it is sensitive both to molecules adsorbed at the interface and to molecules dissolved in the medium. This latter 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.

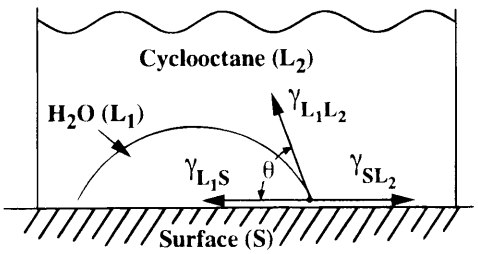

Figure 2. Schematic representation of a drop of water (L_1) in contact with surface (S) and cyclooctane (L_2) with contact angle θ. The vectors represent the balance of surface tensions at the edge of the drop.

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 signal due to analyte that *adsorbs* to the interface is the *difference* between the two curves.

Results and Discussion.

Measurement of the Wettability of SAMs of Alkanethiolates on Gold. Much of the previous work on the effect of surface wettability on the adsorption of proteins has used the contact angle of water under air as an index of wettability. Because contact angles under air are dominated by the interfacial free energies of the solid–air interfaces[22] (an energy term that does not influence protein adsorption occurring in aqueous solution), they do not provide an appropriate wettability scale for studies of protein adsorption. Figure 2 is a schematic representation of the surface forces acting on a drop of water (L_1) on a surface (S) immersed in an organic solvent (L_2). The contact angle under organic solvent (θ_s) is related to the interfacial free energies (γ) according to Young's equation (eq 1).[23] The measurement of contact angles of water under an

$$\gamma_{SL_2} - \gamma_{L_1S} = \gamma_{L_1L_2} \cos \theta_s \quad (1)$$

organic solvent gives a parameter, $\cos \theta_s$, that is proportional to the free energy of transferring the surface from water to the organic solvent. To a rough approximation, this process could be considered analogous to the replacement of water at the surface with a layer of adsorbed protein (i.e., a protein molecule containing hydrophobic groups is modeled crudely as a drop of organic solvent). The values of $\cos \theta_s$ for different surfaces, therefore, should provide a scale for the comparison of the energetics of protein binding to different surfaces.

We prepared SAMs of alkanethiolates on gold by the adsorption of alkanethiols from ethanolic solution. Table 1 lists the thiols used in this study and the functional groups displayed at the surface of the corresponding SAMs; throughout the paper, we use the structure of the terminal functional group to represent the SAM prepared from a given thiol. Table 1 also lists the values we measured for the advancing contact angle of water on the SAMs under cyclooctane (θ_{co}) and under air (θ_{air}). Despite the absence of charge on the functional groups presented on the surface, the wide range of values of θ_{co} indicates that the different SAMs covered a wide range of wettability. Figure

(15) Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*,.
(16) Mrksich, M.; Whitesides, G. M. *Trends Biotechnol.* **1995**, *13*, 228–235.
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Table 1. Contact Angle of Water on SAMs of Alkane Thiols on Gold under Cyclooctane and Air[a]

surface moiety	thiol	θ_{co} (deg)[b]	cos θ_{co}	θ_{air} (deg)[c]	cos θ_{air}
$-CH_3$	$HS(CH_2)_{10}CH_3$	165	−0.97	112	−0.37
−OPh	$HS(CH_2)_{11}OPh$	156	−0.91	85	0.09
$-CF_3$	$HS(CH_2)_2(CF_2)_9CF_3$	154	−0.90	118	−0.47
−CN	$HS(CH_2)_{11}CN$	146	−0.83	63	0.45
$-OCH_3$	$HS(CH_2)_{11}OMe$	106	−0.28	85	0.09
$-CONHCH_3$	$HS(CH_2)_{10}CONHMe$	94	−0.07	76	0.24
−OH	$HS(CH_2)_{11}OH$	65	0.42	<15	>0.97
$-EG_6OH$	$HS(CH_2)_{11}(OCH_2CH_2)_6OH$	52	0.62	38	0.79
$-CONH_2$	$HS(CH_2)_{10}CONH_2$	20	0.94	<15	>0.97

[a] Advancing contact angles were measured in triplicate. The values of all the replicates were within ±3° of the mean. [b] Advancing contact angle of water under cyclooctane. [c] Advancing contact angle of water under air.

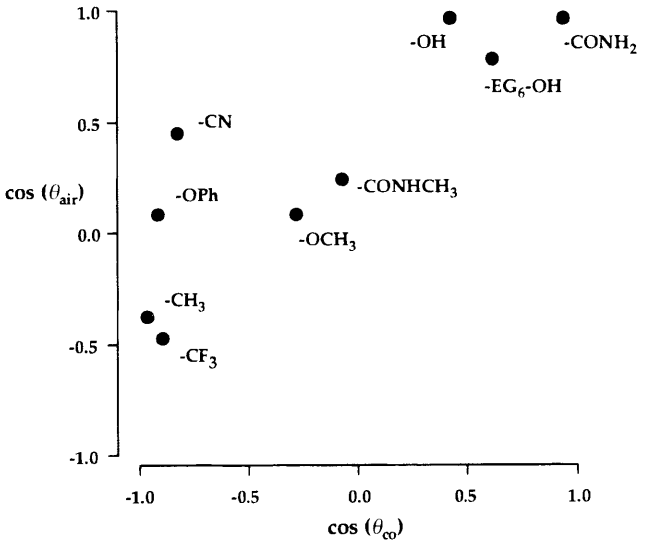

Figure 3. Advancing contact angles of water on the SAMs used in this study. The cosines of the values measured under air are plotted as a function of the values measured under cyclooctane. The plotted values are the averages of three measurements. All replicates were within ±0.05 of the mean.

3 compares the contact angles measured under air and cyclooctane. There are considerable differences observed in the ordering of the contact angles under air and cyclooctane: in particular, the $-OCH_3$ and $-CONHCH_3$ surfaces wet considerably better than the −CN surface under cyclooctane but worse under air. These differences show that interpreting interfacial phenomena in solution on the basis of contact angles under air requires caution.

