

Monitoring Multilayer Film Growth with the Atomic Force Microscope. Aluminum(III) Alkanebisphosphonate Multilayer Films and DNA Immobilization

Kai Hu, Radha Pyati,[†] and Allen J. Bard*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712

The growth of aluminum(III) alkanebisphosphonate multilayer thin films on gold surfaces in aqueous solutions was investigated by probing the surface charge following alternate treatments with anionic phosphonate and cationic Al(III). This was accomplished by determining the force between a modified silica tip of an atomic force microscope (AFM) and the film-covered gold substrate. The AFM force measurements revealed that the formation of the films followed a regular layer-by-layer growth mechanism as evidenced by the occurrence of surface charge reversal with each adsorption step. However, the quantitative surface charge data, obtained by theoretical fits of the force data to solutions of the complete nonlinear Poisson–Boltzmann equation with a knowledge of the silica probe surface potential, indicated that the films became less ordered with an increase in the number of layers. The AFM force measuring technique was also employed to monitor the immobilization of both single-stranded (ss) and double-stranded (ds) DNA on positively charged surfaces (i.e., aluminum(III)- and ammonium-terminated surfaces) and their subsequent interactions with a transition metal chelate, Ru(phen)₃²⁺. The force measurement results showed that both the ss-DNA and ds-DNA could be immobilized on positively charged surfaces, while only the ds-DNA showed interaction with Ru(phen)₃²⁺.

Mallouk and co-workers first described the technique in which films were formed by the sequential adsorption of the components of di-, tri-, or tetravalent metal phosphonate salts from aqueous and nonaqueous solutions. Films assembled in this way are structurally analogous to layered metal–organic compounds, in which the metal–oxygen–phosphorus network is held together by strong ionic and covalent bonds.^{1,2} An aluminum(III) alkanebisphosphonate multilayer film represents an example of these systems where the interlayer connections are established through strong ionic–covalent bonds. Characterization of the structure

of these multilayer films and monitoring of film growth are currently carried out by quartz crystal microbalance, reflectance infrared, ellipsometric, or electrochemical measurements. While each of these techniques is very useful for characterization of a range of surface processes, none of them directly yields information about film surface charge state. However, recent studies with an atomic force microscope (AFM) have shown that surface charge can be determined by direct force measurements between a sphere and a flat surface.^{3–10} This direct measurement of surface force has allowed the in situ measurement of surface charge and the study of interfacial processes. In a typical AFM force measurement, the deflection of a microfabricated cantilever is measured as a function of its separation from a surface. In this work, we are particularly interested in the change of surface charge with each adsorption step and its relationship with the growth of the film. The principle of the measurement is shown in Figure 1. The deflection of the AFM cantilever was monitored by the movement of a laser beam reflected by the end of the cantilever. This deflection was caused by interfacial forces between the negatively charged silica sphere attached to the cantilever and the surface under study. In this experimental study, the growth of aluminum(III) alkanebisphosphonate multilayer thin films on gold surfaces in aqueous solutions was investigated.

The immobilization of DNA on a self-assembled thin film is of interest both in studies of molecular recognition of DNA and in various applications.^{11–13} For example, the DNA must be firmly anchored to a surface to minimize the effects of tip perturbations when scanning probe techniques are applied to the study of DNA.¹⁴ Immobilization of DNA on an electrode is also used to

[†] Current address: Department of Chemistry, University of Colorado at Colorado Springs, Colorado Springs, CO 80933-7150.

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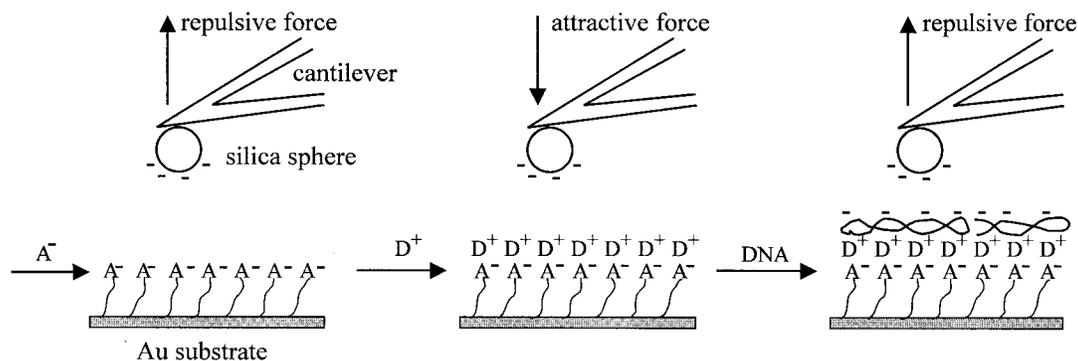


