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Titration of Ionizable Monolayers by Measurement of the Electric Double-Layer Force

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We have determined the pK_a of surface-bound primary amine groups by determining the surface potential as a function of solution pH from the magnitude of the electric double-layer force. Using colloid-probe atomic force microscopy (AFM), we measured the force as a function of separation between a particle of radius $R = 10\ \mu$m and a planar surface, each coated with a self-assembled monolayer of $HS(CH_2)_2CONH((CH_2)_2O)_8(CH_2)_2NH_2$. The force was measured from pH 3 to 7, and the surface potential was determined by fitting the results to solutions of the nonlinear Poisson−Boltzmann equation. The surface pK_a of the primary amine group was found to be 5.0 ± 0.2, in agreement with the results of contact-angle and chemical-force titrations on similar surfaces with primary amine groups. The surface charge density indicates that less than 1% of the NH_2 groups are dissociated at pH 3, suggesting that ionization is very unfavorable in the local environment of the ethylene oxide chains. This noncontact method should be of general applicability to surfaces with ionizable groups and avoids the possible complications of large contact forces on the surfaces under study.

Introduction

The properties of surface-bound ionizable groups are usually not the same as for entities that are free in solution. In particular, their ionization may be significantly affected by differences in solvation, in the dielectric constant of the surrounding medium, in the molecular mobility, and in the electrostatic charge of surrounding molecules. This results in a significant shift in the pK_a of surface-bound groups compared to that of the same groups in solution.[1−9] An understanding of this pK_a shift is especially important for organic and inorganic multilayer and multicomponent systems, where electrostatic interactions may play a key role.

Long-chain organothiol molecules bind covalently to gold surfaces to form highly ordered self-assembled monolayers (SAMs),[10,11] and many titration studies have been concerned with this class of surface.[3,5−9] The terminal groups of the organothiol molecules can be selected to form a SAM with an exposed surface of well-defined surface energy and chemical functionality. The ease of preparation and stability of these SAMs have led to their widespread use in applications such as crystal growth, molecular electronics, electrochemistry, bioattachment, protein-resistant surfaces, photopatterning, protective coatings, and the control of colloidal interactions.[10−12]

pK_a shifts of ionizable groups in monolayers have been measured for alkanethiol SAMs using contact-angle goniometry[1−3] and chemical force microscopy[3−9] (i.e., pull-off force and friction force measurements with the atomic force microscope (AFM)[4−9]). Contact-angle titration relies on a significant change in the surface free energy (and hence the wetting of the surface) as the pH of the wetting medium is varied. This method can be applied to surfaces that are partially wet, but is less sensitive for higher-energy hydrophilic surfaces. For the latter types of surfaces, AFM adhesion and friction-force methods have been applied, although these methods also suffer from limitations. Adhesion and friction AFM measurements are sensitive to changes in the loading rate and applied load, respectively. Contact measurements with monolayers are also susceptible to physical and chemical changes caused by the indentation of the surfaces. The irregular geometry of commercially available AFM tips leads to an uncertainty in tip radius and a significant variation between tips, which decrease the accuracy of these measurements.

In this letter, we show how measurements of the force versus separation as a function of pH may be used to determine the pK_a of ionizable surface groups. Borosilicate microspheres of well-defined radius were attached to AFM cantilevers coated with gold and then functionalized with an NH_2-terminated oligoethylene oxide SAM. By fitting to DLVO theory the measured repulsive electric double-layer force and attractive van der Waals force between the spherical probe and a similarly functionalized flat substrate, we are able to determine the pK_a of the surface-bound amino groups as well as the absolute surface charge density of the monolayer.

