Complex Chemical Force Titration Behavior of Amine-Terminated Self-Assembled Monolayers

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The adhesion force between two 11-amino-1-undecanethiol self-assembled monolayers has been measured as a function of pH in solutions of two ionic strengths, high (10^{-1} M) and low (10^{-4} M). In high ionic strength solutions, the force titration curve approximates to a sigmoidal step, centered at ca. pH 3, with negligible adhesion at low pH and a high adhesion (20 nN) at higher pH but with a previously unobserved drop in adhesion almost to zero above pH 12. In low ionic strength solutions, the shape of the force titration curve is radically different, comprising two *peaks* centered at ca. pH 6 and 10. The length of the alkyl spacer has a strong effect on the magnitude of the peak at pH 6 which increases significantly with decreasing spacer length, whereas the peak at pH 10 is unaffected. We propose that the pH 6 peak in low ionic strength conditions and the very high adhesion in high ionic strength buffer are due to a hydrophobic interaction between two disordered monolayers and that *in-plane* hydrogen bonding between neutral and charged amine groups increases the surface ordering and reduces this effect in low ionic strength solutions. The drop in adhesion at high pH in both ionic strength solutions is unexpected. However, Fourier transform infrared data provide evidence for the presence of sulfur-containing groups in the monolayer surface, and the ionization of these groups at high pH resulting in electrostatic repulsion may explain the zero adhesion. In support of this hypothesis, a long-range, presumably electrostatic, repulsion is indeed observed in the approach part of the force–displacement curves.

Introduction

Intermolecular and surface forces[1-5] such as van der Waals forces, electrostatic interactions, and hydrogen bonding play key roles in a wide variety of chemical and biochemical interactions involved in phenomena such as catalysis, membrane transport, colloids, molecular recognition, cell adhesion and cell signaling, DNA/protein interactions, and the control of skeletal mineral growth.

The ionization state of molecular groups such as carboxylic acids and amines plays a critical role in these interfacial interactions, and it is of great importance to develop methods to measure the pK_a of such surface groups and ideally to map their distribution with high resolution. Established methods of measuring surface ionization states are unable to map the distribution of charged groups with anything approaching molecular resolution. For example, contact angle titration[1] is limited to a spatial resolution of millimeters and also experiences problems with highly hydrophilic surfaces that are completely wetted in both ionization states.

The development of atomic force microscopy (AFM) has provided a new tool for the study of interfacial phenomena with subnanometer resolution,[6-7] and chemical force microscopy (CFM) is a recent innovation which allows one to map chemical heterogeneity in surfaces[8,9] by using a functionalized AFM probe. Such functionalization has generally been achieved by the formation of ω-functionalized self-assembled monolayers (SAMs) on the AFM tip.[2] The introduction of the "force titration" technique,[10,11] in which the chemical adhesion between tip and sample is monitored as a function of pH, permits the p$K_{1/2}$ of surface-bound groups to be mapped with nanometer resolution. (The p$K_{1/2}$ is defined as the bulk pH at which half of the surface groups are ionized.[12])

Previous force titration experiments have been used to characterize the interactions between tips and substrates modified with carboxylic acid, hydroxyl, amine, and phosphonic acid groups.[10,11,13-17] Our own studies with carboxylic and phosphonic acid terminated SAMs[16,17] have

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revealed that the shape of the force titration curves is highly dependent on the ionic strength of the solutions in which the measurements are performed and on the spacer length of the SAM used (i.e., the length of the alkyl chain which spaces the functional group from the substrate). We have shown that the force titration curves change from a sigmoidal step (or steps for the diprotic acid) at high ionic strength to a peak (or two peaks for the diprotic acid) in very low ionic strength solutions. We have attributed the peaks in the low ionic strength force titrations to the formation of strong ionic hydrogen bonds between neutral and ionized groups which are of the order of 20 times stronger than a neutral hydrogen bond.