Figure 1. Schematic representation of the AFM force measurement between a negatively charged silica sphere and the gold as the thin film was grown.

produce biosensors, e.g., those that can recognize the base sequence on a complementary strand of DNA.¹⁵ In this latter application, a single strand of DNA is attached to an electrode surface in a manner that still permits access to the bases and allows hybridization with a complementary DNA strand in solution. In previous studies, we demonstrated that an aluminum(III) alkanebisphosphonate film can be used to immobilize both single-stranded (ss) and double-stranded (ds) DNA, based on the interaction of the film's positive metal center (Al^{3+}) with the phosphate group of DNA, and that $\text{Ru}(\text{phen})_3^{2+}$, which only associates with immobilized ds-DNA, can be detected by electro-generated chemiluminescence (ECL).^{16,17} Noting the possible surface charge changes that accompany these processes, we revisit these systems and employ AFM force measurements to probe the surface charge. More specifically, with the aluminum(III)- and ammonium-terminated thin films on gold as the substrates, an AFM was used to monitor the immobilization of both ss-DNA and ds-DNA on these positively charged substrates and their subsequent interactions with a metal chelate, $\text{Ru}(\text{phen})_3^{2+}$. To our knowledge, this is the first demonstration of AFM to measure quantitatively the surface charge of immobilized DNA.

EXPERIMENTAL SECTION

Materials. $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Spectrum Chemical MFG), NaH_2PO_4 (Baker), $\text{Ru}(\text{phen})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ (Aldrich), 2-mercaptoethanesulfonic acid, sodium salt (Aldrich), and diethyl methylphosphonate (Aldrich) were used as received without purification. The bisphosphonic acid, $\text{H}_2\text{O}_3\text{P}(\text{CH}_2)_4\text{PO}_3\text{H}_2$ (C_4BPA), was generously provided by the Mallouk group (Pennsylvania State University). Mercaptomethylphosphonic acid ($\text{HSCH}_2\text{PO}_3\text{H}_2$) was prepared from diethyl (mercaptomethyl)phosphonate by refluxing in concentrated HCl, and diethyl (mercaptomethyl)phosphonate was prepared from diethyl methylphosphonate.¹⁸ (2-Mercaptoethyl)trimethylammonium chloride ($\text{HS}(\text{CH}_2)_2\text{N}(\text{CH}_3)_3\text{Cl}$) was prepared from (2-mercaptoethyl)trimethylammonium bromide acetate (Aldrich) by refluxing in methanol solution with 1 equiv of concentrated HCl for 6 h.

The eight-base ss-DNA sample, 5'GCCGCCAT3', was donated by Boehringer Mannheim Co. The calf thymus ds-DNA was purchased from Sigma. Deionized water from a Millipore Milli-Q (18 M Ω) system was used to prepare all aqueous solutions and to rinse the film surfaces.

Silica substrates were prepared from commercial glass cover slips (M6045-2, Baxter Healthcare Corp., McGaw Park, IL). Before each experiment, the silica substrates were cleaned in a concentrated nitric/sulfuric acid (1:1) solution and rinsed thoroughly with deionized water followed by exposure to condensing steam vapor for 30 min. AFM images obtained from the silica surfaces indicated a mean roughness of 1.1–1.4 nm/ μm^2 with a maximum peak to valley height of 3.5–4.7 nm over a 1 μm square.

Large, flat, template-stripped gold surfaces were prepared following the method of Hegner et al.¹⁹ Briefly, gold was vacuum-deposited onto freshly cleaved mica sheets. The vapor-deposited gold surface (200 nm in thickness) was then glued (Epo-tek No. 377, Polyscience) to a piece of precleaned Si wafer. After chemical stripping (in THF) of the gold from the mica, a smooth gold surface on Si was obtained. AFM images of this type of template-stripped gold surfaces showed a mean roughness of 0.20–0.33 nm/ μm^2 with a maximum peak to valley height of 2.5–2.8 nm over a 1 μm square. All multilayer film preparation and force measurements were conducted on the gold surfaces prepared in this way.

