J|A|C|S
ARTICLES

pH-Dependent Force Spectroscopy of Tri(ethylene Glycol)- and Methyl-Terminated Self-Assembled Monolayers Adsorbed on Gold

Christian Dicke[†] and Georg Hähner*

Contribution from the School of Chemistry, University of St. Andrews, North Haugh, St. Andrews, Fife KY16 9ST, U.K.

Received June 25, 2002

Abstract: Self-assembled monolayers (SAMs) of methoxy-tri(ethylene glycol)- (EG_3-OMe) and methyl-terminated alkanethiols (C_{16}) adsorbed on polycrystalline gold were investigated by chemical force spectroscopy. Measurements were performed in aqueous electrolyte solutions depending on ionic strength and pH value. Charged and hydrophobic tips were employed as probes to mimic local patches of proteins and to study the interaction at the organic/liquid interface in detail. Force−distance curves reveal information about the origin of the observed interaction and the underlying mechanisms. The measurements confirm an effective negative surface charge to be present at the oligo(ethylene glycol) (OEG) and the methyl interface and suggest that the charges are due to the adsorption of hydroxyl ions from aqueous solution. pH-dependent measurements further support the robustness of the established charge associated with the OEG films. Its sign does not change over the whole range of investigated values between pH ~3.5 and ~10. In contrast, the hydrophobic self-assembled hexadecanethiol films on gold show an isoelectric point (IEP) around pH 4. While the mechanism of charge establishment appears to be similar for both SA films, the strength of hydrogen bonding to interfacial water, which acts as a template for hydroxyl ion adsorption, is likely to be responsible for the observed difference.

Introduction

Understanding the mechanisms by which biomolecules adsorb to material surfaces, and controlling these interactions at the nanometer scale, will be essential if “smart (bio)materials” are to be successfully developed for biomedical applications. The interaction of biomolecules with organic interfaces is therefore of significant importance not only in natural environments and biological organisms but also for technological applications such as biosensors. An understanding of the forces acting between proteins and organic interfaces is highly needed, particularly in view of the rapidly growing field of “life sciences”.

Proteins can, taking a reductionist view, be thought of as being composed of hydrophobic and charged patches. It is necessary to determine the interaction of hydrophobic and charged entities with organic surfaces *independently* in order to understand the importance of the contributing factors. Chemically functionalized (charged and hydrophobic) scanning force microscope probes can mimic local structures of proteins and hence allow the influence of these parameters on the overall observed interaction to be studied separately. The nanometer scale contact between probe and surface resembles the size of the “typical” contact area between single biomolecules and surfaces and is therefore central to a fundamental understanding of these systems.

Of particular interest in the field of biosensors are films that have the capability to bind certain biomolecules specifically, while showing resistance to *nonspecific* adsorption at the same time. Nonspecific adsorption often screens the specific signal to be detected and hampers reasonable signal intensity.

The current trend to miniaturization and the related recent boost in nanotechnology have enhanced the need for ultrathin films of only a few nanometer thickness with reliable functional properties. A promising candidate for a monolayer film with resistance to nonspecific protein adsorption are oligoethylene glycol-terminated alkane based self-assembled monolayers (SAMs).[1] Their protein repelling properties have been at the center of interest in several studies.[1−6] The mechanism underlying inertness, however, is still being debated. The knowledge of this basic property is nevertheless a prerequisite for a tailoring of functional coatings with high reliability and reproducibility as well as potential applications. Experiments performed with the corresponding polymer poly(ethylene glycol) (PEG), which also shows inertness to nonspecific protein adsorption, and EG_3 functionalized SAMs on gold revealed that there is a fundamental difference in the repulsion mechanism.[1,3] The polymer

---

* Corresponding author. Phone: +44 (0)1334 463889. Fax: +44 (0)-1334 463808. E-mail: gh23@st-and.ac.uk.

[†] Also at: Department of Materials, ETH Zurich, ETH Zentrum, 8092 Zurich, Switzerland.

(1) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714.
(2) Harder P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis P. E. *J. Phys. Chem. B* **1998**, *102*, 426.
(3) Feldman, K.; Hähner, G.; Spencer, N. D.; Harder, P.; Grunze. M. *J. Am. Chem. Soc.* **1999**, *121*, 10134.
(4) Pertsin, A. J.; Grunze, M. *Langmuir* **2000**, *16*, 8829.
(5) Zolk, M.; Eisert, F.; Pipper, J.; Herrwerth, S.; Eck, W.; Buck, M.; Grunze, M. *Langmuir* **2000**, *16*, 5849.
(6) Dicke, C.; Hähner, G. *J. Phys. Chem. B* **2002**, *106*, 4450.

10.1021/ja027447n CCC: $22.00 © 2002 American Chemical Society

shows “steric repulsion”, which comprises high conformational flexibility of the chains and tightly bound water.[7−9] It is obvious that the flexibility of the OEG tails is restricted in a densely packed SA monolayer.[1] A repulsive force that is due solely to tightly bound water is usually of short range with a decay length of a few nanometers only.[10] In contrast, the forces that were observed on the SAMs are of long range (tens of nanometers) and strongly dependent on the ionic strength, which suggests that charges are contributing significantly.[3,6] Recent streaming potential and streaming current measurements show the preferential adsorption of OH^- on hydrophobic methyl-terminated self-assembled monolayers on gold.[11,12] A similar mechanism could be responsible for the charges found on EG films.