We are interested in the interactions of proteins with surfaces, particularly inorganic mineral surfaces in the context of the control of skeletal mineral tissue growth and desorption. Amine groups are one of the ionizable species within amino acids,[18] and as such their ionization state plays a critical role in the determination of many biomolecular structures and interactions. In addition to the measurement of the pK_a of surface-immobilized groups such as amines which is of fundamental interest, AFM tips modified with ionizable groups can also be used to map charge density distributions on surfaces, which may help in our understanding of the driving processes behind protein binding. Previous force titration work concerning amine surfaces has been performed by both Vezenov et al.[10] in high ionic strength (0.01 M) buffers using APTES-derived monolayers on silicon and van der Vegte et al.,[13] also in high ionic strength (0.01 M) buffers but using thiolated monolayers on gold. The shape of the force titration curves in both cases was a sigmoidal step with negligible adhesion at low pH and a finite adhesion of magnitude 15−30 nN at high pH. The surface $pK_{1/2}$ derived from these measurements has been seen to vary from 3.9 to 7.0.[10,13] These shifts are of the order of 7−4 pH units lower than the pK_a values of free organic primary amines in aqueous solutions.[19] The magnitude of the adhesion forces between NH_2 groups is larger in both cases than the adhesion between $COOH$ groups found previously, which would not be expected to be the case from their calculated relative hydrogen bond strengths ($NH\cdots N$ bonds are of the order of 3 kcal/mol, whereas $C=O\cdots HO$ are of the order of 7 kcal/mol.[20]) It was proposed that the large shifts in dissociation constant and large adhesion forces were due to a hydrophobic environment caused by disorder at the monolayer−liquid interface[21,22] revealing the alkyl chains.

To elucidate further the nature of the interaction between two amine-terminated SAMs, we report new results from force titration experiments performed with a much longer alkyl spacer (and therefore more ordered) amine SAM (11 carbon atoms). By using combinations of long- and short-spacer SAMs on the tip and substrate, we have been able to investigate the effect of SAM disorder on the interaction between the two surfaces. We also report on the significant effect that ionic strength has on the shape of the force titration curves and present evidence to suggest that the monolayer−liquid interface is not

chemically uniform but comprises both amine and sulfur-containing groups which can themselves ionize at high pH giving rise to long-range electrostatic forces between the two surfaces.

Experimental Section

Materials. All solvents used were reagent grade or better (Sigma, U.K.). Low ionic strength solutions (10^{-4} M) were prepared from dilutions of phosphate buffers (10^{-2} M), KH_2PO_4 (A), and Na_2HPO_4 (B) in doubly distilled water. Low pH solutions were prepared by titrating solution A with dilute HCl, and high pH solutions were prepared by titrating solution B with dilute NaOH. Intermediate pH values were obtained by mixing A and B in different amounts, while keeping the ionic strength approximately constant. High ionic strength buffers (10^{-1} M) were prepared by the addition of 10^{-1} M KCl to either A or B.

Amine-terminated SAMs were prepared from commercially available alkanethiols: 11-amino-1-undecanethiol (Dojindo Chemicals, Japan) and 2-aminoethanethiol (Sigma-Aldrich, U.K.).

Preparation of Chemically Modified Substrates and AFM Probes. Flat, template-stripped gold surfaces were prepared by the template stripping method of Wagner et al.[23] A silicon wafer surface was cleaned in water, methanol, and dichloromethane and then blown dry with nitrogen. Gold (100 nm) was then deposited onto the surface by thermal evaporation (Edwards Auto 306) at pressures $<2 \times 10^{-6}$ mbar. Epo-Tek 377 adhesive (Promatech, U.K.) was used to bond a clean glass slide to a gold-coated silicon wafer surface. The glue was then cured at 140 °C for 1 h. The silicon wafer was peeled away from the glass, revealing a gold template of the silicon surface with a roughness of ∼3 nm per square micron. Commercially available silicon nitride AFM cantilevers (NP cantilevers, DI, U.K.) were coated with 100 nm of gold using the same thermal evaporation method as above. Prior to the gold evaporation, however, the surfaces were coated with 10 nm of chromium to improve adhesion of the gold.

Template-stripped gold surfaces and gold-coated cantilevers were immersed in a 1 mM ethanol solution of 11-amino-1-undecanethiol for 2−12 h at room temperature immediately after preparation. The gold-coated cantilevers and substrates were then rinsed extensively with absolute ethanol and gently blown dry with nitrogen prior to force−distance measurements.