Materials and Methods

The surface-preparation procedure prior to self-assembly of the monolayers was identical to that used in a recent study of cholesterol insertion into a lipid bilayer.[13] Briefly, the colloid probes (Duke Scientific, NIST-certified calibration standards) of radius 10.15 ± 0.70 μm were coated with a 10-nm-thick adhesion layer of chromium, followed by a 120-nm-thick layer of gold. The planar substrates were prepared by template stripping—gold-coated silicon wafers were peeled off of gold-coated glass slides to which they had previously been glued with an epoxy resin. The gold surfaces were functionalized in a 1 mM isopropanol solution of *N*-[26′-amino-

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3′,6′,9′,12′,15′,18′,21′,24′-octaoxahexacosyl]-3-mecapto-propionamide ($HS(CH_2)_2CONH((CH_2)_2O)_8(CH_2)_2NH_2$), henceforth referred to as OEO-NH_2 (Seiko-Epson, Japan), for 18 h at room temperature.

The colloid probes have a rms roughness <1nm over a 500 nm × 500 nm area, as determined by AFM reverse imaging against a tip calibration grid, and the rms roughness of the planar template-stripped gold surfaces is 0.7 ± 0.2 nm over a 500 nm × 500 nm area.[13]

XPS measurements were performed using a Thermo VG ESCA Lab 250 (Thermoelectron Corporation Inc.) UHV spectrometer. A monochromated Al Kα X-ray source (15 kV, 150 W) producing X-rays of 1487 eV energy was used, with a spot diameter of approximately 0.5 mm. A magnetic analyzer was operated in constant energy mode, with a pass energy of 150 eV for survey spectra and 20 eV for high-resolution spectra. All spectra were taken with a 90° takeoff angle.

Contact angles were measured with the sessile drop method, using 1.25 mM NaCl solutions with the pH adjusted by mixing 1.25 mM aqueous solutions of NaOH (Sigma-Aldrich, 99.99%) and HCl (Sigma-Aldrich, 320331) and a pH meter (Fisherbrand Hydrus 300).

Measurements of the force as a function of separation *D*, including the pull-off (adhesion) force, were carried out in the pH-adjusted solutions with an Asylum MFP-SA (Asylum Research Inc.) at room temperature. Cantilever (Veeco, NPS) spring constants were calibrated using the resonance frequency method.[14] The loading rate for each probe was set to 240 nN s^{-1} by adjusting the approach/retract rate, which was nominally 400 nm s^{-1}. The practical force resolution was ca. 0.1 nN at a digitization rate of 2 kHz. The optical lever sensitivity of the probe cantilever was determined against a clean silicon substrate before measurements were made.

The hydrodynamic drag of the cantilever and probe, due to the nominal probe velocity of 400 nm s^{-1}, generated an offset in the zero of the separation of ca. 0.5 nm, which was corrected for with a resulting uncertainty of 0.1 nm. The hydrodynamic drag also results in an offset in the zero position of force. This was corrected by using a linear fit to the baseline of the deflection trace, with an uncertainty in the zero of the force of ca. 2.5%.

At each pH value, 50 force curves and adhesion measurements were made at 10 different points on a planar SAM surface with each of five different probes, each on a different substrate. Prior to averaging, the measured forces were corrected for the repulsive hydrodynamic force $F_h(D)$

$$F_h(D) = \frac{6\pi\eta R^2 V}{D} \qquad (1)$$

where η is the viscosity of the medium, *R* is the radius of curvature of the sphere, and *V* is the probe velocity.[15] The magnitude of the hydrodynamic force was typically less than $^1/_{10}$ the measured force. The corrected and averaged force $F(D)$ was converted to the free energy per unit area $G_f(D)$ between flat surfaces via the Derjaguin approximation,[16]

$$F(D) = 2\pi R G_f(D) \qquad (2)$$

The resulting free-energy data were fitted to DLVO (Derjaguin−Landau−Verwey−Overbeek) theory[16] with the nonlinear Poisson−Boltzmann equation for identically charged surfaces and the attractive van der Waals force using a program based on algorithms by Chan.[17] The Hamaker constant of 4 × 10^{-20} J was taken as that between hexadecanol SAMs in water from force measurements[18] and calculations based on Lifshitz theory.[19] As a check of our method, force measurements were carried out between glass probes and planar glass substrates coated with bilayers of the cationic surfactant cetyltrimethylammonium bromide (CTAB) and immersed in aqueous NaCl solutions. Fits of these data to DLVO theory gave surface potentials within 2.5% of previously published results.[20,21]