Effect of the Wettability of the SAMs on the Adsorption of Detergents. The presence of hydrophobic groups on a surface should be reflected in its ability to interact with small hydrophobic molecules in aqueous media; this interaction provides the basis for reverse phase chromatography.[24] We examined the partitioning of detergent molecules between the solution and the surface of the SAMs. The detergent chosen for this study was sodium dodecyl sulfate (SDS), an anionic detergent with a high critical micelle concentration (cmc = 1.0 mM). The adsorption of SDS to the SAMs was examined by SPR; the procedure has been described in a separate report.[25]

Figure 4 shows the response we observed using SPR when the SAMs presenting CH_3, OCH_3, OH, and EG_6OH groups were treated with solutions containing SDS. During these experiments, phosphate-buffered saline (PBS) was passed over the surfaces. This flow was periodically replaced with solutions of the same buffer containing detergent at increasing concentrations, each for a period of 3 min. We report values of $\Delta\Theta_m$ ($\Delta\Theta_m = \Theta_m - \Theta_m^\circ$), which is the *change* in Θ_m during the experiment (relative to the clean surface in PBS at the start of the experiment). We have previously shown that the SAM presenting EG_6OH groups resists the adsorption of these detergents;[25] the values of $\Delta\Theta_m$ observed on treatment of this surface with detergent are due to the increase in the refractive index of the bulk solution on addition of the detergent. Subtraction of the curves obtained on the surface presenting EG_6OH groups from the curves obtained for the other surfaces gives the shift in resonance angle caused only by the adsorption of detergent on the surfaces. We use the superscript c (as in $\Delta\Theta_m^c$) to refer to values of resonance angles that have been corrected in this manner.

The number of adsorbed detergent molecules per unit of surface area (Γ, in units of pmol/cm^2) can be calculated according to eq 2,[26] where n_f is the refractive index of the close-

$$\Gamma = 0.1[d_{sat}(n_f - n_s)]/R_n \quad (2)$$

packed film (with an assumed value of 1.45), n_s is the refractive index of the buffer solution in the absence of detergent (1.3346), d_{sat} is the thickness of the film (in nm), and R_n is the incremental change in the refractive index of the solution with the concentration of SDS (3.1×10^{-5} mM^{-1}).[25] For thin (<100 nm) films of refractive index 1.45, the dependence of $\Delta\Theta_m^c$ on d is linear and described by eq 3.[27] Combining eqs 2 and 3 gives Γ as a function of the experimentally determined value of $\Delta\theta_m$ (eq 4).[28]

$$\Delta\Theta_m^c/d = 0.071 \text{ deg/nm} \quad (3)$$

(24) Bidlingmeyer, B. A. *Practical HPLC Methodology and Applications*; Wiley: New York, 1992.

(25) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *Langmuir* **1997**, *13*, 2749−2755.

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

(27) 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 et al.: 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 organic film) between two semi-infinite media (glass and solution). The complex indices of refraction for the gold (0.17 + 4.93i) and glass (1.511) were taken from ref 21. The index of refraction of the buffer (1.3346) 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.

(28) As described in ref 26, the value of the surface excess as calculated by eq 4 is relatively insensitive to errors in the assumed value of the refractive index for the organic film. The actual value may vary from 1.4 to 1.5 without leading to errors in the calculated surface error of more than ±5%.

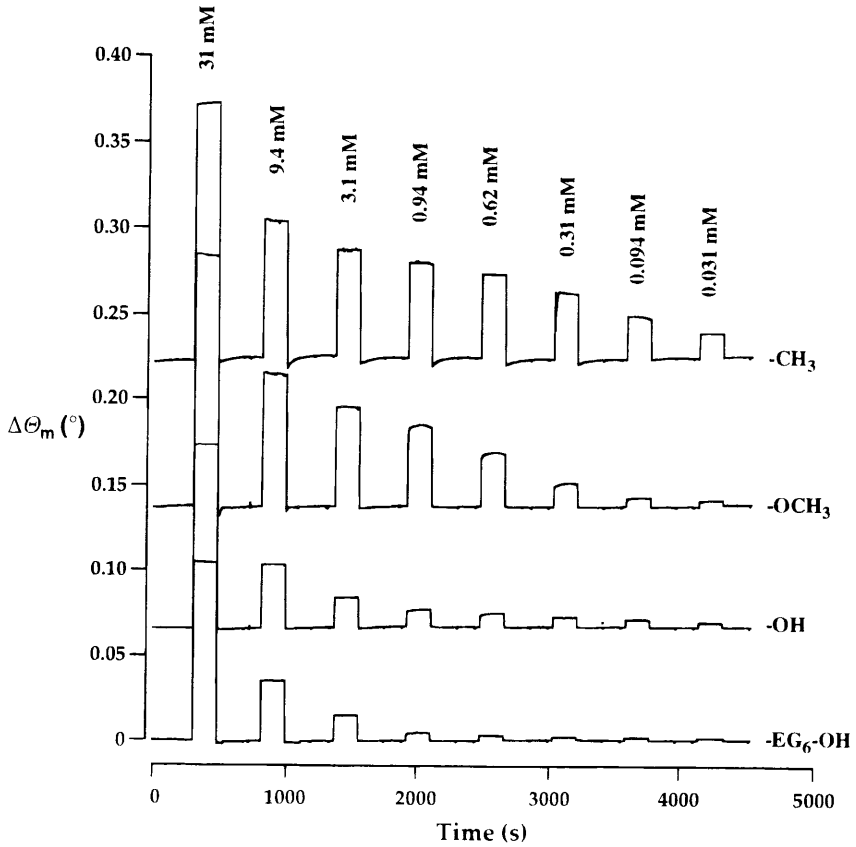

Figure 4. SPR response on the passage of solutions containing varying concentrations of SDS over SAMs presenting $-CH_3$, $-OCH_3$, $-OH$, or $-EG_6OH$ groups. Solutions of the detergent in PBS were passed over the surfaces at a flow rate of 10 μL/min. The surface was washed with PBS between each injection of the solution of detergent. The concentration of detergent in each injection is listed at the top of the graph.

$$\Gamma = \frac{\Delta\Theta_m^c}{0.071 \text{ deg/nm}} \frac{(n_f - n_s)}{R_n} \quad (4)$$

Figure 5 shows a plot of $\Delta\Theta_m^c$ and Γ versus the concentration of SDS passed over the surfaces presenting CH_3, OCH_3, and OH groups. The most wettable surface ($-OH$) adsorbed only small (although measurable) amounts of SDS even at very high concentrations of SDS in solution. We have not yet established whether this adsorption is intrinsic to a surface presenting OH groups or whether it represents hydrophobic defects in the SAM. The binding isotherms for the $-CH_3$ and $-OCH_3$ surfaces were characterized by a transition at the cmc. For concentrations of SDS above the cmc, these two surfaces behaved similarly: (i) the surface concentration of adsorbed detergent was roughly comparable and (ii) the surface concentration of adsorbed detergent was independent of the concentration of detergent in solution (i.e., the surface concentration reached a maximum at the cmc). At concentrations of SDS below the cmc, the two surfaces behaved markedly differently. The binding isotherm for the $-CH_3$ terminated SAM showed a linear increase in adsorption with increasing concentrations of SDS; this surface adsorbed SDS at concentrations significantly below the cmc. In contrast, the $-OCH_3$ terminated SAM did not adsorb significant amounts of detergent until the concentration of SDS approached the cmc.