Aluminum(III) Alkanebisphosphonate Thin-Film Preparation. The freshly prepared gold surface was immediately immersed in a 5 mM mercaptomethylphosphonic acid or a 5 mM (2-mercaptoethyl)trimethylammonium chloride ethanolic solution for over 24 h. Prior to use, the thiol-derivatized gold substrate was rinsed with EtOH for 30 s and dried under argon. The strategy for fabrication of the aluminum(III) alkanebisphosphonate multilayer film by sequential adsorption/reaction steps followed previous practice^{16,17} and is illustrated in Figure 2A. The phosphonic acid-terminated surface was alternately immersed in a 5 mM $\text{Al}(\text{NO}_3)_3$ (pH 4.0) aqueous solution for at least 30 min and in a 5 mM C_4BPA (pH 5.0) aqueous solution for at least 30 min. After each immersion step, the substrate was rinsed with copious amounts of water. For ex situ AFM force measurements, films prepared in this way were used as substrates after drying under argon. For in situ force measurements, a thiol-derivatized, phosphonic acid-terminated gold substrate was mounted in an

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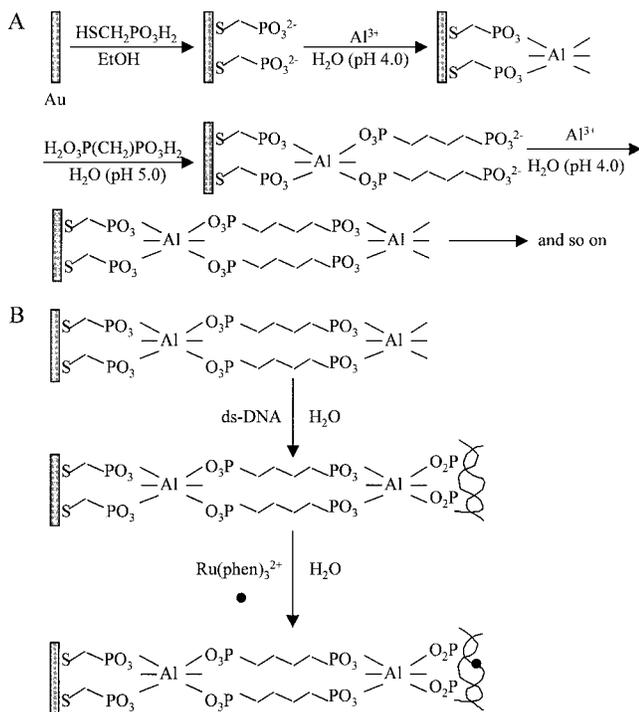


Figure 2. Schematic representation of (A) sequential steps in the formation of aluminum(III) alkanebisphosphonate multilayer films on a gold surface and (B) immobilization of DNA on the film and interaction of Ru(phen)₃²⁺ with the immobilized DNA.

AFM liquid cell (Digital Instruments, Santa Barbara, CA). After the force curves for this first layer were collected in a 1 mM KCl (pH 6.0) aqueous solution, the KCl solution was replaced by a 5 mM Al(NO₃)₃ (pH 4.0) aqueous solution. After 30 min of adsorption, the Al(III) solution was removed and the cell was washed by passing at least 10 mL of deionized water. Force curves were collected for this aluminum(III)-terminated surface in the same KCl solution. This was followed by adsorption of C₄BPA for 30 min. Force measurements were repeatedly conducted after each adsorption and washing step.

DNA Layer Preparation. For ss-DNA layers, an eight-base DNA strand was dissolved in 0.2 M phosphate buffer (pH 7.0) to give a solution of 275 μM (base pair) DNA. The aluminum(III)- or ammonium-terminated substrates and the DNA solution were placed in a Petri dish and agitated in a laboratory shaker (Gyrotory Shaker Model G2, New Brunswick Scientific Co., Edison, NJ) for 30 min at 50 rpm. The substrates were then removed from the Petri dish, rinsed with H₂O, and gently dried with argon.

For ds-DNA layers, calf thymus double-strand DNA was dissolved in 5 mM Tris buffer with 50 mM NaCl by stirring gently overnight. The DNA concentration of this solution was 1.34 mM (base pairs) as measured by UV absorbance of a 1:10 dilution and use of the molar absorptivity (at 260 nm) of 6600 M⁻¹ cm⁻¹ per mole of nucleotide phosphate to calculate concentration.²⁰ The substrates were agitated in the 1.34 mM DNA solution in the shaker for 30 min at 50 rpm, rinsed with H₂O, and dried with argon.