Force Measurements. All adhesion force measurements were obtained on a Molecular Imaging picoSPM equipped with a Teflon fluid cell and controlled by Nanoscope IIIa electronics (DI). All measurements were carried out with a 40 μm × 40 μm (xy) scanner and long (200 μm), wide AFM cantilevers (manufacturer's quoted spring constant of 0.12 N/m) at room temperature. The adhesive interaction between amine SAM modified tips and substrates was determined by recording force versus displacement curves under conditions of high and low ionic strength at varying pH. The fluid cell was rinsed through three times with each pH solution before measurements were made to ensure that only buffer of the required pH was present. Adhesion measurements at each pH value were obtained at slightly different positions on the SAM surface because of the difficulty in positioning the tip in exactly the same location each time a set of measurements was to be obtained. A full cycle of tip approach and retract was recorded, and the point at which the tip separates from the sample on retract is known as the pull off point. By measuring the deflection of the cantilever from the pull off point to the steady-state, equilibrium position of the cantilever (zero interaction force with the surface), it is possible to calculate the pull off force or adhesion between the tip and sample.

The pull off force was obtained for ∼100 force−distance curves per pH value in several different places on the sample. Each force−distance curve was obtained with a z scan size of ∼1 μm and at a rate of 2 cycles/s. All force−distance curves were captured using the Nanoscope IIIa software (version 4.23) and the pull off deflection determined with that software for each curve. The average deflection at each pH was obtained from the mean of each data set and the error derived from the standard deviation

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of the mean. Deflection measurements were subsequently converted to an average adhesion force by using the gold-coated cantilever's spring constant as determined from the Fourier transform of the thermal vibrations according to method of Hutter et al.[24] Adhesion measurements made with different tips but the same SAMs exhibited a variation in measured adhesion of up to 20% because of differences in tip shape and the quality of the SAMs. We use this figure as a rough indication of the level of quantitative comparison that can be made between force titration data from different experiments.

In addition to the adhesion measurements, the approach curves of the force spectroscopy measurements were also studied at each pH. Previous studies have shown that for a neutral tip and substrate the approach curve contains an attractive component due to the van der Waals interaction between the two surfaces.[25] This attractive component can be measured only for forces less than a critical "snap-in" force at which point the force derivative exceeds the spring constant of the cantilever and the cantilever jumps to contact.[26] For two like-charged surfaces, the appearance of a long-range repulsion leads to the eventual disappearance of the jump to contact feature in the approach curve when the electrostatic repulsion exceeds the attractive van der Waals forces at a given separation. Therefore, by monitoring the approach curves with pH we were able to determine the presence or absence of any long-range repulsive forces at a given pH.

FTIR. To assist in the characterization of the 11 carbon spacer amine-terminated SAMs, Fourier transform infrared spectroscopy (FTIR) was performed. The spectra were obtained on a Bruker IFS48 spectrometer equipped with a DTGS detector. A SPECAC variable angle reflection accessory was used for reflection adsorption measurements at a grazing incidence[27] angle of 83°. Spectra were obtained on both an 11-amino-1-undecanethiol SAM on gold and on the same material in a KBr pellet. A bare gold background spectrum was first obtained, followed by the amine terminated SAM spectrum. Two thousand sample scans were obtained at 2 cm^{-1} resolution. The sample chamber was purged with dry air to eliminate the spectral interference of ambient water vapor.

Results and Discussion

Figure 1 shows the chemical force titration for tip and substrate *both* modified with 11-amino-1-undecanethiol at high and low ionic strengths (10^{-1} and 10^{-4} M, respectively). At low pH, a small adhesion (<2 nN) is observed in both high and low ionic strength solutions. At high ionic strength, as the pH is increased the curve follows a sigmoidal step, rising to a maximum adhesion force of 20 nN at ca. pH 5 consistent with previously reported results.[10,11,13] At low ionic strength, the titration exhibits a peak and a shoulder centered around approximately pH 6 and pH 9–10, respectively. It is important to note that the shoulder in the low ionic strength titration is *highly reproducible* both between experiments with the same alkyl spacer length SAM and with SAMs of different spacer lengths (see Figure 4, data to be discussed in detail below). However, the scatter in the high ionic strength force titration is completely random and cannot be reproduced between experiments. At pH > 11, the adhesion force decreases in both ionic strength solutions and approaches zero above pH 12. This drop in adhesion force above pH 12 can be reproduced under both ionic strength conditions by cycling the pH in the range pH 8–12 and was observed on all samples that were studied.