Figure 6 plots the limiting value of the surface concentration of SDS (Γ_{sat}) as a function of $\cos\theta_{co}$ for each surface listed in Table 1. Significant adsorption of detergent to the surfaces only occurred if $\cos\theta_{co} < 0$ (that is, $\theta_{co} > 90°$). The values of Γ_{sat} measured on the surfaces that adsorbed SDS ranged from 220 to 350 pmol/cm^2 and correspond to values of areas of surface occupied per molecule of SDS ranging from 48 to 76 Å^2. The packing density is, therefore, considerably less than would be expected for a tightly packed monolayer (~28 Å^2 per molecule of SDS, based on the cross-sectional area of a hydrated sulfate ion,[29] or ~21 Å^2 based on the cross-sectional area of alkanethiolates in a closely packed SAM[30]).[31] For the surfaces that adsorb SDS, there was no clear trend in the magnitudes of Γ_{sat}. The capacity of the surfaces to adsorb detergent may be a function of the packing geometry, as opposed to the wettability. Figure 6 also plots the surface concentration of adsorbed SDS that we observed when the concentration of SDS in solution was $c = 90\ \mu M \approx$ cmc/10 ($\Gamma_{1/10}$). The magnitude of $\Gamma_{1/10}$ provides an indication of the sharpness of the binding isotherm. At this low concentration of detergent, adsorption only occurred on the least wettable surfaces: $\cos\theta_{co} \leq -0.9$.

The drastic differences in the binding isotherms on SAMs having values of $\cos\theta_{co} \leq -0.9$ and SAMs having values of $\cos\theta_{co}$ of $-0.9 \leq \cos\theta_{co} \leq 0.0$ suggests that the adsorption of SDS on these two classes of surfaces proceeds by different mechanisms. The adsorption of SDS onto the least wettable SAMs ($\cos\theta_{co} \leq -0.9$) from solutions containing SDS at concentrations far below the cmc is consistent with the

(29) Tajima, K.; Muramatsu, M.; Sasaki, T. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1991–1998.

(30) Strong, L.; Whitesides, G. M. *Langmuir* **1988**, *4*, 546–558.

(31) The submonolayer coverage we observed is consistent with the values of molecular surface area determined for SDS adsorbed on other hydrophobic surfaces: Studies determining the density of SDS at the air–water interface by radiolabeling (Tajima, K. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3063–3066) and on the surface of methylated silica by in situ ellipsometry (ref 9) gave molecular surface areas of 40 and 68 Å^2, respectively. The large molecular surface area at saturation suggests that the detergent is present on the surface in a fluidlike 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 makes a closer packing unfavorable.

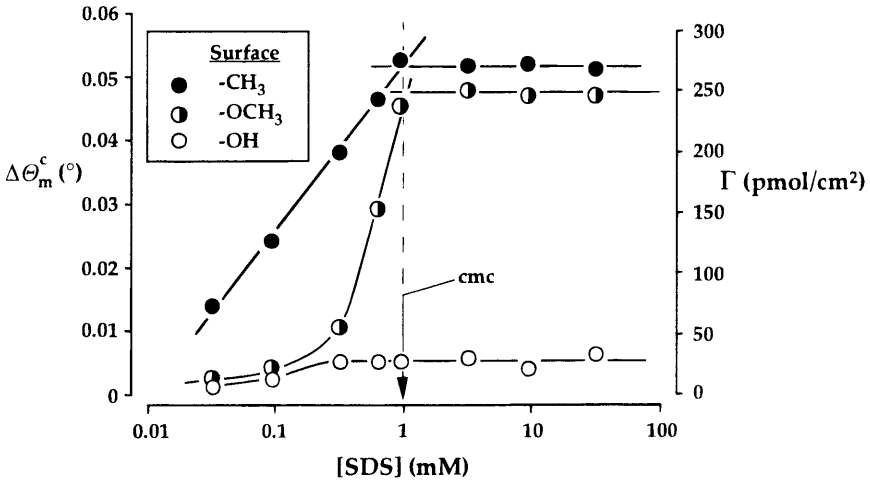

Figure 5. Adsorption isotherms for SDS on SAMs presenting surfaces with hydrophobic, hydrophilic, or intermediate character. The y axis on the left gives the SPR response (corrected for the effect of bulk refractive index). The y axis on the right gives the surface density of detergent molecules on the surfaces.

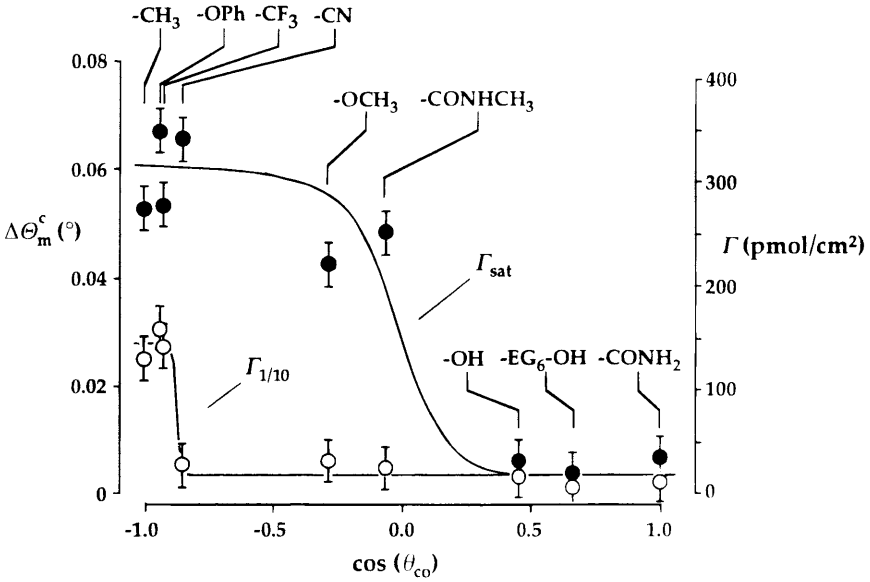

Figure 6. Adsorption of SDS to a variety of SAMs as a function of the contact angle of water on the SAMs under cyclooctane. The y axes on the left and right give, respectively, the SPR response due to the adsorption of SDS and the surface density of SDS molecules. Data are shown for the adsorption of detergent under saturating conditions (Γ_{sat}, filled circles) and from solutions containing SDS at concentration of 90 μM $\approx$ cmc/10 ($\Gamma_{1/10}$, open circles).