It is generally accepted that also water plays a central role in the observed interaction.[3] The exact mechanism, however, is not yet clear. It has been suggested that water molecules in the interfacial surface region are associated to the ethylene oxygen via hydrogen bonding.[4] The strength of the interaction of the water with the oxygen atoms in the ethylene glycol entities, which act as hydrogen bond acceptors, has also been emphasized.[13−15] Because of the dense and highly ordered SAM film, an overall average orientation of the water molecules associated with the EG interface leading to an effective surfaces dipole moment was proposed.[3,6]

To study the mechanism of charge establishment at the OEG interface of the SAMs in some more detail, we have undertaken the present investigation. We employed charged and hydrophobic atomic force microscope (AFM) probes, which can mimic local patches of proteins. Force spectroscopy, i.e., recording force profiles for chemically well-defined probes and model organic surfaces, can reveal the dominant interaction. It was recently reported that the pH value of an aqueous solution is a crucial parameter for the orientation of water molecules at a hydrophobic/liquid interface.[16] By changing the pH from very low to very high values, a “flip” in molecular orientation and the corresponding molecular dipole of about 180° was observed for weakly bonded interfacial water molecules at a hydrophobic surface.[16] This was attributed to a change in interfacial potential caused by a change in interfacial OH^- concentration. It is known that interfacial water can be highly ordered in comparison to bulk water.[17] The particular structure depends on the prevailing pH.[17,18] Ionic strength, charges, and pH have influence on interfacial water structures.[19]

In the present study we have mainly focused on the pH dependence of the force between functionalized probes and organic films. This should give some clue to the underlying mechanism, i.e., reveal the presence and importance of the strength of hydrogen bonds and on possible preferred adsorption of hydroxyl or hydronium ions from aqueous solution. In an earlier study we have shown that the force is independent from the type of added ions in solution.[6]

The contact area between two bodies is another important parameter in view of the relevant interactions. In fact the observed force behavior can, for example, in the case of hydrophobic forces, be quite different for nanoscopic and macroscopic contacts and depends on the actual size of relevant structures.[20] Force measurements with AFM might hence be more relevant for understanding protein adsorption than measurements with techniques that probe macroscopic contact areas. We therefore expect the phenomena described in the following to be of high relevance in biochemical and biophysical systems.

Experimental Section

Materials. EG_3-OMe (1-mercaptoundec-11-yl)tri(ethylene glycol) methyl ether was dissolved in ethanol (>99.8%, p.a., Merck AG, Dietikon, Switzerland) at a concentration of 2 mM.

Electrolyte solutions were prepared by dissolving the salts KNO_3 (>99.5%, p.a., Merck) and KCl (≥ 99%, Fluka Chemie AG, Buchs, Switzerland) in ultrapure water (EASYpure, Barnstead, 18 MΩ cm) at concentrations between 0.1 and 100 mM. These “neutral” solutions were around pH 6. Similar values are reported in the literature.[21]

Aqueous solutions of KOH (Ph. Eur. III, Siegfried Synopharm, Zofingen, Switzerland) and HCl (37%, Fluka) at a concentration of 1 mM were used to adjust series of pH values in the acidic and alkaline regions.

Preparation of Samples. Plasma-cleaned pieces of singly polished silicon (100) wafers (MEMC Electronic Materials, Inc., St. Peters, MO) were coated with a 5 nm thick chromium layer (99.99%, Unaxis, Materials Divison, Balzers, Liechtenstein) by electron beam evaporation to promote adhesion. Subsequently, 80 nm of polycrystalline gold (99.99%, Unaxis) were deposited via thermal evaporation in a Balzers BAE 370 coating system at a base pressure of 5×10^{-7} mbar and a deposition rate of 0.5−1 nm/s. The substrates were then immersed in the ethanolic solution of EG_3-OMe for typically 24 h.

C_{16} films were prepared by immersion of gold-coated Si wafers into 2 mM ethanolic solutions of 1-hexadecanethiol (Aldrich Chemical Co., Inc., Milwaukee, WI) for typically 24 h.

Preparation of SFM Probes. Oxide-sharpened silicon nitride AFM tips (Digital Instruments, Inc., Santa Barbara, CA) with nominal force constants of 0.06−0.12 N/m and nominal radii of curvature of ∼30 nm were employed with different functionalization for force measurements.

Before use, the tips were treated in oxygen plasma for 30 s to enhance the native oxide layer. Some tips were additionally covered with amorphous alumina (∼25 nm) using a sputter process (PSI Villigen, Switzerland). Prior to their use both silica and alumina probes were treated with “piranha solution” at ∼120 °C for 5 s. (Piranha solution is a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at a ratio of 7:3. *Caution!* It has to be handled with extreme caution, because it reacts strongly with many organic materials!) This treatment yields highly hydrophilic surfaces.