A simple explanation for the high ionic strength force titration data, which initially seems reasonable, is as
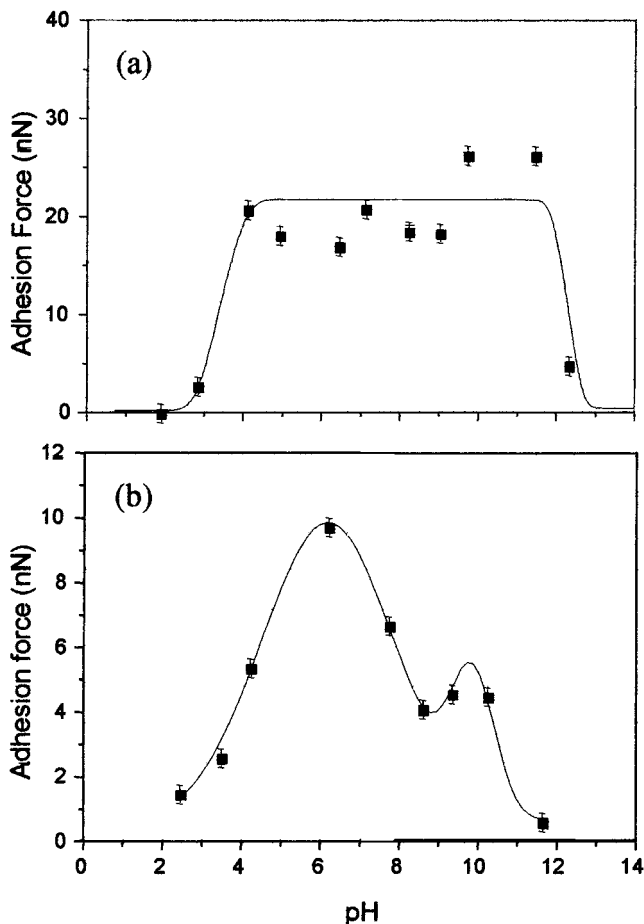
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Figure 1. Chemical force titration curves for tip and substrate both modified with 11-amino-1-undecanethiol SAMs acquired under solutions of two ionic strengths: (a) 10^{-1} M and (b) 10^{-4} M. (The curves have been added as a guide to the eye.)

follows. At low pH, both tip and sample are protonated (NH_3^+) and the net positive charge results in the formation of electric double layers on both surfaces which leads to an electrostatic repulsion and a low adhesion force. As the amine groups become deprotonated with increasing pH, a larger adhesion arises because of the hydrogen bond formation between neutral groups on the tip and sample. If the p$K_{1/2}$ of the ionizable groups is taken as the pH at which the adhesion force is half its maximum value, a value of pH 3.0 is obtained, which is approximately 7 pH units *less* than the pK_a of amine free in solution.[19]

However, when considered in greater detail there are three noticeable inconsistencies in this explanation, especially taken in the context of recent observations made with acid SAMs:[11,13,16,17]

(1) The adhesion (~20 nN) between predominantly neutral amine surfaces above pH 6 is greater than that observed for neutral acid surfaces (~6 nN) which should not be the case.[20] In addition, the ionic strength is seen to dramatically affect the interaction between amine surfaces at pH > 6, which would not be expected to be the case if the interaction arose purely from hydrogen bonding between neutral surfaces.

(2) The maximum adhesion force measured for the amine SAMs at high ionic strength is approximately twice the size of the maximum force observed in low ionic strength solutions. (The opposite trend is observed for acid SAMs; i.e., the peak adhesion force in low ionic strength solutions is much greater than the maximum force observed under high ionic strength conditions.)

(3) At very high pH, the adhesion force falls to zero.

These observations are not consistent with a simple model of electrostatic repulsion between charged surfaces and hydrogen bond formation between neutral surfaces. It is pertinent to note that the large adhesion force observed above pH 5 in the high ionic strength force titration is similar in magnitude to the adhesion between methyl terminated SAM surfaces shown independently by Vezenov et al.[10] and van der Vegte et al.[13] and repeated in our laboratory (data not shown). In addition, recent force titration work performed on patterned SAMs comprising COOH and CH_3 regions has shown that the hydrophobic interaction in the CH_3 areas varies greatly as a function of lateral position. This behavior was attributed to heterogeneity in the local environment surrounding the methyl groups in these regions.[15] The COOH regions exhibited no such scatter in the measured adhesion force. Our results obtained with the amine SAMs in high ionic strength also show a large scatter in the adhesion force (in the region pH 4−11) which we have never observed with carboxylic or phosphonic acid SAMs. It seems reasonable therefore that the scatter and high adhesion force observed for these amine SAMs are due to the interaction of two hydrophobic surfaces. We therefore propose that even the surface of our longer 11 carbon chain amine SAM is in fact quite disordered and that the exposure of the alkyl chains results in a strong hydrophobic interaction as observed for short-chain SAMs.[10,13] This interaction appears to vary greatly with position over the monolayer surface because of local changes in order, leading to a large scatter in the adhesion data also observed for methyl SAMs.[15]

