

Single Complexation Force of 18-Crown-6 with Ammonium Ion Evaluated by Atomic Force Microscopy

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Abstract: The cation complexation force of 18-crown-6 has been measured in ethanol by means of atomic force microscopy using probe tips and mica substrates modified chemically with 18-crown-6 and ammonium ion, respectively. The specific complexation force was suppressed by free potassium ion in the measurement solution, indicating a blocking effect based on the competitive complexation of the 18-crown-6 moiety between the free ion and the ammonium ion bound to the substrate. The single complexation force of 18-crown-6 with ammonium ion was evaluated to be about 60 pN in ethanol by autocorrelation analyses of the histograms for observed forces.

Introduction

In recent years, atomic force microscopy (AFM) has attracted considerable attention due to its capability of sensing interactions between its cantilever tip and a substrate as well as acquiring topographic images of sample surfaces.^{1–5} There has been growing recognition that AFM is a versatile tool for the direct measurements of intermolecular forces on a molecular scale, since chemical functionalization of its probe tips and substrates by self-assembled monolayers (SAMs) allows the observation of various chemical interactions with high force and spatial resolutions. In fact, the forces for several chemical interactions have been measured by means of AFM using chemically modified probe tips,^{6–13,15} which is termed chemical force microscopy.⁶ In addition, the evaluations of single (individual) forces of various noncovalent specific interactions, such as those of antigens–antibodies,^{14,20,21,23} DNA strands,²⁴ DNA nucleotide bases,²⁵ carbohydrates,²⁶ and β -cyclodextrin–ferrocene,²⁷ have also been reported so far. These studies on measuring the

specific forces at a single molecule level are of fundamental and practical interest for nanotechnology and development of molecular devices.²²

Specific intermolecular forces play important roles in molecular recognition processes in the biological and chemical systems. Specific interactions between metal ions and ligands have been of great interest. A number of works have been reported on molecular recognition of synthetic receptors.^{35–38} Their thermodynamic constants, such as stability constants of

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macrocyclic host molecules with guest ions, have been also extensively investigated by several titration techniques using NMR, calorimetry, potentiometry, and so on.³⁴ Recently, direct measurements of coordination bondings with heavy metal ions, e.g. Ag^+ –methyl sulfide²⁸ and histidine– Ni^{2+} nitrilotriacetate,²⁹ by using AFM have been also carried out, since the specific interactions are comparably strong.

Crown ethers are typical synthetic macrocyclic compounds that possess specific cation-complexing abilities based on the multiple ion–dipole interactions. In the present study, we attempted to make direct measurements of the complexation force of 18-crown-6 with ammonium ion by AFM using probe tips modified chemically with an alkanethiol derivative having a terminal group of 18-crown-6, which is one of the well-known artificial macrocyclic compounds that complex alkali metal and ammonium ions. We here demonstrate the applicability of AFM to the study of cation-binding processes on a molecular scale in host–guest chemistry that exhibits weaker interactions than the biological and coordination-chemical systems. Furthermore, the single complexation force of 18-crown-6 with ammonium ion has been evaluated with the histogram treatment by an autocorrelation method. To the best of our knowledge, this is the first example of the direct measurement of the single host–guest interaction in the cation complexes of crown ethers.

Experimental Section

Reagents. 6-Mercaptohexyloxymethyl-18-crown-6 (**1**), used for the chemical modification of AFM tips, was synthesized according to the literature.^{30,31} (3-Aminopropyl)triethoxysilane (APTES) (Shin-Etsu, Tokyo, Japan) was used as received. Ethanol of analytical grade was used for the solvent both for the chemical modifications and force measurements. Other chemicals were of reagent grade and used as received.

Cantilever Spring Constants. AFM cantilevers used in the force measurements were commercially available V-shaped Si_3N_4 cantilevers (Olympus, Tokyo, Japan) that were coated with Au/Cr on their tip side as well as the back side. It was reported by the manufacturer that the cantilevers had a spring constant of 0.09 N m^{-1} and a resonant frequency of 32 kHz. We separately determined the spring constants of cantilevers by measuring their unloaded resonant frequency³² in dynamic force mode (DFM), which is based on the mechanical vibration of cantilevers, by using a scanning probe microscope (SPA300, Seiko Instruments, Tokyo, Japan). The average resonant frequency was 32–33 kHz, which corresponds to a spring constant of $0.09\text{--}0.10 \text{ N m}^{-1}$.³²