Hydrophobic tips were prepared by coating Si_3N_4 probes with chromium and gold simultaneously with the Si wafer. Subsequently, a SAM was adsorbed by exposing the cantilevers to a hexadecanethiol-enriched gas phase (∼24 h) in a metal desiccator.[3]

Instrumentation. Force measurements were performed with a commercially available scanning force microscope, Nanoscope IIIa (Digital), equipped with a liquid cell. Force−distance curves were collected using the “force−volume software” of DI at a cycle frequency

---

(7) Taunton, H. J.; Toprakcioglu, C.; Fetters, L. J.; Klein, J. *Nature* **1988**, *332*, 712.
(8) Jeon, S. I.; Andrade, J. D. *J. Colloid Interface Sci.* **1991**, *142*, 159.
(9) Harris, J. M. *Poly(Ethylene Glycol) Chemistry*; Plenum: New York, 1992.
(10) Israelachvili, J. N. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: London, 1992.
(11) Schweiss, R.; Welzel, P. B.; Werner, C.; Knoll, W. *Langmuir* **2001**, *17*, 4304.
(12) Schweiss, R.; Welzel, P. B.; Werner, C.; Knoll, W. *Colloid Surf. A* **2001**, *195*, 97.
(13) Ide, M.; Yoshikawa, D.; Maeda, Y.; Kitano, H. *Langmuir* **1999**, *15*, 926.
(14) Huang, L.; Nishinari, K. *J. Polym. Sci. B* **2001**, *39*, 496.
(15) Dreesen, L.; Humbert, C.; Hollander, P.; Mani, A. A.; Ataka, K.; Thiry, P. A.; Peremans, A. *Chem. Phys. Lett.* **2001**, *333*, 327.
(16) Scatena, L. F.; Brown, M. G.; Richmond, G. L. *Science* **2001**, *292*, 908.
(17) Du, Q.; Freysz, E.; Shen, Y. R. *Phys. Rev. Lett.* **1994**, *72*, 238.
(18) Kim, J.; Cremer, P. S. *J. Am. Chem. Soc.* **2000**, *122*, 12371.
(19) Kim, J.; Kim, G.; Cremer, P. S. *Langmuir* **2001**, *17*, 7255.
(20) Lum, K.; Chandler, D.; Weeks, J. D. *J. Phys. Chem. B* **1999**, *103*, 4570.
(21) Kokkoli, E.; Zukovski, C. F. *Langmuir* **2000**, *16*, 6029.

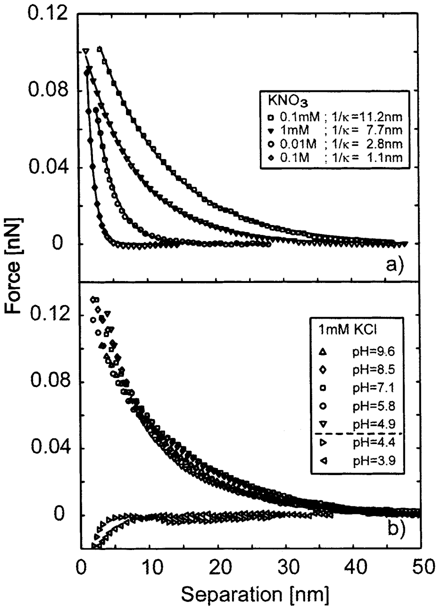

Figure 1. Force between an oxidized Si_3N_4 tip and an EG_3-OMe film on gold depending on (a) the ionic strength in aqueous KNO_3 solution at constant of pH ~ 6 and (b) the pH value in aqueous KCl solution at constant ionic strength of 1 mM. The dashed line in the legend of pH values indicates an attraction/repulsion transition.

of 0.3−0.5 Hz. Low-protein-binding PTFE filters (Semadeni AG, Ostermundigen, Switzerland) with 0.2 and 0.45 μm pore size were used to introduce electrolyte solutions into the liquid cell. pH values were adjusted and controlled with a pH meter (pH2001, Sentron, 9300 AC Roden, Netherlands) providing an accuracy of ~0.1 pH units. Due to a slight drift of the electrolyte solutions' pH value during exposure to air the overall error is estimated to be 0.2 pH units.

Results

Force between Oxidized Si_3N_4 Probe and EG_3-OMe Film.

Oxide-sharpened silicon nitride probes were employed for force measurements on EG_3-OMe films under aqueous KNO_3 solution at (neutral) value of pH ~ 6. The isoelectric point (IEP) of Si_3N_4 probes is typically between 5.5 and 7,[22] depending on the "history of the tips" and the amount of oxide being present. The IEP of SiO_2 has been reported to be around 2.[23] For oxidized Si_3N_4 tips IEPs decrease to values between 3.5 and 5.[24] At pH 6 such tips exhibit a total negative surface charge.[22]

Figure 1a shows averaged force-versus-distance curves recorded for salt concentrations ranging from 0.1 mM to 100 mM. Data processing was performed for 64 force−distance curves. At the lowest value of 0.1 mM a long-range repulsive force is detectable. The decay length of this force is 11.2 nm determined by fitting an exponential to the experimental data.[6] For the higher ionic strength of 1 mM the repulsive force shows a smaller decay length of 7.7 nm. Increasing the salt concentration further to 10 mM reduces the decay length of the repulsive force to 2.8 nm. At the highest concentration (100 mM), a short-range repulsive interaction is detectable with a decay length of 1.1 nm. Errors in the decay lengths are below 15%.[6] Errors in the force within a single force curve did not exceed 0.01 nN.[6]

The force curves recorded were very stable and did not show large variation over one sample. Different samples and probes did show deviating force curves in the sense that absolute force values can vary. The repulsive character, however, remained unaffected and the decay lengths did only differ within the limits given above. This is true for all repulsive force curves recorded.