Organic molecules are less soluble in high ionic strength solutions, an effect usually referred to as "salting out". A similar effect could lead to an increase in adhesion force under high ionic strength conditions; however, it cannot explain the drop to zero adhesion at high pH, and therefore this effect alone cannot explain the full force titration curve. It has been postulated that amine groups compete with thiol groups for binding to the gold surface[28−32] during SAM formation. If this is the case, then this would result in a surface containing both amine and sulfur groups (which would probably oxidize under the experimental conditions to form sulfonic acid groups), which may contribute to the greater disorder than expected in the monolayer. The drop in adhesion at very high pH in our data could therefore be explained by an electrostatic repulsion between two surfaces containing ionized acid groups as well as neutral amines. If such a long-range electrostatic repulsion is present, then it should be observed clearly in the approach part of the force−distance curves. The approach curves for the high ionic strength force titration are shown in Figure 2. At very low pH, there is an electrostatic repulsion between electric double layers formed on positively charged NH_3^+ surfaces that dominates the approach curve as expected. In the intermediate pH range, the approach curves contain a jump to contact, which suggests that an attractive interaction dominates over electrostatic repulsion at this tip−sample separation. Because of the magnitude of the measured adhesion, we suggest that this is principally as a result
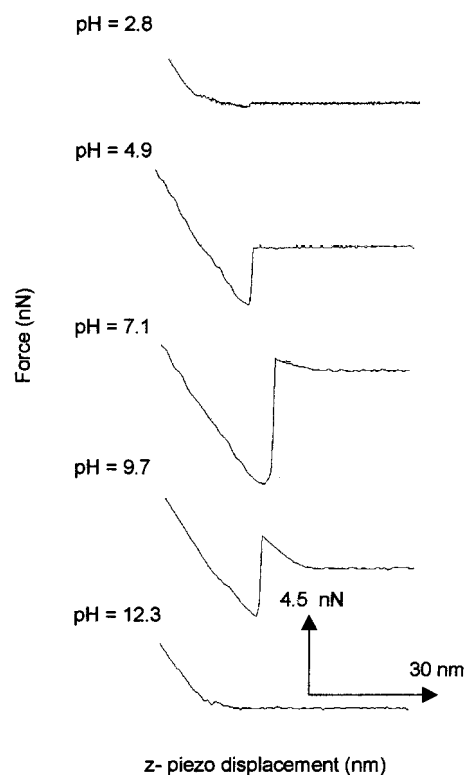
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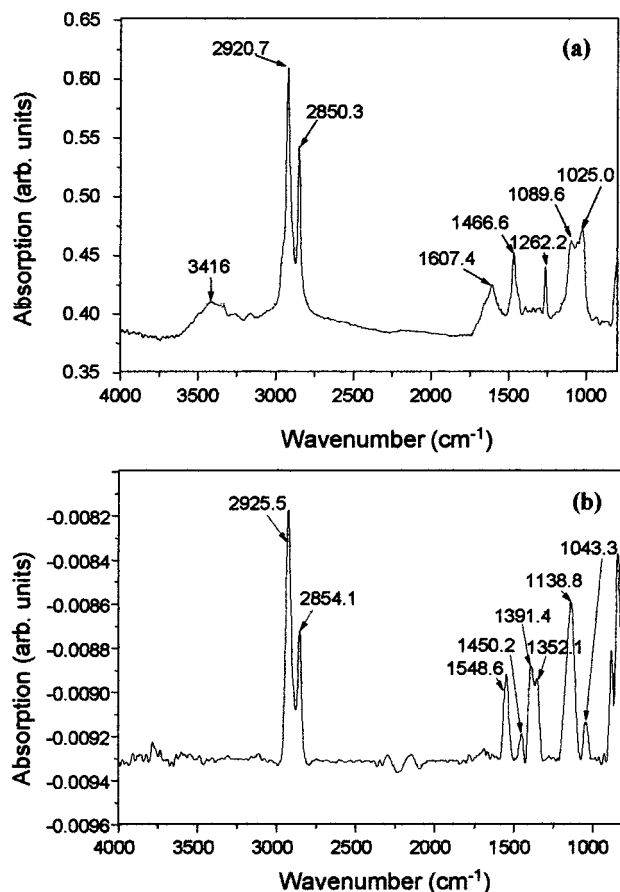
Figure 2. The approach sections of representative force−displacement curves at different pH values for an 11-amino-1-undecanethiol SAM modified tip and similarly modified substrate in high ionic strength buffer (10^{-1} M).