Chemical Modification. The formation of SAM of **1** on probe tips was carried out as follows. Cantilevers were cleaned prior to modification by immersion into a piranha solution (concentrated H_2SO_4 /28% H_2O_2 , 7/3, v/v) for 20 min. **Caution:** Piranha solutions react violently with organic compounds and should be handled with great care. The cleaned tips were rinsed successively with deionized water and ethanol and were then immersed in an ethanol solution containing 1 mmol dm^{-3} of **1** overnight. The tips were rinsed with ethanol and dried in air. The sheets ($14 \text{ mm} \times 14 \text{ mm}$) of natural mica (Nilaco, Tokyo, Japan) were modified with APTES and treated with a HCl aqueous solution to obtain surface ammonium ions ($-\text{NH}_3^+$) according to the same procedure as described in our previous paper.³³ The resulting tips and substrates are denoted as 18-crown-6/tip and APTES/mica, respectively.

Force Curve Measurements. Force curves were measured at room temperature (ca. 298 K) with the scanning probe microscope on which a 18-crown-6/tip and an APTES/mica were mounted using a liquid cell. Force curve measurements were made more than 300 times at different positions of the substrate for each tip/substrate combination. The noise level of forces measured with the apparatus was estimated to be $\pm 15 \text{ pN}$ from the baseline of force curves near its pull-off point.

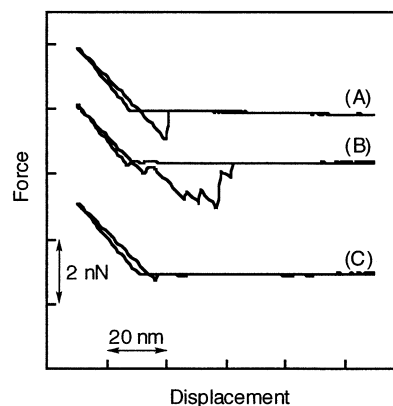


Figure 1. Force curves observed on APTES/mica substrate using 18-crown-6/tip: (A) a typical force curve, (B) a less typical force curve showing multiple pull-off events observed in ethanol and (C) a force curve showing smaller adhesion force due to blocking in an ethanol solution containing $10 \text{ mmol dm}^{-3} \text{ CF}_3\text{SO}_3\text{K}$.

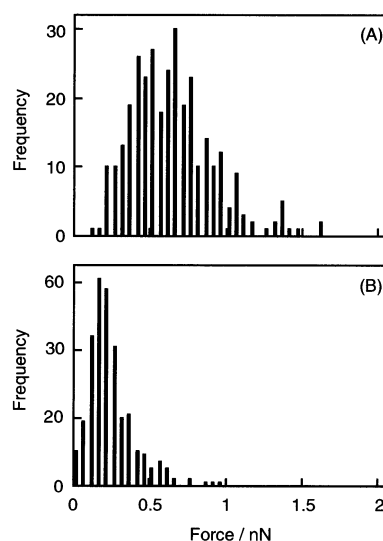


Figure 2. Histograms for adhesion forces obtained from each set of repetitive force measurements in ethanol using APTES/mica substrate and 18-crown-6/tip (A) in the absence of and (B) in the presence of potassium ion. The histograms were drawn at a force interval of 50 pN.

The scanning rate of cantilever during the force measurements was 40 nm s^{-1} . The measurement liquid was prepared using ethanol as a solvent. Aqueous solutions of 10 mmol dm^{-3} HCl or tetramethylammonium hydroxide (TMAOH) and deionized water were used for the force titration.

Results and Discussion

Specific Complexation Force. We have adopted 18-crown-6, a typical crown ether, as a terminal group of the alkanethiol used for the chemical modification of AFM probe tips, taking advantage of its simple structure and high stability constant with ammonium ion.^{34,35} The SAM of **1** was investigated by an electrochemical impedance spectroscopic method and was found to possess a selective complexing ability of its crown ether moiety with alkali metal cations.^{30,31}

Figure 1A shows a typical force curve observed on an APTES-modified mica substrate in ethanol using a 18-crown-6 modified tip. The APTES/mica substrate was pretreated with a 0.1 mol dm^{-3} HCl aqueous solution and dried in air in order to ensure protonation of the surface amino groups. In addition to the single pull-off event (Figure 1A), there were multiple pull-

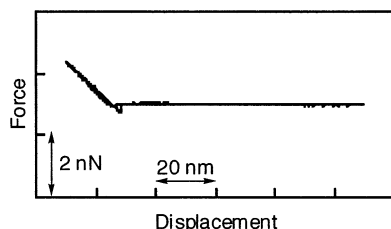


Figure 3. A typical force curve observed on ODS/mica substrate using 18-crown-6/tip in ethanol.