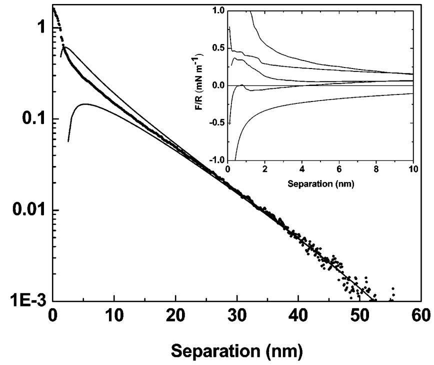

Figure 1. Mean force (points and thick solid line) measured on approach (normalized by the radius of curvature *R* of the probe) between OEO-NH_2 SAM surfaces immersed in a 1.25 mM NaCl solution at pH 3. The thinner lines are forces calculated from solutions to the nonlinear Poisson−Boltzmann equation with a surface potential of 30.5 mV and a Debye length of 8.7 nm with constant charge boundary conditions (upper curve) and constant potential boundary conditions (lower curve). The nonretarded Hamaker constant of 4 × 10^{-20} J was used (text). The inset shows the reduction in force as the pH of the aqueous medium increases from pH 3 to 7 (top curve to bottom curve). Each curve represents the average of 50 measurements.

Results and Discussion

The contact angle of NaCl solutions on monolayers of OEO-NH_2 on gold increased from 34° at pH 3 to 38° at pH 7. Given the ±1 to 2° accuracy of the contact angle measurements, it is difficult to determine accurately a pK_a value from these data (not shown).

The normalized adhesion force at pH 3 was less than 0.1 mN m^{-1}, increased to 0.9 ± 0.2 mN m^{-1} at pH 4, and was within the error constant at 1.2 ± 0.2 mN m^{-1} from pH 5 to 7. As with the contact angle data, a force titration of these monolayers would be rather inaccurate.

Figure 1 shows the mean repulsive force measured during the approach of the OEO-NH_2 surfaces at pH 3 plotted against surface separation. The surface potential from the fit was 30.5 ± 4.5 mV, and the Debye length was 8.7 ± 0.4 nm (within 1% of that calculated from the solution concentration). The increased repulsion below about 2.5 nm may be due to a steric or a hydration repulsion between the NH_2 groups and/or oligoethylene oxide chains, but we did not attempt to investigate this further. The force in this regime will be affected by the surface roughness because the probe must slide laterally when in repulsive contact with the planar surface. We believe that this is the main origin of the fine structure below 2.5 nm, which is evident even after averaging the force curves, but it does not affect the fits made at much larger surface separations.

As the pH of the electrolyte increased, the repulsive double-layer force decreased in magnitude, and the increased repulsion at short range disappeared by pH 5, until at pH 7 the force was attractive between 30 nm and contact (inset to Figure 1). The surface potential decreased from 30.5 mV at pH 3 to 0 mV at pH 7, where no repulsion was found between 0 and 100 nm. The surface potential titration graph is shown in Figure 2, with the data fitted using a sigmoidal function, which gave a pK_a of the

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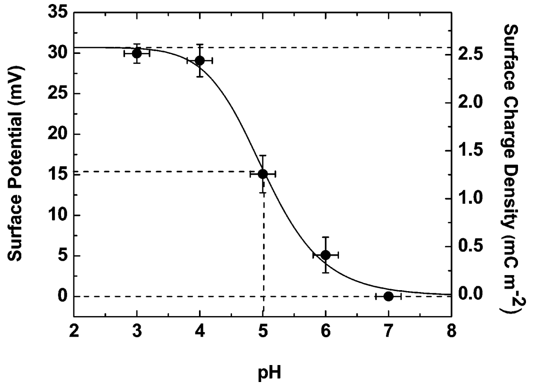

Figure 2. Surface potential titration of OEO-NH_2 surfaces in 1.25 mM NaCl. The solid line shows a sigmoidal fit to the data. The dashed lines show the upper and lower limits of the surface potential of the fit and the 50% surface potential position corresponding to the pK_a.