of two hydrophobic surfaces excluding water when brought very close together. As the pH is increased further, an electrostatic repulsion is indeed observed and at very high pH (pH = 12.3) the approach curve is once again entirely repulsive. It seems likely then that there are sulfonic acid groups in the surface of our "amine" SAM as a result of competition with the amine groups for binding to the gold substrate which clearly has serious implications for the modification of AFM probes with amine ω-functionalized thiols for CFM, even with long alkyl spacers.

If sulfonic acid groups are present in the SAM surface, then a second possible explanation for the shape of the force titration curves is as follows. At low pH and high pH, the low adhesion is due to the repulsion of two charged surfaces as discussed above. The rise in adhesion between pH 2 and 6 could be due to the neutralization of the electrostatic repulsion of the charged amines and an adhesive interaction between two neutral zwitterionic surfaces. However, we favor the former explanation, that is, a combination of hydrophobic and electrostatic interactions, because the magnitude of the adhesion under high ionic strength conditions is considerably larger, by at least a factor of 2, than adhesion forces attributed to purely electrostatic interactions in the literature.[10,11,13−17]

To confirm our hypothesis concerning the origins of the measured adhesion behavior, FTIR spectroscopy was used to characterize the amine SAM surface. Reflection FTIR spectra in the frequency region 4000−1000 cm^{-1} of 11-amino-1-undecanethiol in a KBr pellet (bulk) and in the form of a SAM are shown in Figure 3. The first feature of note in the SAM spectrum (Figure 3b) is the absence of the absorption due to the N−H stretching mode. In the isotropic sample (Figure 3a), this vibration gives rise to a broad peak around 3416 cm^{-1}. According to the surface selection rule of grazing angle reflectance FTIR on a smooth metal surface, only the molecular vibrations

Figure 3. Grazing angle FTIR spectra of 11-amino-1-unde-canethiol (a) in a KBr pellet and (b) formed as a self-assembled monolayer on a gold substrate.

possessing a nonzero projection of their dipole moment in the direction of surface normal can absorb.[27] The disappearance of the N–H stretching mode in the SAM spectra indicates that the N–H bond direction is parallel or nearly parallel to the surface. A second important observation concerns the positions of the asymmetric and symmetric CH$_2$ stretching vibrations.[33] In the KBr pellet, these vibrations occur at 2921 and 2850 cm^{-1} for the asymmetric and symmetric vibrations, respectively. In the SAM surface, these peak positions are shifted by a few wavenumbers to 2925 and 2854 cm^{-1}, respectively. The peak positions of the CH$_2$ stretching modes are sensitive indicators of the degree of packing of the alkyl chains in the SAM spacer.[34] For crystalline SAMs (i.e., highly ordered alkyl chains), the asymmetric peak usually occurs at 2920 cm^{-1}, but in a liquidlike phase the peak shifts several wavenumbers to a higher frequency. The FTIR spectrum of the SAM studied here therefore indicates a more liquidlike environment for the alkyl chains. When the two surfaces are brought together in the force titration measurement, this is more likely (than highly crystalline ordering) to lead to deformation of the SAM and exposure of the alkyl chains leading to hydrophobic forces in agreement with our explanation of the large adhesion force observed. The third important feature in the SAM spectrum is the appearance of peaks at 1352 and 1391 cm^{-1} that are not observed in the KBr pellet spectrum.

These peaks are typical of S–O containing moieties[35] which supports the hypothesis that some of the thiol groups become exposed at the monolayer–liquid interface and have subsequently become oxidized. The FTIR data therefore appear to lend some support, albeit far from conclusive, that the SAM surface contains sulfonic acid groups that would be ionized at high pH and cause the electrostatic repulsion observed in the force–distance experiments.