To scrutinize the type and origin of the observed repulsive force, the pH value of the electrolyte solution was varied systematically. The ionic strength of the KCl solution was kept at a constant value of 1 mM during these measurements. Values between pH ~ 3.5 and ~ 10 could easily be adjusted by adding 1 mM KOH for the alkaline range and 1 mM HCl for more acidic conditions. The measurement cycles were started with the "standard" pH ~ 6, without any addition of KOH or HCl. The pH value was then reduced to acidic values. Subsequently, the standard value was set up again, and a test measurement was made to check for any possible film instabilities. All films showed reproducible results at this value. The pH value was then increased to the more alkaline regime. Finally another measurement with the standard pH value was performed for a further check, which confirmed good reproducibility and stability.

Figure 1b displays the pH-dependent force−distance measurements recorded on an EG_3-OMe film with an oxidized silicon nitride tip at constant ionic strength of 1 mM. All interaction forces for pH values above 4.4 are repulsive and of long range. The two lowest pH values (3.9 and 4.4) only display attractive forces. The repulsive curves show comparable absolute force values and similar decay lengths at constant ionic strength of the electrolyte solution for the different pH values.

Force between AlO_x Probe and EG_3-OMe Film. Measurements were also performed with probes that are positively charged at neutral pH to probe the electrostatic properties of the organic film independently. Literature values for the isoelectric point of alumina surfaces are between 8 and 10,[23,25] but values down to 6.7 have also been reported.[26] In consequence these tips carry a net positive surface charge at pH ~ 6.[23,25]

Force−distance curves recorded with alumina-coated probes depending on ionic strength at constant of pH ~ 6 are shown in Figure 2a. For all concentrations an attractive interaction of constant range was detectable between the EG_3-OMe sample and the positively charged alumina probe.

The pH-dependent measurements with the alumina probe at constant ionic strength of 1 mM are shown in Figure 2b. They display—as expected—a behavior different from that observed with Si_3N_4 tips: for "high" pH values (8.3 and 9.8) the probe experiences a long-range repulsion of about 10 nm. All force curves recorded at lower and more acidic pH values indicate attractive interactions.

Force between Hydrophobic C_{16} Probe and EG_3-OMe Film. Measurements with hydrophobic probes on EG_3 films depending on the ionic strength and the type of electrolyte have been reported earlier.[6] Figure 3a displays the forces between the EG_3-OMe sample and a hexadecanethiol-covered tip for different salt concentrations exemplarily. The observed interaction resembles a typical DLVO-like behavior with both surfaces carrying similar charges.[3,6]

---

(22) Lin, X.; Creuzet, F.; Arribart, H. *J. Phys. Chem.* **1993**, *97*, 7272.
(23) Parks, G. A. *Chem. Rev.* **1965**, *65*, 177.
(24) Marti, A. Ph.D. Thesis, ETH Zurich, Dissertation No. 12320, 1997.
(25) Sposito, G. *The Environmental Chemistry of Aluminium*, 2nd ed.; CRC Press: Boca Raton, FL, 1995.
(26) Yopps, J. A.; Fuerstenau, D. W. *J. Colloid Sci.* **1964**, *19*, 61.

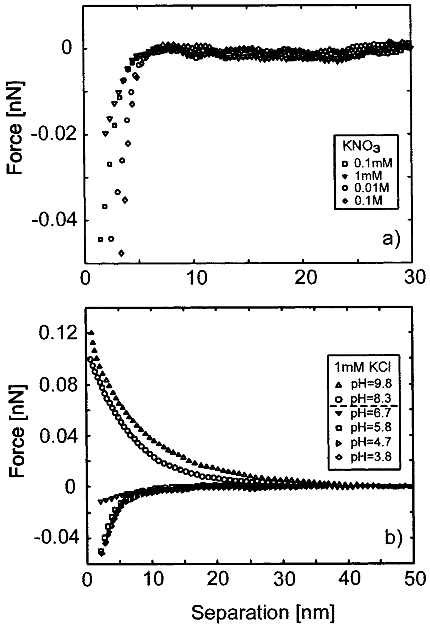

Figure 2. Force between an AlO_x tip and an EG_3-OMe film on gold depending on (a) the ionic strength in aqueous KNO_3 solution at constant of pH ~ 6 and (b) the pH value in aqueous KCl solution at constant ionic strength of 1 mM. The dashed line in the legend of pH values indicates an attraction/repulsion transition.

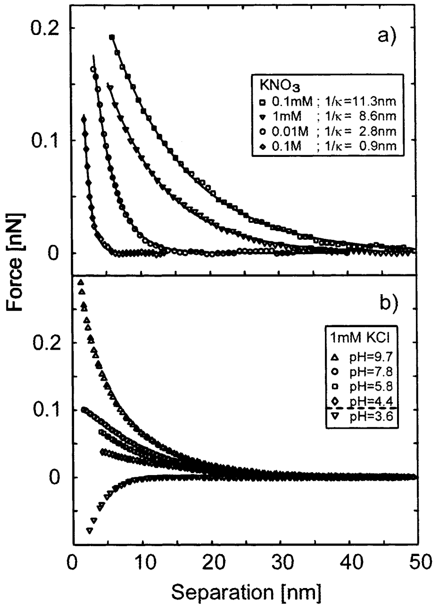

Figure 3. Force between a hydrophobic C_{16} coated tip and an EG_3-OMe film on gold depending on (a) the ionic strength in aqueous KNO_3 solution at constant of pH ~ 6 and (b) the pH value in aqueous KCl solution at constant ionic strength of 1 mM. The dashed line in the legend of pH values indicates an attraction/repulsion transition.

pH-dependent measurements with hydrophobic C_{16} probes at constant ionic strength of 1 mM are shown in Figure 3b. Long-range repulsive forces for pH values above 3.6 are observed. Solely for the lowest and most acidic value of 3.6 an attractive interaction is found.