After force measurements on both the ss-DNA and ds-DNA-coated substrates, they were soaked for 1 h in 1 mM Ru(phen)₃²⁺

aqueous solution, rinsed with H₂O, and dried with argon. The procedure for DNA layer formation is illustrated in Figure 2B.

AFM Force Measurements. All force measurements were performed with a Nanoscope III AFM (Digital Instruments) equipped with a piezoscanner having a maximum scan range of 15 μm × 15 μm × 2 μm. The standard AFM silicon nitride tip was modified by the attachment of a spherical silica bead. The spring constant of the silica sphere modified cantilever, determined using the method of Cleveland et al.,^{21,22} was 0.65 ± 0.12 N/m. The AFM force measuring technique is well-documented,^{3–10} and the experimental details have been described elsewhere.^{10,23–25} Typically, the AFM was operated in the force mode, in which the xy-raster motion of the sample on the piezoelectric crystal was turned off and the substrate was moved toward and away from the cantilever in the z direction by applying a sawtooth voltage. This allowed the force between a surface and the AFM tip to be measured as a function of the separation between the surface and tip.

AFM Data Analysis. Force–separation curves were obtained by recording the voltage from the split photodiode detector and the substrate displacement as given by the applied piezovoltages. The photodiode voltages and piezovoltages were converted via calibration standards to a normalized force (force/radius) versus tip–substrate separation for further analysis. Derjaguin–Landau–Verwey–Overbeek (DLVO) theory^{26–29} was employed to calculate the surface electrostatic potentials between the similarly charged surfaces, i.e., the interaction between two silica surfaces. The electrical double-layer interaction energy between dissimilarly charged surfaces such as the silica–gold interaction was calculated for the constant-charge limit of the complete nonlinear Poisson–Boltzmann equation using the method of Hillier et al.¹⁰ The Hamaker constants (A_H) used for the theoretical calculations were 0.88 × 10⁻²⁰ J^{30–32} and 1.1 × 10⁻¹⁹ J^{10,23,24} for the silica–silica and silica–gold interactions, respectively.

RESULTS AND DISCUSSION

Silica Probe Calibration. Typically, to measure quantitatively surface charge of a substrate, the surface electrostatic potential of the probe must be known in an AFM force measurement. Force measurements between a silica sphere and a silica substrate were conducted as described previously^{3,4,10,23–25} to determine the silica surface potential (ψ_p) under solution conditions similar to those used while probing the diffuse double layers at the gold substrates covered with aluminum(III) alkanebisphosphonate thin films. The silica surfaces, isoelectric point about pH 2.0, are

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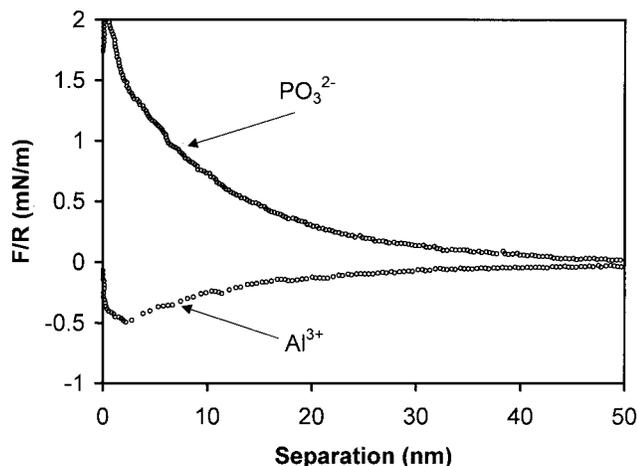


Figure 3. Forces obtained in 1 mM KCl aqueous solution of pH 6.0 between the silica probe and the thiol-derivatized phosphonate-terminated gold substrate before and after immersing the substrate into the aluminum(III) aqueous solution. The force is scaled to the probe radius ($R = 8.0 \mu\text{m}$).

negatively charged in a 1.0 mM KCl solution of pH 6.0 and diffuse double layers form at the silica–solution interfaces. AFM force measurements can probe the double-layer structure and thickness as the tip penetrates the double layer. Generally, these force–distance curves exhibit an exponential dependence with distance that is well predicted by standard DLVO theory.^{26–29} The measured silica surface potential was -41 mV under these conditions. The corresponding Debye length, κ^{-1} , of the diffuse double layer was 9.6 nm, in good agreement with that calculated from the salt concentration of the solution.³³