If the interaction between the amine SAMs is mainly hydrophobic in origin, why is the adhesive force above pH 5 considerably reduced in low ionic strength solutions? If SAM disorder is responsible for the large adhesion in high ionic strength solutions, then a lower adhesion under low ionic strength conditions implies that the SAM surface is more ordered. In the case of carboxylic acid SAMs,[17,12] the effect of high ionic strength on SAM ordering has been attributed to the prevention of strong *in-plane* ionic hydrogen bond formation, COO$^-\cdots$HOOC, because of the association of ions from solution with the SAM. Under low ionic strength conditions, these strong in-plane hydrogen bonds stabilize the monolayer surface, increasing the ordering and reducing hydrophobic interactions. The FTIR data discussed above indicate that the N–H bond lies in the plane of the monolayer and therefore intramonolayer hydrogen bonding may occur. The formation of neutral in-plane hydrogen bonds would not be affected by solution ionic strength. However, the possibility of *ionic* hydrogen bond formation in the amine SAMs studied here is supported to some extent by the FTIR data. The peak at 1607 cm^{-1} in the KBr spectrum results from contributions from N–H bending (60%) and C–N stretching (40%).[36–37] This peak position is sensitively affected by hydrogen bonding, and in the SAM spectrum its position is shifted to 1549 cm^{-1}. This is a much larger shift than can be explained by the formation of neutral NH$\cdots$N hydrogen bonding, but it could be caused by NH$\cdots$O$^-$ bond formation.[36–37] We therefore tentatively suggest that the formation of ionic in-plane hydrogen bonds between neutral and charged groups in the monolayer surface is responsible for increased SAM order under low ionic strength conditions. Under high ionic strength conditions, the formation of these stabilizing bonds is prevented by association of ions from solution which results in greater disorder and a large hydrophobic interaction.

To further investigate the contribution from hydrophobic forces, the experiments were repeated with shorter carbon spacer SAMs (2-amino-ethanethiol). The length of the carbon spacer has been shown previously to strongly affect the low ionic strength force titrations of carboxylic acid terminated SAMs.[17] The data displayed in Figure 4 show the chemical force titration curves for combinations of carbon chain length amine SAMs (11:11, 11:2, and 2:2) on tip and substrate, respectively. Figure 4a shows the case already discussed where both tip and substrate are terminated with SAMs of 11 carbon spacer length. The peak adhesive forces observed are 9.8 and 5.4 nN at pH 6.1 and 9.7, respectively. A combination of 11 and 2 carbon spacer SAMs (Figure 4b) results in peaks at essentially the same position (pH 6.3 and 9.8), but the adhesion of the first peak is slightly larger (11.0 nN) whereas the second remains approximately constant. When both tip and

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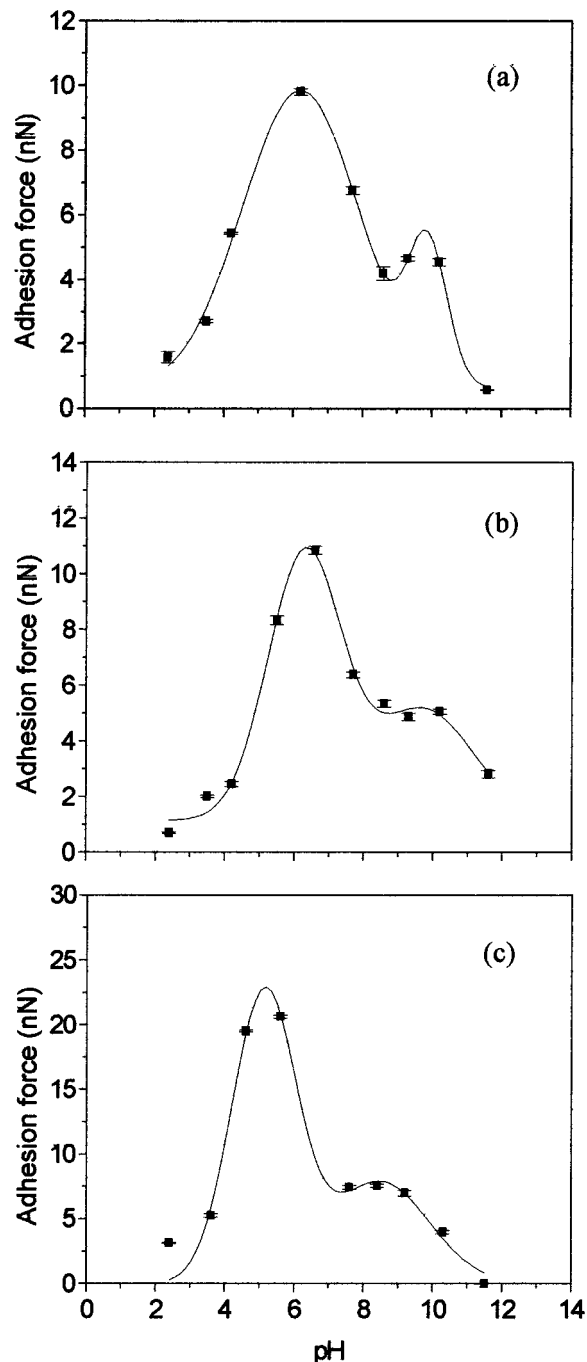(37) Stuart, B. *Biological Applications of Infrared Spectroscopy*; John Wiley and Sons: New York, 1980.