NH_2 group of 5.0 ± 0.2. This is about 5 pH units lower than that seen for primary amines in solution[22] but similar to the pK_a of other primary amine groups in monolayers, measured by adhesion force[3,5] and contact angle titration.[3] Simulations of surface NH_2 groups show large pK_a shifts when these are poorly solvated,[23] and it is clear that the local environment of the amino groups in the present SAM is very different from that of an aqueous solution.

The surface potential at pH 3 translates to a surface charge density of 2.57 mC m^{-2}, or one charge per 62.3 nm^2. We can estimate the surface density of OEO-NH_2 groups, which is equal to the maximum possible surface charge, from the XPS measurements. Figure 3 shows the Au 4f and C 1s peaks, and their integrated intensity gave an elemental C/Au ratio of 0.77, compared to ca. 1.8 seen in measurements of octadecanethiol SAMs on gold.[24] This indicates that the percentage of occupied binding sites on the gold surface is about 40% of that of an alkanethiol SAM. This is reasonable in view of the helical conformation of the ethylene oxide chain, which would increase the surface area per molecule. Taking this reduction in binding density into consideration and assuming that the alkanethiols bind to all available sites on the gold substrate, we estimate that only about 1% of the amino groups in OEO-NH_2 are ionized at pH 3. The environment of the NH_2 groups on the oligoethylene oxide chains obviously does not favor ionization. To further investigate the reasons for this, it would be necessary to have information on factors such as the effect of other ionic species (these experiments were carried with 1.25 mM NaCl as the background electrolyte) and of the surface density of OEO-NH_2.

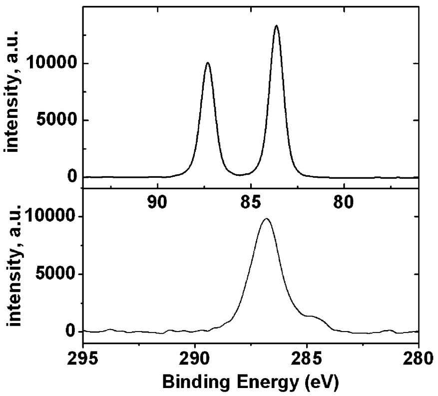

Figure 3. High-resolution XPS spectra of the OEO-NH_2 SAM surface showing the Au 4f (top) and C 1s (bottom) spectra. The Au 4f spectrum is a spin−orbit coupling doublet at 83.6 eV (Au $4f_{5/2}$) and 87.3 eV (Au $4f_{7/2}$) with a 3/4 intensity ratio, and the C 1s spectrum has a primary peak at 286.8 eV due to carbon in the ethylene oxide units and a secondary peak at 284.7 eV due to carbon in the alkyl chains. From the integrated intensities, the C/Au ratio was calculated to be 0.77.

The absence of a significant transition in the measured contact angle between pH 3 and 7 is not surprising in light of the small degree of ionization—the wetting properties of the SAM would hardly vary across the studied pH range. The increase in the adhesion force from 0.1 mN m^{-1} at pH 3 to approximately 1 mN m^{-1} from pH 4 to 7 is most likely related to the disappearance of the double-layer repulsion and the additional short-range repulsion across this range. The repulsion close to contact of ca. 1 mN m^{-1} at pH 3 is close to the increase in the adhesion force as the repulsion disappears with increasing pH.

We have shown how measurements of the electric double-layer force between surfaces may be used to determine the pK_a of surface-bound charged groups. As an advantage over other titration methods, one also obtains the absolute surface charge from the measurements. The method should be generally applicable as long as both a colloid probe and a planar surface can be functionalized to yield identical surfaces.

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