It is also noteworthy that the absolute value of the repulsive force at a distance close to the sample surface is maximal at the highest pH value and decreases successively with lower hydroxyl concentrations until the interaction force becomes finally attractive, i.e., there is a gradual transition from repulsion to attraction.

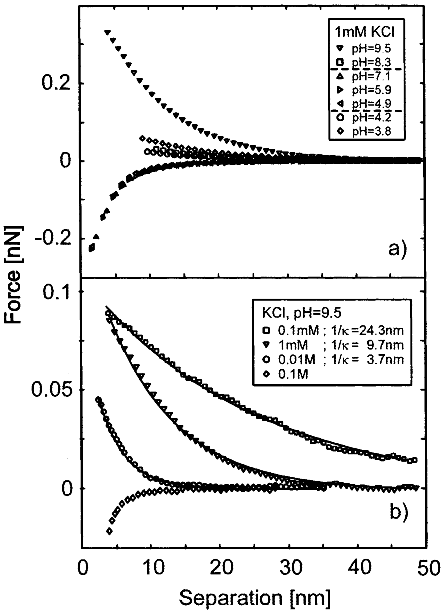

Figure 4. Force between hydrophobic C_{16} tip and a C_{16} film on gold depending on (a) the pH value in aqueous KCl solution at constant ionic strength of 1 mM and (b) the ionic strength in aqueous KCl solution at constant of pH 9.5. The dashed lines in the legend of pH values indicate attraction/repulsion transitions.

Force between Hydrophobic C_{16} Probe and C_{16} Film. To learn more about the observed interaction and the behavior of hydrophobic films under different pH conditions, additional measurements were performed for the symmetric system of two hexadecanethiol films adsorbed on gold.

A pH-dependent series of force curves for hexadecanethiol-covered tip and surface recorded at constant ionic strength of 1 mM is shown in Figure 4a. The force curves are attractive at "neutral" pH values and become repulsive for pH values below ~4 and above ~8. Jump-into-contact distances for the repulsive force curves measured for pH values 3.8, 4.2, and 8.3 were large compared to those found in the systems described above. Solely at pH 9.5, where the highest normal force can be observed, a repulsive interaction with shorter jump-into-contact distance was detectable similar to the systems involving EG_3-OMe.

In addition both the interaction at neutral pH of ~6 and at high and constant pH of 9.5 were studied depending on the ionic strength. While the force at pH 6 showed no dependence on ion concentration (as discussed in ref 6), the force at pH 9.5 exhibits a strong dependence on ionic strength and corroborates its electrostatic nature (see Figure 4b).

pH Values of Vanishing Force. Figure 5 presents the nominal values of the interaction forces at a constant distance between AFM probe and "hard wall"-potential depending on the pH value of the solution for the different probe/surface combinations investigated.

All graphs in Figure 5 show a transition from attraction to repulsion with increasing pH values for those systems involving EG_3-OMe films. The exact pH value of vanishing force varies with the probe material.

Remarkably, solely the symmetric C_{16}/C_{16} system displays two of these prominent pH values where the resulting overall interaction cancels. The measurements elucidate that in addition to pH 4.5 also at pH 7.6 the overall interaction force is zero. The lower value of pH 4.5 is similar to the one found for

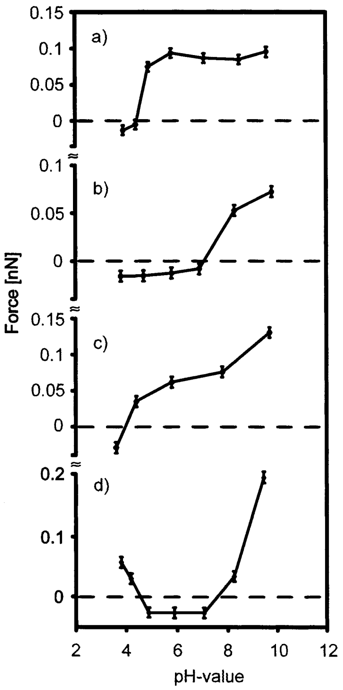

Figure 5. Force at constant distance between tip and surface in 1 mM aqueous solution depending on pH for (a) oxidized Si_3N_4 tip and EG_3-OMe film, (b) AlO_x tip and EG_3-OMe film, (c) C_{16} tip and EG_3-OMe film, and (d) C_{16} tip and C_{16} film. Tip−surface distances are 5 nm for a−c and 10 nm for d.