Aluminum(III) Alkanebisphosphonate Multilayer Thin Films. Multilayer thin films of divalent metal (Zn and Cu) alkanebisphosphonates prepared on gold surfaces modified with (4-mercaptopbutyl)phosphonic acid by sequential adsorption/reaction steps have been previously characterized by quartz crystal microbalance, reflectance infrared, and ellipsometric measurements.³⁴ The interlayer connections that assemble the monolayers into ordered multilayers are based upon ionic–covalent interactions. With the gold surfaces modified with mercaptomethylphosphonic acid as the substrates, we obtained an electrostatic repulsive force–separation curve (Figure 3) in a 1 mM KCl aqueous solution of pH 6.0, indicating that the gold substrate was negatively charged. As reported in early studies,^{10,23} an attractive interaction is exhibited between a silica probe and a clean gold surface in deionized water at neutral pH. Thus, the negative charge on the gold substrate can be attributed to the anchored terminal phosphonate ions. After the gold substrate was immersed in a 5 mM $\text{Al}(\text{NO}_3)_3$ solution of pH 4.0 for 30 min followed by thorough washing with water, force measurements in the same aqueous solution (1 mM KCl, pH 6.0) produced an attractive force curve, showing that the gold substrate became positively charged after immersion in the aluminum(III) solution. Apparently, aluminum(III) ions bound to the surface through the aluminum–

oxygen–phosphorus network render the surface positive charge and effect the observed charge reversal of the substrate. Repetitive force measurements following each adsorption–reaction–washing step consistently showed alternating changes between positive and negative surface charge (up to 10 layers), suggesting that the formation of the multilayer films follows a regular layer-by-layer growth mechanism.

During the force measurements, although electrostatic repulsive and attractive forces were repeatedly observed during alternating anion and cation treatments, the magnitude of both types of forces gradually and continuously decreased with the number of layers. In situ and ex situ force measurements were both conducted and yielded essentially the same results. However, in situ force measurements did show better reproducibility. While aluminum(III) worked very well for the multilayer growth, other metal ions showed a poorer binding to the phosphonate surface. For example, in separate experiments, both Ba^{2+} and Pb^{2+} ions did not show any binding ability to the phosphonate surfaces (i.e., did not show any decrease in the phosphonate repulsive force). Similarly, unlike the phosphonate functionality, a sulfonate-terminated surface showed a much weaker anchoring ability to aluminum(III).

The diffuse double layer reflects the magnitude of surface charge of a substrate immersed in an aqueous solution. In this work, the silica probe interrogated the gold surface covered with multilayer films. The attractive force between the negatively charged silica tip and the aluminum(III)-terminated gold surface shown in Figure 3 can be explained in terms of the nature of the diffuse double layer. In the force measurement, the silica spherical tip probes the diffuse double layer near the gold substrate, which consists of counterions that compensate the charge residing at the interface. For the aluminum(III)-terminated gold surface immersed in a 1 mM KCl aqueous solution of pH 6.0, the net surface charge of the gold substrate is positive, and a diffuse double layer, consisting of a higher local concentration of Cl^- and OH^- and lower local concentration of K^+ and H^+ , with a net negative charge forms near the gold substrate. Therefore, as the negatively charged silica probe (with its own positively charged diffuse double layer) penetrates this double layer near the gold substrate, the overlap and interaction of the two oppositely charged double layers results in an electrostatic attractive force.

Theories describing the forces between interacting double layers in electrolyte solutions are well-developed. Generally, for similarly charged surfaces at low surface potentials, the linearized form of the Poisson–Boltzmann equation yields the same results as DLVO theory. For dissimilarly charged surfaces, the complete nonlinear Poisson–Boltzmann equation must be solved due to the presence of an additional Maxwellian stress term that represents an induced image charge. In this work, the method of Hillier et al.¹⁰ was used to calculate the electrical double-layer interaction between silica and gold surfaces. This model of the interaction force between two dissimilarly charged surfaces has proven extremely successful in matching experimental observations for a variety of systems.^{10,23–25} With the knowledge of silica probe surface potential, the surface electrostatic potentials (ψ_s) of the thin-film-covered gold substrates were obtained by theoretical fits of the force data to solutions of the complete nonlinear

(33) For dilute aqueous solution containing 1:1 electrolyte at 25 °C, the Debye length is given by $\kappa^{-1} = 0.3045/C^{1/2}$, where C is the salt concentration of the 1:1 electrolyte.