adsorption of individual detergent molecules on the surface. In contrast, the sharp transitions in the binding isotherms measured on the moderately hydrophobic SAMs ($-0.9 \leq \cos\theta_{co} \leq 0.0$) suggest a cooperative mechanism of adsorption on these surfaces; adsorption requires the formation of aggregates of SDS in solution or on the surface. We note that cooperative mechanisms have been postulated for the adsorption of detergent to charged hydrophilic surfaces (for example, the adsorption of hexadecyltrimethylammonium salts on mica and the adsorption of poly(ethylene glycol) alkyl ethers on silica).[32,33] There is, however, an important distinction between the results of these studies and our observations on uncharged SAMs: the interaction of the detergent headgroups with charged groups on the surface is an important driving force for the adsorption of detergent molecules on charged surfaces. In contrast to our results, the adsorption of detergent on charged surfaces occurs even when they are very hydrophilic and leads to surface densities of detergent molecules that are consistent with bilayer or multilayer films.

Effect of the Wettability of the SAMs on the Adsorption of Proteins. We used SPR to quantitate the adsorption of protein on the SAMs from solutions. We examined the six proteins listed in Table 2. Figure 7 shows the adsorption of the proteins to the surface presenting $-CH_3$ groups. The introduction of protein (0.05 mg/mL) to the solution flowing over a CH_3 surface leads to a shift in the value of $\Delta\Theta_m$ as the protein adsorbed on the surface. The shift has two components, a rapid increase due to the refractive index in bulk solution ($\sim$0.001$-$0.002° at this concentration of protein), followed by a slower, but much larger, increase corresponding to the adsorption of protein at the interface. On reintroduction of buffer to the flow cell, the value of $\Delta\Theta_m$ remained relatively constant; this observation indicates that the protein layer was kinetically stable. Figure 7 also shows the SPR signals resulting from the introduction of the proteins to the solution flowing over an EG_6OH surface, a surface known to resist the adsorption of protein.[14,15] In this experiment, only the shift in $\Delta\Theta_m$ due

(32) Chen, Y. L.; Chen, S.; Frank, C.; Israelachvili J. *Colloid Interface Sci.* **1992**, *153*, 244–265.

(33) Tiberg, F.; Jönsson, B.; Lindman, B. *Langmuir* **1994**, *10*, 3714–3722.

Table 2. Proteins Used in This Study

protein	source	MW (kD)[a]	pI	$h \times w \times l$ (nm)[b]	ref
RNAse A	bovine	14	9.5	3.8 × 2.8 × 2.2	*d*
lysozyme	chicken egg	14	11.1	4.5 × 3.0 × 3.0	*d*
serum albumin (BSA)	bovine	69	4.8	14 × 4 × 4	*c*
γ-globulins (BGG)	bovine	~170 (mixture)	~6.0	24 × 4.4 × 4.4	*c*
pyruvate kinase	rabbit muscle	237 (dimer)	8.9		*e*
fibrinogen	human	340 (tetramer)	5.5	47 × 5 × 5	*c*

[a] Molecular weight of the protein. [b] Approximate molecular dimensions of the protein. [c] Reference 33. [d] Shirahama, H.; Lyklema, J.; Norde, W. *J. Colloid Interface Sci.* **1990**, *139*, 177–187. [e] Ibsen, K. H.; Marles, S. W.; Lopez, T. P.; Wilson, S. E.; Basabe, J. R. *Int. J. Biochem.* **1976**, *7*, 103–106.

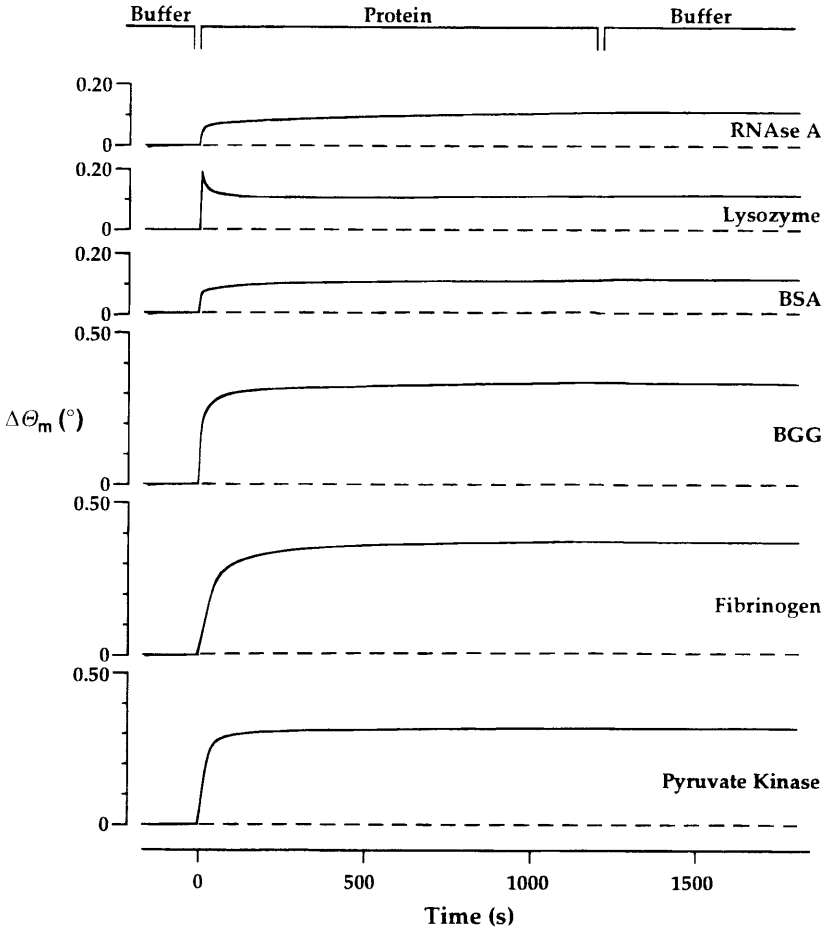

Figure 7. Kinetics of the adsorption of protein to SAMs presenting $-CH_3$ groups. PBS was passed over the SAMs at a flow rate of 10 μL/min. At time = 0 s, the solution was replaced with a solution containing the indicated protein at a concentration of 50 μg/mL in PBS. After 1200 s, PBS was reintroduced over the surfaces. The graphs show the SPR response as a function of time. The dashed line shows the SPR response on passage of the same solutions of protein over SAMs which resist the adsorption of protein ($-EG_6OH$).