C_{16}/ EG_3 (pH ~ 4). In contrast to that combination, C_{16}/C_{16} shows an attractive interaction for the range of pH ~ 4−8, which at least at pH 6 does not depend on the ionic strength.[6]

Discussion

Dependence of Force on Ionic Strength at Constant (Neutral) pH ~ 6. All force-versus-distance curves recorded at constant neutral pH involving EG_3 films show a strong dependence on ion concentration, indicating that the dominant component of the observed interaction is electrostatic. The total interaction force between a charged sphere with radius, R, and a plane in electrolyte solutions can be described by the following equation, which is derived from DLVO theory assuming low surface potentials (ψ < 25 mV):[27]

$$F_{\mathrm{el}} = \frac{2\pi R}{\kappa\epsilon\epsilon_0}[(\sigma_1^{\,2} + \sigma_2^{\,2})e^{-2\kappa D} + 2\sigma_1\sigma_2 e^{-\kappa D}] \qquad (1)$$

with σ_1 and σ_2 being the surface charge densities, $1/\kappa$ the so-called Debye length, and D the separation between the two bodies. Note that an overall electrostatic interaction, and a finite force results even if one of the surfaces does not carry a net charge and hence the second term cancels. The resulting force is then repulsive. In such a case, however, the decay length of the exponential force would be half the value of the Debye length. In contrast, the decay lengths we found correlate quite well with the Debye lengths (except for the lowest concentration[6]), and hence those expected if both surfaces are charged. In consequence, the EG_3-OMe film must carry an effective surface charge. Its sign can be derived from the interaction with the negatively and positively charged probes. Since there is a repulsive interaction with negatively charged and attraction to positively charged tips, the effective surface charge of the OEG interface is negative.

In contrast to the systems where EG_3 is involved, the symmetric C_{16}/C_{16} combination displays a typical hydrophobic attraction at pH 6 and shows no dependence on ion concentration at this pH value.[6] It can be concluded from the C_{16}/EG_3 combination, however, that C_{16} also carries a negative surface charge at pH ~ 6. This is confirmed by recent streaming current measurements.[11,12] Since there is no sign of any ion concentration dependence at pH 6, the electrostatic component for C_{16}/C_{16} is negligible and the observed force is dominated by attractive hydrophobic interactions.

pH Dependence of Interaction at Constant Ionic Strength of 1 mM. Oxide surfaces such as silica and alumina display characteristic isoelectric points, or points of zero charge, if there is no specific adsorption of ions other than H_3O^+ or OH^-. The values of pH 4.4 and 7.1 found in Figure 5a,b for vanishing force when approaching the EG_3 film with oxidized Si_3N_4 and AlO_x probes, respectively, are in reasonable agreement with IEPs reported in the literature.[23,25,26] We therefore assign these pH values to the IEPs of the amphoteric silica and alumina probes.

Remarkably, such a distinctive pH value can also be found in the C_{16}/EG_3 system. At a pH of 4.0 the overall force vanishes and changes sign (Figure 5c), i.e., C_{16} appears to have an IEP at this pH. Recent streaming current measurements show a similar value for the IEP of these methyl-terminated alkanethiol films[11,12] confirming this assignment.

For the symmetric C_{16}/C_{16} system there are two such prominent pH values. The lower one is similar to the value determined for C_{16}/EG_3. In addition the force also vanishes around pH 7.6. At pH 9.5 there is a strong dependence of the repulsive force on ion concentration. This typical DLVO behavior indicates the main interaction force to be electrostatic under these conditions, in contrast to pH ~ 6, where hydrophobic attraction dominates any electrostatic component.[6]

If pH ~ 4 corresponds to the IEP of the methyl-terminated film, then at pH ~ 8 there must be a finite negative charge density present which counterbalances and then overcomes hydrophobic attraction. Consequently the charging mechanism is not symmetric. This is reflected by the ζ-potentials of these films determined by streaming current measurements[11,12] and those of drops of hexadecane and dodecane in aqueous solutions.[28]

The overall force at pH 8 would be simply a linear superposition of attractive hydrophobic and repulsive electrostatic forces if both are competing and are not affected by each other. What seems more plausible, however, is a more complex and nonlinear relation where the charge established at the surface can be viewed as a kind of chemical surface modification which alters the hydrophobic properties. Therefore it is difficult to extract a detailed picture at this pH without additional information.

Mechanism of Charge Establishment. One of the main questions that arises is for the origin of the electric surface potential at the organic/liquid interfaces and the mechanism of charge establishment at the organic films. Several scenarios can be imagined which describe a mechanism leading to negative surface charges. It was reported that hydrophobic oil droplets

---

(27) Butt, H.-J. *Biophys. J.* **1991**, *60*, 777.

(28) Marinova, K. G.; Alargova, R. G.; Denkov, N. D.; Velov, O. D.; Petsev, D. N.; Ivanov, I. B.; Borwankar, R. P. *Langmuir* **1996**, *12*, 2045.

of hexadecane also acquire a negative surface charge in aqueous solutions.[28] We follow a similar argumentation here:[28]

First, the negative charge could be due to the depletion of hydronium ions at the organic/liquid interface leading to an excess of (negative) hydroxyl ions close to the organic surface. Second, the orientation of water molecules and their corresponding dipoles in close vicinity of the organic surface could result in a dipolar field, and third, the adsorption of hydroxyl ions at the interface could be responsible for a negative surface charge density.