Figure 4. Chemical force titration curves obtained in low ionic strength (10^{-4} M) buffer for (a) both tip and substrate modified with an 11-amino-1-undecanthiol monolayer (alkyl spacer length 11 carbons), (b) tip modified with an 11-amino-1-undecanethiol monolayer and substrate with a 2-aminoethanethiol monolayer (alkyl spacer length 2 carbons on substrate), and (c) both tip and substrate modified with a 2-aminoethanethiol monolayer. (The curves have been added as a guide to the eye.)

sample are terminated with 2 carbon spacer SAMs (Figure 4c), the position of both peaks shifts to slightly lower pH

(pH 5.1 and 8.5) and the second peak is almost unaltered in strength ($\sim$7.9 nN) but the magnitude of the first peak almost doubles ($\sim$22 nN). We therefore conclude that the peak at lower pH is due to a hydrophobic interaction arising from SAM disorder because it increases in magnitude with a decrease in spacer length that is known to increase SAM disorder. The second peak is almost unaffected in magnitude or position by the carbon spacer length and therefore is probably electrostatic in origin. The precise origin of this second small peak in the force titration remains unclear, but one may postulate that it arises because of an electrostatic interaction between ionized acid groups and protonated amine groups on the two SAM surfaces. This attractive interaction would decrease as the number of ionized acid groups was reduced at lower pH and would also decrease as the number of protonated amine groups was reduced at higher pH, resulting in the peak observed.

Conclusions

We have reported, for the first time, on the effect of ionic strength and alkyl spacer length on the chemical force titration behavior of amine-terminated self-assembled monolayers. The force titration curves display a complex ionic strength and pH dependent behavior which we suggest is attributable to the presence of sulfonic acid groups in the SAM surface because of a competition between amine and thiol for binding to the gold substrate during SAM formation. Thiols exposed in the SAM surface would be oxidized rapidly, forming sulfonic acid which ionizes at higher pH.

An electrostatic repulsion between two electric double layers is responsible for the zero adhesion at low pH, and the large adhesion at intermediate pH is attributed to a hydrophobic interaction between exposed alkyl chains because of disorder in the SAMs. This hydrophobic force is very much reduced in low ionic strength conditions, and we tentatively propose that this is due to increased ordering in the SAM caused by strong *in-plane* ionic hydrogen bonds formed between ionized acid groups and neutral amines. Under high ionic strength conditions, strong association of ions from solution with the ionized acid groups prevents the formation of ionic hydrogen bonds, resulting in disorder and a large hydrophobic interaction when the two SAMs are brought together. The rapid drop in adhesion above pH 12 appears to be due to an electrostatic repulsion which is clearly observed in the approach part of the force–distance curve. This is attributed to the presence of the ionized sulfonic acid groups in the SAM surface.

Two peaks in the force titration were observed for these amine SAMs under low ionic strength conditions. By use of a range of different alkyl spacer length SAMs on the tip and substrate to control the degree of disorder in the SAM surface and hence the hydrophobic interaction, it was shown that the lower pH peak could be attributed to the hydrophobic interaction whereas the higher pH peak appeared to be electrostatic in origin, although further work is required to completely characterize this complex behavior.