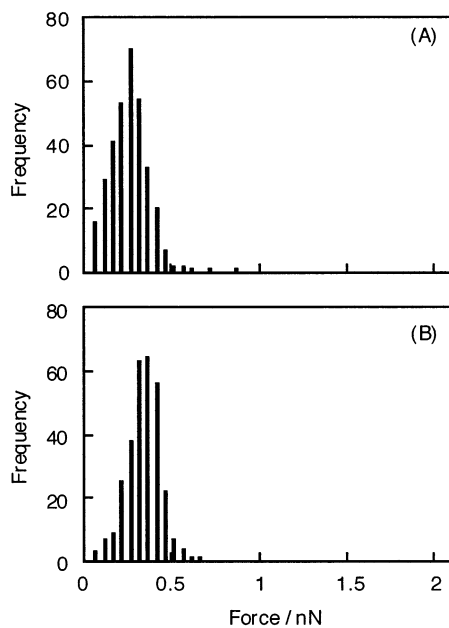


Figure 4. Histograms for adhesion forces obtained from each set of control force measurements on ODS/mica substrate using 18-crown-6/tip (A) in ethanol and (B) in an ethanol solution containing $10 \text{ mmol dm}^{-3} \text{ CF}_3\text{SO}_3\text{K}$. The histograms were drawn at a force interval of 50 pN.

off steps in some of the force curves, as exemplified in Figure 1B, which may be attributed to the sequential ruptures of discrete molecular interactions between the probe tip and substrate. Similar stepwise shapes in the force curve also have been reported in the AFM studies on measuring other specific interactions.^{14,20,21,26,27}

Competitive cation-complexation experiments were also carried out in the same system to confirm that the observed forces are based on the specific interaction between 18-crown-6 and ammonium ion, which is in turn easily blocked by another cation. Taking into account the high stability constant of 18-crown-6 with potassium ion,^{34,35} we have selected the metal ion as the blocking guest ion. After changing the measurement liquid from pure ethanol to an ethanol solution containing $10 \text{ mmol dm}^{-3} \text{ CF}_3\text{SO}_3\text{K}$, the force curve showed a much smaller adhesion force (Figure 1C). The typical histograms for each set of adhesion forces are shown in Figure 2.

Obviously, the adhesion force was drastically decreased in the presence of potassium ion in the solution. The effect of potassium ion is characteristic of the adhesion forces observed on APTES/mica substrates, as discussed later. In the presence of potassium ion in the solution, most of the available 18-crown-6 moieties on the tip would complex potassium ions in the solution due to the high stability constant for 18-crown-6–potassium ion complex.^{34,35} Therefore, the complexation with

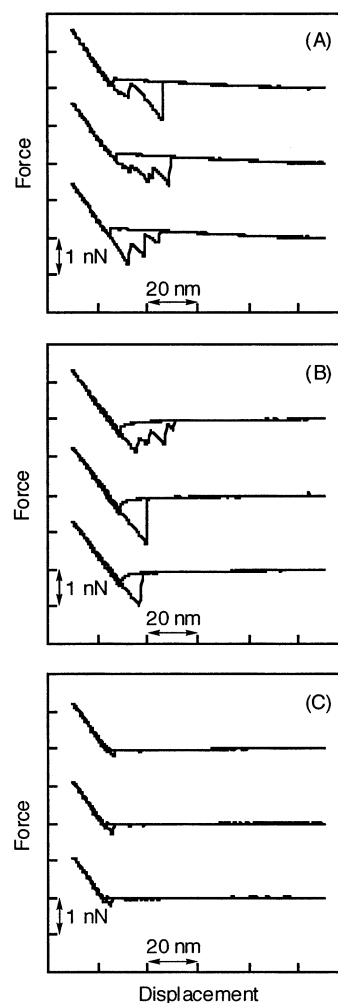


Figure 5. Force curves observed on APTES/mica using 18-crown-6/tip (A) in $10 \text{ mmol dm}^{-3} \text{ HCl}$ aqueous solution, (B) in deionized water, and (C) in 10 mmol dm^{-3} tetramethylammonium hydroxide aqueous solution.

potassium ion interferes with the complexation interactions of 18-crown-6 moieties on the tip with ammonium ions on the APTES/mica substrate, thus leading to the decrease in the observed adhesion forces. The small residual adhesion forces observed in the presence of potassium ion (Figure 1C) may be attributed to the ion exchange between potassium ions bound to the 18-crown-6 moieties on the tip and the ammonium ions on the substrate.