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Table 1. Force Data Analysis Results of the Aluminum(III) Alkanebisphosphonate Multilayer Thin Films as a Function of Layer Number^a

| layer no. | surface potential, ψ_s (mV) | | surface charge, σ ($\mu\text{C}/\text{cm}^2$) | |
|-----------|----------------------------------|------------------|--|-------------------|
| | PO_3^{2-} | Al^{3+} | PO_3^{2-} | Al^{3+} |
| 1 | -65 ± 2 | 12 ± 2 | -0.605 ± 0.029 | 0.087 ± 0.015 |
| 2 | -65 ± 2 | 11 ± 2 | -0.605 ± 0.029 | 0.080 ± 0.015 |
| 3 | -63 ± 3 | 11 ± 2 | -0.578 ± 0.041 | 0.080 ± 0.015 |
| 4 | -60 ± 3 | 9 ± 3 | -0.539 ± 0.040 | 0.065 ± 0.022 |
| 5 | -56 ± 4 | 10 ± 2 | -0.489 ± 0.050 | 0.073 ± 0.015 |
| 6 | -55 ± 3 | 8 ± 3 | -0.477 ± 0.037 | 0.058 ± 0.022 |
| 7 | -56 ± 2 | 7 ± 2 | -0.489 ± 0.025 | 0.051 ± 0.015 |
| 8 | -53 ± 3 | 5 ± 3 | -0.454 ± 0.035 | 0.036 ± 0.022 |
| 9 | -52 ± 4 | 6 ± 3 | -0.443 ± 0.047 | 0.043 ± 0.022 |
| 10 | -48 ± 4 | 6 ± 4 | -0.399 ± 0.044 | 0.043 ± 0.029 |

^a Force data were obtained between a silica probe and gold substrate covered with aluminum(III) alkanebisphosphonate multilayer thin films in a 1 mM KCl aqueous solution at pH 6.0 as a function of layer number. The average of four separate force measurements with standard deviation is reported. In each force measurement, 15 force curves were collected from 5 different locations of the film-covered gold substrate. The silica probe surface potential $\psi_p = -41$ mV. All calculations include both electrostatic and van der Waals interactions with $A_H = 1.1 \times 10^{-19}$ J and $\kappa^{-1} = 9.6$ nm.

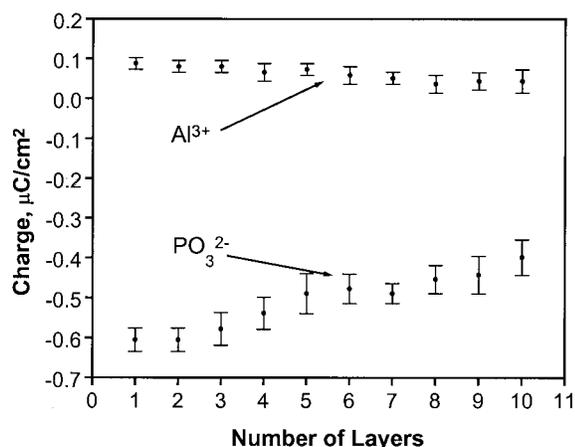


Figure 4. Surface charge of the aluminum(III) alkanebisphosphonate multilayer films as a function of layer number.

Poisson–Boltzmann equation with the boundary condition of assuming a constant surface charge. All calculations include both electrostatic and van der Waals interactions, with $A_H = 1.1 \times 10^{-19}$ J and $\kappa^{-1} = 9.6$ nm. The best-fit parameters for all force data are given in Table 1. The surface charges (σ) in Table 1 were calculated from the corresponding surface potentials and the measured Debye lengths of the diffuse double layers.^{35,36}

The surface charge of a substrate immersed in an electrolyte solution reflects the number of ions (aluminum(III) or phosphonate ions in this case) anchored on the substrate. Figure 4 is a plot of the surface charge versus the number of layers. As shown, the force measurements of the film revealed the regular layer-by-layer growth mechanism as shown by the surface charge

(35) The surface charge (σ) in Table 1 is calculated from the following relationship,³⁶ $\sigma = \epsilon_0 \epsilon_s \kappa (2kT/e) \sinh(e\psi/2kT)$, where κ is the reciprocal Debye length of the electrolyte solution with a dielectric constant, ϵ_s , taken to be 78.49 and assumed to be independent of surface charge change, and ϵ_0 is the permittivity of free space.