The adsorption of ions other than hydroxyl or hydronium can be ruled out based on an earlier study that we performed with different ions from the Hofmeister series.[6]

The depletion of (positively charged) hydronium ions is not realistic. Measured ζ-potentials of approximately -50 and -70 mV at pH 6 for C_{16} and EG_3, respectively,[11,29] correspond to excess ionic surface densities on the order of 4×10^{-9} and 7×10^{-9} mol/m^2. The calculation is based on the Grahame eq 2,[10] assuming that the excess charges are solely due to OH^- and that the surface potential, Ψ_S, is equal to the ζ-potential:

$$\sigma = \sqrt{8\epsilon\epsilon_0 kTC_{el}}\sinh\left(\frac{ze\Psi_S}{2kT}\right) \tag{2}$$

The latter assumption is satisfactory for low and moderate electrolyte concentrations.[30] C_{el} is the total bulk ion concentration. A simple estimation of the thickness, δ, of the depletion layers for the hydronium[28] then results in $\delta = 4$ μm and $\delta = 7$ μm, respectively. Similarly, a potential of -80 mV at pH 8 for both EG_3 and C_{16} films in 1 mM solutions[11,29] results in a thickness of the depletion layer of 1 cm. From a physical point of view, however, δ should not exceed the Debye screening length which is about 10 nm for 1 mM solutions.[10] This underlines that depletion can effectively be ruled out.

A long-range structured water interface was proposed to explain the observed long-range repulsion and the negative surface charge density.[3,31] A long-range directional ordering of water molecules occurring at the boundary to a nonpolar organic face is associated with a loss in entropy. The close approach of two such surfaces would lead to the expulsion of some amount of the oriented molecules into the bulk water phase, which is an entropically favored process.[10] If such a long-range parallel ordering of molecules takes place, it should lead to a potential difference, Ψ, between the bulk water and the interface. Different ions from the Hofmeister series should have a different effect on such a structured water interface and hence the resulting range and surface charge density. The measurements performed earlier, however, did not show any systematic trend for different ions.[6] Therefore this model is also not very likely.

The measured strong pH dependence of the forces involving EG_3 suggests that the most probable mechanism for the negative interfacial charging is the “adsorption” of hydroxyl ions at the organic/liquid interface. From an electrostatic point of view, however, the close approach of an ion to the boundary between water and another phase of low dielectric constant is energetically not favorable.[32] In consequence, ion adsorption can take place only if it is promoted by some specific (not long-ranged electrostatic) interaction of the ion with the interface. This could originate either from the particular structure of the water molecules at the interface or the specific interaction of the ions with the organic molecules.

In the case of methyl-terminated films the first hypothesis is supported by the fact that the boundary water molecules are highly ordered at the interface with hydrocarbon phases.[10] Theoretical considerations of the boundary between water and nonpolar fluids have shown that the interfacial water molecules are preferentially oriented with the oxygen atoms toward the hydrophobic phase.[33,34] The adsorption of hydroxyl ions can then be explained with strong dipole or hydrogen bonding of the OH^- with the hydrogen atoms of the interfacial water molecules. The hydrogen bond between OH^- and water molecules is usually classified as “strong” with almost 60 kT per bond.[35] In the bulk aqueous phase fractions of the H-bonds between the water molecules are broken due to Brownian motion. Therefore, the specific adsorption is a result of the restricted mobility of water molecules in the interface establishing a stagnant layer, which allows more pronounced hydrogen bonding of the OH^- and the neighboring water molecules accompanied by a respective free energy gain.

For the OEG containing molecules direct adsorption appears possible since similar to water also H_3O^+ and OH^- could establish hydrogen bonds to the oxygen of the EG units. However, in case of direct adsorption of ions the polymer should display a behavior similar to the monolayer films, which was not observed.[3] We interpret the fact that the potentials for EG_3 and C_{16} are similar at pH 8[11,29] as an indicator that the mechanism of charge establishment is also similar for both systems.

Difference between EG_3-OMe and C_{16} Films. The most striking difference between EG_3-OMe and methyl-terminated C_{16} films is the fact that EG_3-OMe does not change the sign of the surface charge density over the whole pH range investigated. A possible reason for the different behavior might be found in the strength of the hydrogen bond of interfacial water to the synthetic organic films. While *weakly* hydrogen bonded water molecules in the interfacial region of the hydrocarbon/water system are emphasized in ref 16, where a pH change was found to cause a flip in orientation of water molecules and hence a reorientation of molecular dipoles, several other studies emphasize the *strong* interaction of water with the oxygen atoms of ethylene glycol entities, which act as hydrogen bond acceptors.[13−15] It has been suggested that this particular influence of strong hydrogen bonds over neutral “weak” hydrogen bonds on interaction forces in electrolyte solutions close to organic model surfaces is due to a reduction of the local dielectric constant of the solvent.[36] Such a strong hydrogen bond at the EG-terminated SAM surface would explain the persistence of the water molecules at the EG_3-OMe surface over the broad pH range investigated in our experiments.

In a study that is concerned with pH-dependent adsorption of polyions on structured COOH/EG_3-OMe terminated gold

---

(29) Grunze, M.; Chan, M. Private communication.
(30) Hunter, R. J. *Zeta Potential in Colloid Science: Principles and Applications;* Academic Press: New York, 1981.
(31) Wang, R. L. C.; Kreuzer, H. J.; Grunze, M. *J. Phys. Chem. B* **1997**, *101*, 9767.
(32) Landau, L. D.; Lifshitz, E. M. *Electrodynamics of Continuous Media*, 2nd ed.; Pergamon Press: Oxford, U.K., 1984.
(33) Conway, B. E. *Adv. Colloid Interface Sci.* **1977**, *8*, 91.
(34) Dubrovich, N. A. *Kolloid Z.* **1995**, *57*, 275.
(35) Spangler, D.; Christoffersen, R. E. *Int. J. Quantum Chem.* **1980**, *17*, 1075.
(36) Smith, D. A.; Wallwork, M. L.; Zhang, J.; Kirkham, J.; Robinson, C.; Marsh, A.; Wong, M *J. Phys. Chem. B* **2000**, *104*, 8862.

surfaces[37] it was found that at low-pH conditions strong hydrogen bonding at the ether and alcohol oxygens of the EG sites give rise to an enhanced adsorption of polyions offering hydrogen bond donor sites. In addition, hydrophobic interactions of the ethylene groups of the EG repeat units with polyions having hydrophobic backbones are proposed to further support the adsorption. The strong tendency of the EG entities for hydrogen bonding in particular at low pH supports our interpretation of a strong association of water molecules to the EG_3-OMe SAM via hydrogen bonds.