to changes in the refractive index of the bulk solution was observed. Subtraction of the two curves gives the SPR response due to the adsorption of protein ($\Delta\Theta_m^c$).[34] The mass of protein on the surface can be determined from $\Delta\Theta_m^c$ using eq 4 (de Feijter et al. give the value of R_n for proteins as $R_n = 0.18$ mL/g;[26] using this value, eq 4 gives eq 5).

$$\Gamma\ (\text{ng/cm}^2) = 900\ (\text{ng/(deg cm}^2)) \times \Delta\Theta_m^c\ (\text{deg}) \quad (5)$$

Figure 8 gives the concentrations of proteins on the surfaces when the adsorption was allowed to proceed to completion (20 min) from relatively high concentrations of protein (1 mg/mL). All the samples showed the expected trend; more protein adsorbed on the less wettable surfaces. Table 3 compares the measured values of Γ for the adsorption of the proteins onto the SAM presenting $-CH_3$ groups with the theoretical value for a complete monolayer of protein;[35] the surface density of proteins on the least wettable surfaces are roughly consistent with a monolayer of protein adsorbed with the long axis of the protein molecules parallel to the surface.

The proteins could be divided into three main groups according to their behavior. The smaller proteins (RNAse A, lysozyme) were extremely sensitive to the wettability of the surface; these proteins adsorbed well to the surfaces presenting CH_3, CF_3, and OPh groups ($\cos\theta_{co} \leq -0.90$), only slightly to the surface presenting CN groups ($\cos\theta_{co} = -0.83$), and not at all to the other surfaces ($\cos\theta_{co} \geq -0.28$). The larger proteins (fibrinogen, pyruvate kinase, γ-globulins) were much

(34) The adsorption of some proteins to the $-EG_6OH$ surface is detectable (see ref 15). The amount of protein that adsorbs on this surface is, however, always small compared to the amount that adsorbs on the $-CH_3$ surface (<2%).

(35) Soderquist, M. E.; Walton, A. G. *J. Colloid Interface Sci.* **1980**, *75*, 386-397.

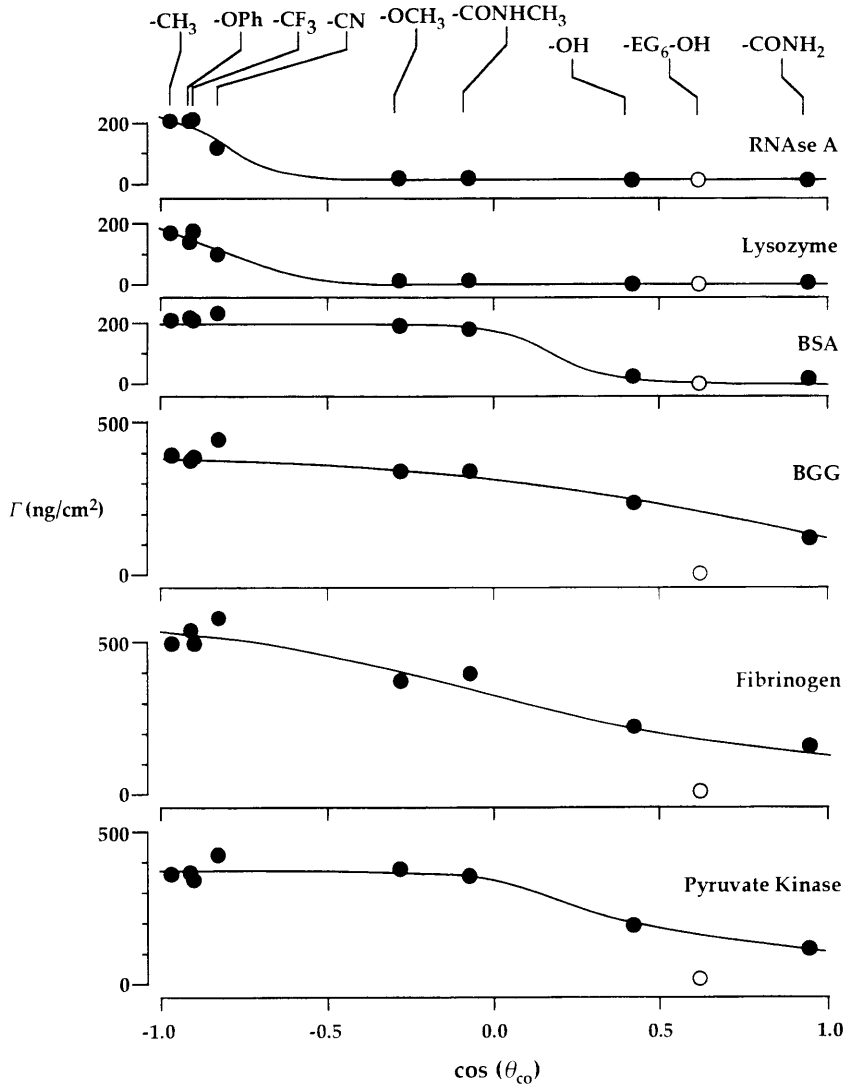

Figure 8. Surface density of adsorbed films of six proteins on a variety of SAMs as a function of the contact angle of water on the SAM under cyclooctane. The adsorption of solutions containing the proteins at a concentration of 1.0 mg/mL in PBS was measured by SPR. The graphs show the surface densities measured after allowing the binding reactions to proceed to completion (20 min). EG_6OH is indicated by an open circle to emphasize its anomalous behavior.