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reversal with each adsorption step. However, the magnitude of the surface charge decreased with the number of layers in the film, suggesting that the order of each layer decreased as the film was grown. Note, however, that the degradation of the order of the multilayer structure found here probably results from the use of short-chain (C_4) bisphosphonic acids as the building blocks for the film deposition. Alkanethiols with short alkyl chains generally do not form well-ordered self-assembled monolayers compared to those made with longer (C_{12} – C_{22}) chains. This behavior is generally in agreement with recent work reported by Decher,³⁷ who used neutron and X-ray reflectometry to study multilayer films fabricated by consecutive adsorption of polyanions and polycations. Interestingly, the measured positive charge was always much lower than the measured negative charge of the film. The reason for this charge difference between aluminum(III)- and phosphonate-terminated surfaces is not clear. However, several factors could be responsible for the charge difference. First, the charge states for the two surfaces are different. For the phosphonate-terminated surface, each phosphonate ion holds a -2 charge, while for the aluminum(III)-terminated surface, each aluminum(III) ion only shows a $+1$ charge after the charge compensation from the binding phosphonate ion. Nevertheless, the argument of different surface charge state does not fully account for the total charge difference between the two surfaces. Second, aluminum(III) and phosphonate ions may have different abilities to form ion pairs and complexes with counterions at the interface, leading to a different surface charge. It is well-known that aluminum(III) ions in an aqueous solution generally form six-coordinate compounds with H_2O and OH^- as the ligands depending upon the solution pH value. Although aluminum(III) ions binding on the phosphonate surface may behave differently compared to those in bulk solutions, some OH^- ions are probably coordinated to the surface-confined aluminum(III) ions, thus contributing to the attenuation of the surface charge of aluminum(III)-terminated surfaces.

DNA Immobilization and $\text{Ru}(\text{phen})_3^{2+}$ Association. The immobilization of DNA on an electronically conductive surface is of interest both in studies of molecular recognition of DNA and in various applications. In the present study, the aluminum(III)-terminated gold substrates were used to anchor both ss-DNA and ds-DNA. In Figure 5, before immersing into DNA-containing solutions, the observed electrostatic attractive force showed that the aluminum(III)-terminated surface was indeed positively charged. However, after immersing in the calf thymus ds-DNA solution, a purely electrostatic repulsive force was obtained, indicating that the surface had become negatively charged. Clearly, as shown previously,^{16,17} the calf thymus ds-DNA was immobilized on the surface, presumably due to the strong interactions between aluminum(III) ions and some of the phosphate groups of the DNA, while other phosphate groups protrude into the solution, thus effecting the surface charge reversal of the substrate. In a separate experiment, the adsorption of an eight-base ss-DNA on an aluminum(III)-terminated surface was also investigated. Quite similar electrostatic repulsive forces were observed, suggesting that both ss-DNA and ds-DNA are immobilized on this type of aluminum(III) film surface. To check if other positively charged surfaces showed a similar anchoring ability for DNA, the thiol-

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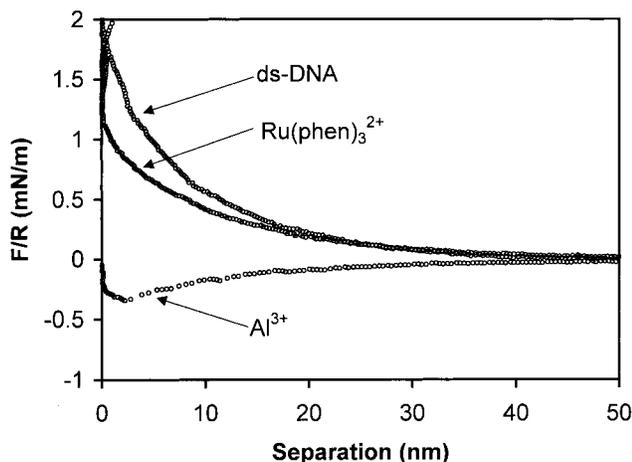


Figure 5. Forces obtained in 1 mM KCl aqueous solution of pH 6.0 between the silica probe and the aluminum(III)-terminated gold substrate before and after sequential immersion of the substrate to the calf thymus ds-DNA and $\text{Ru}(\text{phen})_3^{2+}$ solutions.

derivatized ammonium-terminated surfaces were examined. These ammonium-terminated surfaces showed essentially an equal anchoring ability toward immobilizing both ss-DNA and ds-DNA, suggesting that electrostatic forces are primarily involved.