Control Force Measurements. A control experiment for the force measurements mentioned above was carried out by using a mica substrate modified with octadecyltrichlorosilane (ODS/mica) instead of APTES/mica. In this case, such specific interactions, as observed in the ion–dipole interaction with 18-crown-6 moiety on the tips, are not expected with the resulting substrate, because the substrate is terminated with methyl groups. Figure 3 shows a typical force curve observed for ODS/mica using a 18-crown-6/tip. The force curve exhibits a single pull-off step and the histogram (Figure 4A) indicates that the adhesion forces on the ODS/mica substrate were much smaller than that on the APTES/mica substrate, as shown in Figure 2A.

This suggests that there are no specific interactions between the ODS/mica substrate and 18-crown-6/tip. The small adhesion force in this system may arise from nonspecific interactions owing to the intrinsic forces such as van der Waals forces

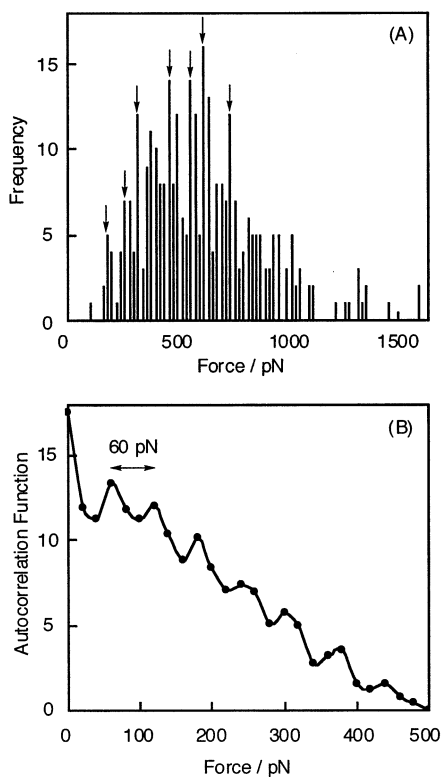


Figure 6. (A) Histogram for adhesion forces obtained from repetitive force measurements between APTES/mica and 18-crown-6/tip in ethanol and (B) its autocorrelation function. The histogram was drawn at a force interval of 20 pN from the same data set as for Figure 2A. The arrows in part A indicate the force maxima corresponding to multiples of the single force.

between the tip and substrate. Figure 4B depicts a histogram of the adhesion forces observed on an ODS/mica substrate in an ethanol solution containing $10 \text{ mmol dm}^{-3} \text{ CF}_3\text{SO}_3\text{K}$. In contrast to the APTES/mica, potassium ion caused no significant decrease in the adhesion forces. Definitely, the decrease in adhesion forces observed in the presence of potassium ion is specific to APTES/mica substrates, as mentioned above. Thus, the blocking effect by potassium ion observed on APTES/mica substrates results from the suppression of interactions between 18-crown-6 and ammonium ion based on the competitive complexation of 18-crown-6 with potassium ion. Therefore, we conclude that the adhesion forces observed on APTES/mica substrates in ethanol can be assigned to the specific complexation forces between the moieties of 18-crown-6 and ammonium ion covalently attached to the tip and substrate, respectively.

Deprotonation Effect of Ammonium Group. Deprotonation of ammonium ions on the APTES/mica substrate was also examined. It is well known that 18-crown-6 derivatives have a much higher complexation stability constant with ammonium ions than their corresponding free amines.^{35,37} There have been several studies on the adhesion force measurements between tips and substrates modified with SAMs having terminal acid/base groups by changing pH of the measurement liquid.^{8–10,28} It has been found that adhesion forces in the experiment are very sensitive to the dissociation of surface acid/base groups and therefore that this technique may be used to determine the acid/base dissociation constants (pK_a/pK_b) of the modified acid/base groups by so-called force titration.⁸ We therefore applied this technique to our force measurement system by changing the proton dissociation of the ammonium groups on the