Table 3. Protein Adsorption on the SAM Presenting $-CH_3$ Groups[a]

protein	Γ (ng/cm^2)[b]	Γ_{side} (ng/cm^2)[c]	Γ_{end} (ng/cm^2)[c]
RNAse A	200	220	380
lysozyme	170	170	260
serum albumin (BSA)	210	250	600
γ-globulins (BGG)	400	270	1500
pyruvate kinase	500		
fibrinogen	370	240	2400

[a] The adsorption from solutions containing the proteins at concentrations of 1.0 mg/mL was allowed to proceed to completion (20 min). [b] The surface concentration of protein as determined by SPR. [c] The theoretical surface density of a complete monolayer of protein, assuming the long axis of the protein is perpendicular (Γ_{end}) or parallel (Γ_{side}) to the surface. These values were calculated from the values listed in Table 2 for the molecular weights and the approximate molecular dimensions of the proteins.

less sensitive to the wettability of the surface; the adsorbance of these proteins was significant even on the most wettable surfaces (the exception being the surface presenting EG_6OH groups; this surface was resistant to the adsorption of all the proteins tested).[36] The behavior of BSA, a protein of intermediate size, showed a sensitivity to wettability that was somewhere between that observed for smaller and larger proteins; no protein adsorbed to surfaces with $\cos\theta_{co} > 0$, but in contrast to the results for RNAse and lysozyme, some adsorption was observed on surfaces presenting $-OCH_3$ and $-CONHCH_3$ groups.

We do not know why the larger proteins adsorb to surfaces that favor water over organic solvent (i.e., $\cos\theta_{co} > 0$). The adsorption may reflect thermodynamically unfavorable interactions of water with hydrophobic surfaces on the protein. Alternatively, the interaction may be due to the adsorption of protein at defects in the SAM.[37] The exceptional ability of the SAM presenting $-EG_6OH$ groups to prevent the adsorption of these proteins is also not well understood.

Despite the general trend of increased adsorption with decreased wettability, some exceptions were observed. For example: (i) the binding of the four larger proteins to the surface presenting $-CN$ groups tended to be 10−20% higher than that on the surface presenting $-CH_3$ groups, (ii) several proteins adsorbed to the $-CONHCH_3$ surface in greater amounts than on the $-OCH_3$ surface, and (iii) the surface presenting EG_6OH groups resisted the adsorption of all the proteins while the more hydrophilic surface presenting $-CONH_2$ groups did not. These

(36) SAMs presenting EG_6OH groups are known to resist the adsorption of proteins (see refs 14 and 15).

(37) Zhao, X. M.; Wilbur, J. L.; Whitesides, G. M. *Langmuir* **1996**, *13*, 3257−3264.

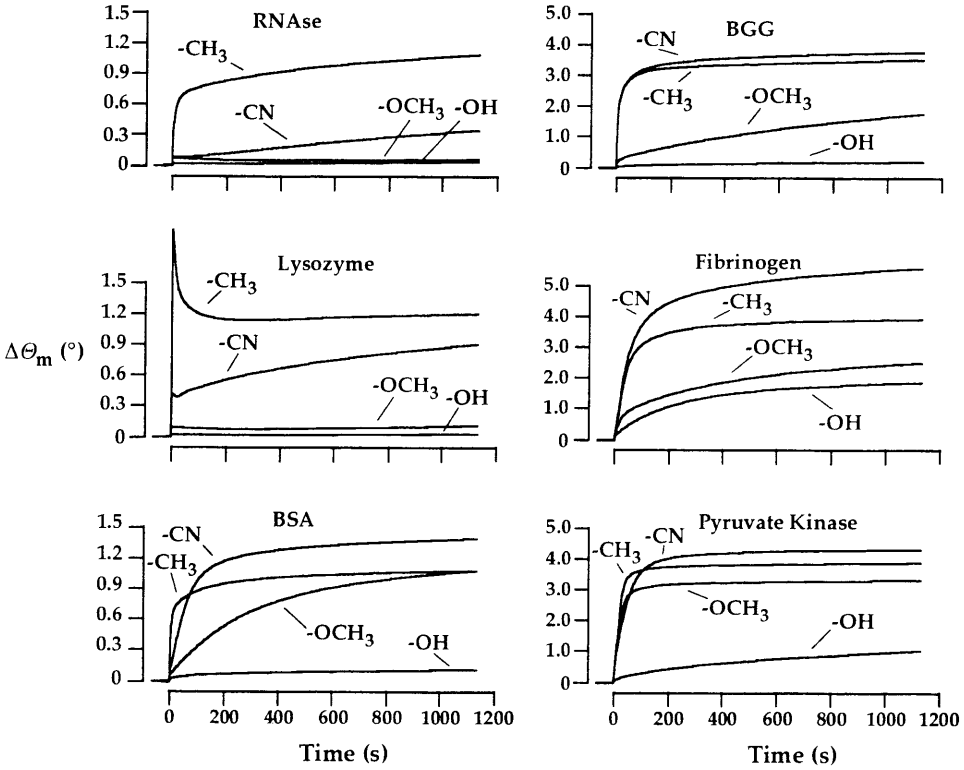

Figure 9. Kinetics of protein adsorption to four SAMs of varying wettability. PBS was passed over the surfaces at a flow rate of 10 μL/min. At time = 0 s, the solution was replaced with a solution containing a protein at a concentration of 50 μg/mL in PBS. The graphs show the SPR response as a function of time.

results indicate that, while surface wettability may be a good general indicator of the propensity of a surface to adsorb proteins, it is also necessary to consider specific structural features—for example, group dipole moment for −CN, hydrogen bonding for $-CONH_2$ and $CONHCH_3$, and conformational disorder for $-EG_6OH$—of each surface.

Effect of the Wettability of the SAMs on the Kinetics of the Adsorption of Proteins. Figure 9 shows the kinetics for the adsorption of the model proteins to four representative surfaces ($-CH_3$, −CN, $-OCH_3$, −OH) from dilute solutions of the proteins (50 μg/mL). We note that there is not much information in the kinetics for the least wettable surfaces; the initial rates are mass transport limited under the conditions of the experiment.[38] We did observe, however, decreases in the association rate below the mass transport limit as the surfaces became more hydrophilic. In general, the decreased kinetics correlated with lower levels of adsorbed protein at saturation. We observed some exceptions to this rule; the initial rate of adsorption of proteins on the SAM presenting −CN was always less than or equal to the rate on the surface presenting $-CH_3$; in some cases, however, more protein adsorbed on the more hydrophilic −CN surface.