The surface charges of the immobilized ss-DNA and ds-DNA immersed in the same 1 mM KCl aqueous solution of pH 6.0, calculated as described earlier, were -0.46 ± 0.05 and $-0.51 \pm 0.04 \mu\text{C}/\text{cm}^2$ for the eight-base ss-DNA and calf thymus ds-DNA, respectively. Note that the surface charge of the eight-base ss-DNA is slightly lower than that of calf thymus ds-DNA. On the other hand, there might be significant amounts of small counterions associated with the highly polymerized calf thymus ds-DNA, as frequently occurs with charged polymer films in electrolyte solutions, thus reducing its total surface charge, so a direct charge comparison is not possible. It would be more interesting to measure and compare the surface charges of an immobilized ss-DNA before and after its hybridization with a complementary strand of DNA in a same solution. The immobilized DNA surface charge difference before and after hybridization, if significant, might find application in DNA diagnostics. Studies of this type are currently under way in this laboratory.

The interaction of transition metal chelates with DNA has been the subject of a number of investigations,³⁸⁻⁴⁰ because metallo-intercalators are useful in probing DNA structure. While most of the studies have focused on investigating the association processes between the chelate and DNA in solution, in some applications such as the development of new DNA biosensors, the DNA is immobilized on a surface. In such cases, the interaction must be between the chelate and surface-immobilized DNA. We study here the interaction of $\text{Ru}(\text{phen})_3^{2+}$ with im-

mobilized ss-DNA and ds-DNA by probing the surface charge. In Figure 5, we show the force curves between the silica sphere and the substrate with immobilized calf thymus ds-DNA before and after immersion of the substrate into the $\text{Ru}(\text{phen})_3^{2+}$ solution. The decrease in electrostatic repulsive force indicated the attenuation of surface charge of the ds-DNA-covered substrate after reaction with $\text{Ru}(\text{phen})_3^{2+}$. Indeed, quantitative calculation revealed that the negative surface charge decreased by 27 (± 2)% after immersion into the intercalator solution. Under similar conditions, the force curves obtained with immobilized eight-base ss-DNA did not show any appreciable decrease after soaking in the same $\text{Ru}(\text{phen})_3^{2+}$ solution, suggesting that its surface charge was unchanged. The surface charge decrease upon exposing the ds-DNA to $\text{Ru}(\text{phen})_3^{2+}$ is a clear indication of the occurrence of the association process, where the $\text{Ru}(\text{phen})_3^{2+}$ charge partially compensates the negative charge of phosphate ions of DNA. The binding of $\text{Ru}(\text{phen})_3^{2+}$ to the surface-immobilized ds-DNA probably follows the same binding mechanism of the corresponding process in solution, where $\text{Ru}(\text{phen})_3^{2+}$ binds in the major groove.³⁸ For the immobilized eight-base ss-DNA, the unchanged surface charge upon exposure to $\text{Ru}(\text{phen})_3^{2+}$ indicates that the intercalation process did not occur, in line with the known lack of association of $\text{Ru}(\text{phen})_3^{2+}$ with ss-DNA.

CONCLUSIONS

An AFM was used to monitor the growth of aluminum(III) alkanebisphosphonate multilayer thin films, DNA immobilization, and $\text{Ru}(\text{phen})_3^{2+}$ intercalation. The occurrence of the surface charge reversal with each adsorption step indicated that the formation of aluminum(III) alkanebisphosphonate multilayer films followed the regular layer-by-layer growth mechanism as previously proposed.^{1,34} However, the quantitative surface charge data also showed that the structure of the multilayer films degraded as the number of layers increased. AFM force measurements confirmed^{16,17} that both ss-DNA and ds-DNA can be immobilized on positively charged film surfaces, while only immobilized ds-DNA showed interaction with $\text{Ru}(\text{phen})_3^{2+}$. As a method complementary to other surface characterization techniques, AFM force measurement is indeed a powerful technique to probe solid/liquid interfacial processes.

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