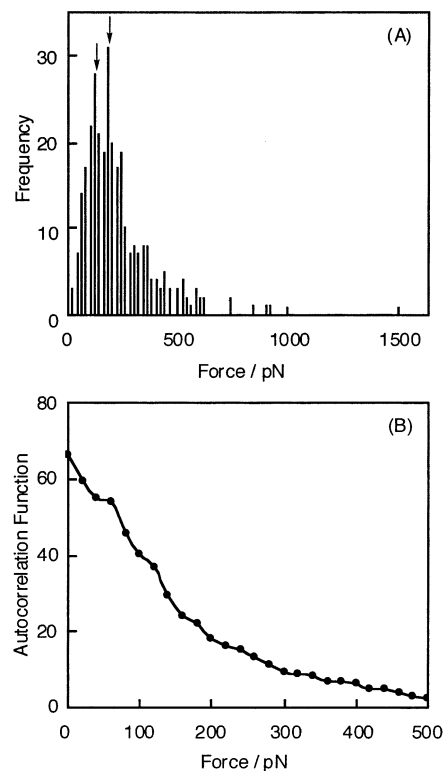


Figure 7. (A) Histogram for adhesion forces obtained from repetitive blocking force measurements using APTES/mica and 18-crown-6/tip in an ethanol solution containing $10 \text{ mmol dm}^{-3} \text{ CF}_3\text{SO}_3\text{K}$ and (B) its autocorrelation function. The histogram was drawn at a force interval of 20 pN from the same data set as for Figure 2B. Two arrows in part A indicate the force maxima corresponding to double and triple values of the single force.

substrate. Force curves observed on the APTES/mica substrate in acidic, neutral, and basic aqueous solutions are summarized in Figure 5. Significant adhesion forces were observed under both acidic and neutral conditions (Figure 5A,B), whereas a much smaller force was observed under basic conditions (Figure 5C).

This observation indicates that the protonation of amino groups on the substrate is essential to the great adhesion forces, as expected. Interestingly, the force curves showing multiple pull-off steps were often found under both acidic and neutral conditions (Figure 5A,B), implying the occurrence of the sequential unbinding of multiple pairs between the crown ether moiety on the tip and the ammonium ion on the substrate. Thus, under basic condition, the deprotonation of ammonium ions on APTES/mica substrate occurs and then results in the decrease in the interaction between the tip and substrate. The pH dependence of the adhesion forces is consistent with the general observation that 18-crown-6 complexes ammonium ion more strongly than the corresponding free amine. This again supports our conclusion that specific complexation forces of 18-crown-6 with ammonium ion can be measured in the force measurements using 18-crown-6/tip and APTES/mica substrate, as discussed above. It should be noted that this is the first observation of a switching in the binding forces for cation complexes of crown ethers by an external signal, i.e., by changing pH.

Single Complexation Force. Two statistical methods have been reported for the evaluation of individual molecular interactions from adhesion force data obtained by repetitive AFM measurements: One is an autocorrelation method^{14,26,27}

and another one is a Poisson method.^{16–21} We adopted the autocorrelation method to evaluate the single complexation force between 18-crown-6 and ammonium ion, since the Poisson method was not applicable to our data because the observed adhesion forces had less variety. The histogram for the adhesion forces on the APTES/mica substrate and its corresponding autocorrelation function are given in Figure 6.

The autocorrelation function (Figure 6B) showed distinct peaks with a periodicity of about 60 pN. In addition, the histogram possesses several maxima corresponding to multiples of the force value, as indicated by arrows in Figure 6A. Thus, the single complexation force between 18-crown-6 and ammonium ion is determined to be 60 pN. Since this value is larger than the noise level of the apparatus, it is of significance. Figure 7 demonstrates the result of the autocorrelation analysis for adhesion forces under blocking by potassium ion. In this case, the autocorrelation function does not reveal such a distinct periodicity, as shown in Figure 7B. The histogram may, however, afford two distinct peaks at the adhesion forces of 120 and 180 pN, as indicated by the arrows in Figure 7A, which correspond to double and triple values of the single force. This finding may suggest that the effective competition of ammonium ion with potassium ion in the solution substantially reduces the number of complexes of 18-crown-6 with ammonium ion between the tip and substrate.

Conclusion

We have demonstrated that the specific intermolecular forces based on ion–dipole interaction between the moieties of 18-crown-6 and ammonium ion attached covalently to the tip and substrate, respectively, can be measured by means of AFM. The complexation forces observed in ethanol decreased substantially in the presence of potassium ion in solution, i.e., by blocking the guest ion. The individual complexation force between 18-crown-6 and ammonium ion was evaluated to be about 60 pN by the autocorrelation function analyses of the histogram for the complexation forces. Additionally, we also observed that the cation binding force in the complex of 18-crown-6 with ammonium ion can be switched by an external signal, i.e., by changing pH.

AFM probe tips modified chemically with a synthetic host compound that can bind a particular guest ion would be useful for measuring specific complexation forces in its complex on a molecular scale. The 18-crown-6-modified AFM probe tips used in the present study might be also applicable to ion-selective mapping devices to visualize the distribution of metal ions, as reported by Zammaretti et al.^{39,40}

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