The concentration of lysozyme on the surface presenting $-CH_3$ groups reaches a maximum within a few seconds after the introduction of the protein. The concentration on the surface then slowly declines to an intermediate value. This behavior has been observed by others (for example, by Soderquist et al. for the adsorption of BSA on siliconized glass surfaces)[35] and has been attributed to a change in the orientation of the protein on the surface;[35,39] a kinetically favored orientation is slowly replaced by a more stable orientation that requires more surface area per molecule of protein.

Desorption of Proteins from the SAMs. The films of protein that adsorbed on the surfaces were kinetically stable: over 1 h, less than 5% of the adsorbed protein desorbed into flowing buffer. The wettability of the surfaces appeared to have no influence over the stability of the films once they were formed. The ability of detergent to elute proteins from surfaces has been used as a probe to characterize the structure of adsorbed proteins.[40,41] All the proteins except pyruvate kinase were easily desorbed from the surfaces (>90% desorption in less than 1 min) by treatment with solutions containing SDS at concentrations greater than the cmc.[42] Solutions containing SDS at concentrations at or below the cmc were much less effective at desorbing proteins from the surfaces (despite our observation that the binding isotherms for SDS on clean hydrophobic surfaces reached saturation roughly at the cmc); these observations suggest that the interaction of proteins with detergent micelles or other aggregates was a requirement for the desorption of the proteins.[25] The nonionic detergent β-octyl glucoside was also effective at desorbing all the proteins (with the exception of pyruvate kinase) from the surfaces. While SDS is a strongly denaturing detergent,[43,44] β-octyl glucoside is reported to be

(38) The initial rates for the adsorption of proteins to the hydrophobic surfaces showed a linear dependence on the cube root of the flow rate; this dependence is consistent with the mass transport limited association of protein with the surface of a thin rectangular flow cell (for a detailed description of the mass transport in the BIACore instrument, see Glaser, R. W. *Anal. Biochem.* **1993**, *213*, 152−161).

(39) Van Dulm, P.; Norde, W. *J. Colloid Interface Sci.* **1983**, *91*, 248−255.

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(42) There was no correlation between the rates of the desorption of proteins in the presence of SDS and the hydrophobicity of the surfaces. In general, the rates were similar with the exception of the −CN surface, which gave exceptionally fast rates of desorption.

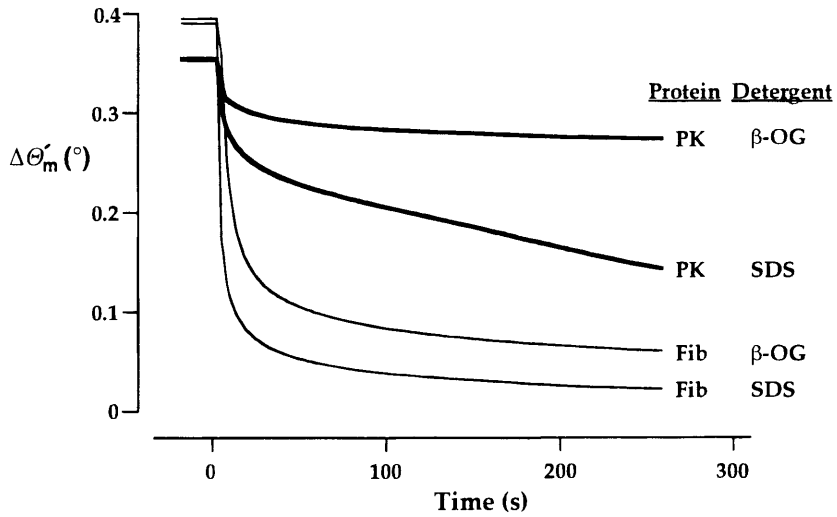

Figure 10. Desorption of pyruvate kinase (PK) and fibrinogen (Fib) from the SAM presenting $-CH_3$ groups. The surfaces were pretreated with solutions containing the proteins at concentrations of 0.1 mg/mL (the adsorption kinetics are not shown). At time = 0, solutions containing SDS (10 mM) or β-octyl glucoside (β-OG, 100 mM) in PBS were passed over the surfaces. To compensate for the SPR response due to the increased refractive index of the solutions containing detergent, as well as for the adsorption of detergent to the surface in the absence of adsorbed protein, the experiment was repeated on surfaces that were not pretreated with protein. The plotted value of the SPR response is the difference, $\Delta\Theta_m' = \Delta\Theta_m$ (protein) $- \Delta\Theta_m$ (no protein).

nondenaturing to many proteins;[43,45,46] for this reason, it has been widely used in applications that are sensitive to changes in protein structure (for example, the purification and crystallization of membrane proteins). The effectiveness of β-octyl glucoside at desorbing the proteins from the surfaces indicates that denaturation of these proteins probably does not play an important role in their solubilization.

Figure 10 shows the desorption of pyruvate kinase from four representative surfaces. Pyruvate kinase desorbs much more slowly than the other proteins in the presence of SDS (for comparison, Figure 10 also shows the desorption of fibrinogen under the same conditions). Almost no desorption of pyruvate kinase is seen in the presence of β-octyl glucoside. The slow rate of desorption and the difference observed between the two detergents suggests that the structure of pyruvate kinase changes during adsorption (for example, by denaturation, aggregation, or dissociation into subunits) to the extent that it is no longer soluble and that the rate-determining step for desorption involves deaggregation or solubilization of the protein by the detergent.

Conclusions

We examined the adsorption by hydrophobic interaction of proteins and detergents to SAMs of alkanethiolates on gold. As was expected, the binding reactions showed a general dependence on the wettability of the model surfaces, as determined by the contact angle of water under cyclooctane (θ_{co}). This trend was observed for both the surface density of the adsorbed films at the completion of the binding reactions (as was observed by Elwing et al. on wettability gradients) and for the rates of adsorption. These studies led us to five conclusions:

(i) Contact angles measured under cyclooctane provide a better measure of the propensity of surfaces to adsorb hydrophobic molecules than contact angles measured under air. In particular, the relative wettability of SAMs presenting $-CN$ and $-OCH_3$ groups is reversed depending on whether the scale used is the contact angle of water under air or under cyclooctane. We believe that the contact angle under cyclooctane is more relevant than the contact angle under air for adsorption occurring under water, and the relative tendencies of proteins to adsorb to the two surfaces were more in keeping with this scale.

(ii) Detergent molecules (SDS) and the three smallest proteins tested (RNAse, lysozyme, and BSA) only adsorbed to the surfaces that have a favorable free energy of transfer from water to cyclooctane (i.e., $\cos\theta_{co} < 0$). This observation is consistent with a model approximating the interaction of the surfaces with hydrophobic patches on the proteins as the transfer of the surfaces from water to an organic solution.