Theoretical studies on the EG_3-OMe/water interface discuss the high surface density of hydrogen bonds between EG units and water molecules as the critical parameter leading to the resistance to protein adsorption of these films.[4,38,39] It is suggested that water molecules are able to penetrate the SAM in such a way that particularly the topmost ethylene and the alcoholic oxygens are involved in hydrogen bonding with water molecules.[4,5]

While from an electrostatic point of view both organic films EG_3-OMe and C_{16} look similar with comparable negative surface potentials for certain pH values,[11,29] the stability of the established charges could make all the difference. A prerequisite for the charges is a stagnant water interface associated with the organic film. Such layers are found on rough and smooth charged surfaces, and it has been speculated that they also persist on uncharged surfaces.[40] A stronger interaction of water with EG units could be responsible for the robustness of the interfacial charges of the OEG films toward pH change. In contrast, more weakly bonded water at the C_{16} interface would lead to a breakdown of the water interface itself and its associated surface charges that are due to hydroxyl ions, resulting in a change of sign when varying the pH.

Finally, the fact that PEG polymers do not show long-ranging electrostatic forces in electrolyte solutions could be due to the size of “typical structures”, i.e., the local density of EG units and the distances between them. The concept of hydrophobicity is closely related to hydrogen bonded networks of water and the ability of water molecules to establish local networks.[20] These depend on the structures established by the organic molecules and how they affect the number of hydrogen bonds that can form. The extended EG structures of the SA films do not allow the same water arrangement as the polymer chains. The extended region created by the densely packed SAM depletes the number of hydrogen bonds that can be established between neighboring water molecules, while hydrogen bonding can simply go around the free PEG polymer chains. As a result OH^- association does not occur or is very weak in the case of the polymer. If the OEG structure, however, is too densely packed such as the EG_3-OMe SAM on silver, a hydrophobic behavior similar to C_{16} films is observed[2,3] accompanied with a loss of protein resistance.[2]

We interpret the stability of the repulsive force curves as an indicator that the density of EG units and not the specific configuration of the molecules is the crucial parameter. The films are likely to have a significant number of defects and domain boundaries, which however do not affect the repulsive force observed.

The differences found for the polymer PEG and SAMs of EG_3-OMe and C_{16} might well be closely related to the “two faces of water” in connection with the specific interaction of water with the organic molecules. These two faces play an important role in hydrophobic interactions and protein folding processes and were recently described by Chandler.[41] The crossover occurs at nanometer length scales, when the local concentration of apolar units is sufficiently high or when an apolar surface is sufficiently large.

Conclusions

Chemical force spectroscopy on tri(ethylene glycol)-containing and methyl-terminated self-assembled monolayers under liquids utilizing differently functionalized tips was employed to mimic the interaction between proteins and model organic surface. The crucial parameters were the tip functionalization as well as pH value and ionic strength of the electrolyte solution. Force-versus-distance measurements performed with hydrophobic and charged probes reveal a difference in the forces observed on the organic films.

We assign the establishment of a negative surface charge on both monolayer films to the adsorption of hydroxyl ions from solution. Crucial for the stability of the surface charge appears to be interfacial water that is associated with the organic films and which acts as a template for hydroxyl adsorption. While charges on C_{16} show a dependence on the pH, its influence on those associated to EG_3-OMe was found to be minor. The measurements suggest that the template for OH^- adsorption created by the immobilized interfacial water is more stable in the case of the OEG interface compared to hydrophobic methyl groups.

A further crucial parameter appears to be the local density of molecular units, which determines the networks that can be established by water molecules. This might well account for the different behavior shown by the polymer and the EG_3-OMe films.

Acknowledgment. The authors thank N. Spencer, ETH Zurich, for use of his AFM instrument and laboratory infrastructure. We also thank S. Herrwerth, W. Eck, and M. Grunze, University of Heidelberg, for providing the (1-mercaptoundec-11-yl)tri(ethylene glycol) methyl ether and M. Chan and M. Grunze for sharing scientific results prior to publication with us. Financial support from ETH Zurich (ETH-Fund No. 0-20778-00) is gratefully acknowledged. G.H. thanks the Deutsche Forschungsgemeinschaft (DFG) for a Heisenberg Fellowship.

JA027447N

---

(37) Clark, S. L.; Hammond, P. T. *Langmuir* **2000**, *16*, 10206.
(38) Wang, L. C.; Kreuzer, H. J.; Grunze, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 3613.
(39) Pertsin, A. J.; Hayashi, T.; Grunze, M. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1598.
(40) Lyklema, J.; Rovillard, S.; De Coninck, J. *Langmuir* **1998**, *14*, 5659.
(41) Chandler, D. *Nature* **2002**, *417*, 491.