(iii) The binding isotherms of SDS on SAMs with values of $\cos\theta_{co}$ of < -0.9 and SAMs with values of $\cos\theta_{co}$ of $-0.9 \leq \cos\theta_{co} \leq 0.0$ are fundamentally different (Figure 6). This result is particularly interesting and unexpected. The sharpness of the transition on the moderately hydrophobic surfaces strongly suggests a cooperative mechanism for the binding of SDS to these surfaces (the formation of detergent bilayers on hard acid surfaces provides a precedent for cooperative binding of detergents to surfaces[32,33]).

(iv) Large proteins (but not small ones) adsorb to surfaces that prefer water over cyclooctane (i.e., $\cos\theta_{co} > 0$). The single exception was the SAM presenting hexa(ethylene glycol) groups at the surface; this surface was resistant to the adsorption of all the proteins we used in this study. We have not established the mechanism of the adsorption of proteins to hydrophilic SAMs, but it is probably due either to the thermodynamic instability of the protein in water (i.e., the unfavorable interaction of hydrophobic amino acids with water) or to the adsorption of protein at defects in the SAM. We note that, while SAMs of alkanethiolates on gold are good model systems for organic surfaces, they are not perfect: the presence of defects in SAMs (as seen by STM)[37] introduces the possibility that some of the behavior we observe depends on surface structure at the molecular level and is not accurately reflected in global physical properties such as contact angles.

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(v) The desorption of proteins from the SAMs by detergent seems to be facilitated by micelles but does not, in general, require denaturation, since β-octyl glucoside, a nondenaturing detergent, is effective. We observed one exception: pyruvate kinase only desorbed in the presence of a strongly denaturing detergent (SDS).

Experimental Section

Materials. All materials and reagents were used as received. Phosphate-buffered saline (P3813), serum albumin (bovine; A7638)), fibrinogen (human; F4883), pyruvate kinase (rabbit muscle; P9136), ribonuclease A (bovine pancreas; R5125), lysozyme (chicken egg white; L6876), and γ-globulin (bovine; G5009) were purchased from Sigma. Electrophoresis grade detergents sodium dodecyl sulfate (Bio-Rad) and β-octyl glucoside (Sigma) were used in this study. Undecanethiol was purchased from Aldrich and purified by silica gel chromatography. The other thiols described in this report, with the exception of $HS(CH_2)_{11}$-OPh, were prepared according to established procedures.[47-49] 11-Bromo-1-undecene was purchased from Pfaltz and Bauer. All buffers and solutions of proteins were filtered through 0.45-μm filters before use.

11-Phenoxy-1-mercaptoundecane. Sodium (0.35 g, 15 mmol) was dissolved in 40 mL of methanol under nitrogen. Phenol (2.1 g, 20 mmol) and 11-bromo-1-undecene were added sequentially, and the solution was heated under reflux for 14 h under nitrogen. The solvent was evaporated to give a crude oil. Purification by silica gel chromatography using 75:1 hexane/ether as the eluent gave 2.0 g (82%) of 11-phenoxy-1-undecene as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.27 (m, 2H), 6.92 (m, 3H), 5.83 (m, 1H), 4.99 (d, 1H), 4.91 (d, 1H), 3.94 (t, 2H), 2.04 (m, 2H), 1.75 (m, 2H), 1.3−1.5 (bm, 12H).

The 11-phenoxy-1-mercaptoundecene (2.0 g, 8.2 mmol) was dissolved in 40 mL of distilled tetrahydrofuran, together with 1.5 mL of thiolacetic acid (20 mmol) and 100 mg of azobis(isobutyronitrile). The solution was irradiated for 4 h under a 450-W medium-pressure mercury lamp (Ace Glass). Evaporation of the solvent gave the thioacetate as an oil. Hydrolysis to the thiol was carried out without further purification. The crude thioacetate was dissolved in 50 mL of dry methanol and the solution purged with nitrogen. A solution containing sodium methoxide in methanol at a concentration of 2 M (7.5 mL, 15 mmol) was added anaerobically, and the solution was stirred for 15 min at room temperature. The solution was neutralized (2 mL of acetic acid) and evaporated to an oil. The residue was taken up in 50 mL of ether; this solution was washed with three 20-mL portions of a saturated solution of sodium chloride, dried over magnesium sulfate, and evaporated to give an oil. Purification by chromatography on silica gel using 75:1 hexane/ether as the eluent to give the thiol (2.3 g, 98%) as a clear oil that solidified on cooling to 4 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.26 (m, 2H), 6.92 (m, 3H), 3.96 (t, 2H), 2.54 (q, 2H), 1.77 (m, 2H), 1.63 (m, 2H), 1.3−1.5 (bm, 15H). ^{13}C NMR ($CDCl_3$, 400 MHz, δ): 159.18, 129.44, 120.49, 114.54, 67.91, 34.09, 29.54, 29.43, 29.34, 29.11, 28.42, 26.11, 24.70. HRMS-FAB $[M]^+$: calcd for $C_{17}H_{28}OS$ 280.1861, found 280.1871.

Surface Plasmon Resonance Spectroscopy. We used the Biacore instrument (Pharmacia) for all studies described here. The BIACore instrument reports Θ_m in resonance units (RU, 10 000 RU = 1°). The resolution is the instrument is ~0.0001°. We modified the manufacture's cassettes to accept our substrates as described previously.[15,50] Briefly, substrates were prepared by evaporation of titanium (1.5 nm) and gold (39 nm) onto glass cover slips (0.20 mm, No. 2, Corning). The metallized substrates were cut into squares 1 cm^2 in size, immersed in solutions of the specified alkanethiol in ethanol (2 mM thiol) for 10 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 manufacturer's instruction. All buffers and samples were degassed under vacuum.

Contact Angles. Contact angles were determined under air- and water-saturated cyclooctane using a Ramé-Hart Model 100 contact angle goniometer. We measured advancing contact angles on a drop of water delivered to the surface using a Matrix Technologies Microelectrapipette. The reported values are the average of three measurements taken at different locations on the SAM.

Acknowledgment. This work was supported by the National Institutes of Health (GM 30367), the Office of Naval Research, and the Advanced Research Projects Agency. M.M. is grateful to the American Cancer Society for a postdoctoral fellowship.

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