AFM Observation of Stimuli-Responsive Stretching Behavior for Spirobenzopyran/Crown-Ether Copolymer

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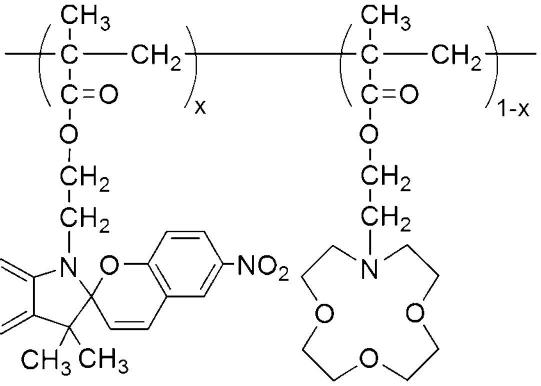
We have observed stretching behavior of a stimuli-responsive polymer chain bearing two functional groups, spirobenzopyran (SP) and crown ether (CR), at the side chain (SP/CR copolymer) by atomic force microscopy (AFM). The spirobenzopyran side chain can undergo photoisomerization from its electrically neutral spiropyran form to zwitterionic merocyanine by UV irradiation. The crown ether side chain can complex with metal ions. These reactions in the side chains would cause changes in their geometric structure and/or physical properties, resulting in the enhanced bifunctionality of SP/CR copolymer based on their stimuli-responsive ability. In this study, SP/CR copolymer, which was adsorbed on mica substrate, was stretched by AFM tip, when external stimuli (i.e., photoirradiation or metal ion addition) were applied. The force–extension curves of SP/CR copolymer were then analyzed by curve fitting based on a theoretical model of polymer chain elasticity to elucidate the external-stimuli effect on the polymer stretching behavior at the single molecule level.

Stimuli-responsive molecules have attracted considerable interest because they can be utilized to develop artificial materials whose functionality can be controlled by external stimuli. Several types of stimuli-responsive molecules have been so far reported for designing functional materials, which can be manipulated efficiently by external stimuli. We have previously reported a stimuli-responsive copolymer carrying spirobenzopyran (SP) and crown ether (CR) moieties at the side chain (SP/CR copolymer) as drawn in Scheme 1.[1–4] The copolymer shows two different stimuli-responsive functions based on photoisomerization and cation complexation resulting from the spiropyran and crown ether side-chains, respectively. As is well-known, spiropyran moieties, when photoisomerized to their zwitterionic merocyanine forms, form molecular aggregates resulting from electrostatic attraction. On the other hand, metal ion complexation of the crown ether moieties would bring about electric charges in the polymer because of the complexed metal ions. The side chain reactions would cause a structural change and a significant increase in the polarity of the molecule, leading to the stimuli-responsive contraction and extension of the polymer chain.[1,2] In a previous study, it has been also reported that such functionality of the SP/CR copolymer can be used to fabricate membranes coated with the polymer for photocontrollable solvent permeation.[3]



Scheme 1. Polymer bearing spirobenzopyran and crown-ether side chains ($x = 0.5$).

In the last decade, it has been reported that rupture forces of various types of chemical interactions and polymer chain stretching at the single molecule level can be measured by atomic force microscopy (AFM).[5,6] As has been reviewed,[7] a number of studies on AFM-based force spectroscopic observation of the single-molecule polymer stretching have been reported widely ranging from biopolymers (e.g., peptides and saccharides) to artificial polymers. In particular, AFM observation of the single-molecule stretching of stimuli-responsive macromolecules has attracted a great deal of attention from the viewpoint of elucidating the changes in their behavior at the single molecule level. For example, pioneering work on photoresponsive single-molecule stretching of an azobenzene polypeptide was reported.[8] Furthermore, similar AFM studies on the stretching behaviors of stimuli-responsive polymers containing ferrocene units have been also reported by several research groups.[9–11] In these previous studies, the target polymers possessed stimuli-responsive functional units in the main chain and their changes in the mechanical properties of the polymer chains by external stimuli were revealed by AFM.

In the present study, we attempted to observe stretching behavior of the bifunctional SP/CR copolymer by AFM. In contrast to the aforementioned previous studies, we focused on the stimuli-responsive polymer-chain stretching related to its functional side chains. It is well-known that side chain groups of functional polymers can substantially affect their character-

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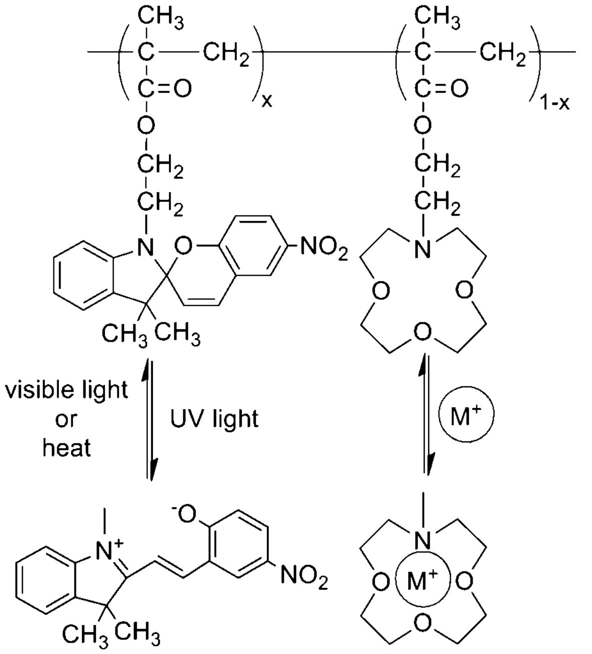

Scheme 2. Photo- and metal-ion-induced reactions of the side chains of SP/CR copolymer.

istics such as mechanical properties and hydrophilicity. Furthermore, conformation of polymer chains under various conditions would be controlled not only by covalent-bonded interactions in the main chain but also by nonbonded interactions between their side chain groups. To the best of our knowledge, the effect of stimuli-responsive side chains on the polymer stretching behavior has not been studied yet. In the case of our SP/CR copolymer, it is expected that external stimuli can induce electric charges on its side chains, resulting in changes of the copolymer stretching arising from the electrostatic interactions between the side chains. Thus, we investigated the effect of photoisomerization and cation-binding reactions of the corresponding side chain groups, as shown in Scheme 2, on the molecular stretching of the polymer chain. The force–extension curves for SP/CR copolymer before and after applying the external stimuli were then analyzed based on a theoretical model for the polymer chain elasticity.

Experimental

Chemicals and Materials. SP/CR copolymer used in this study was synthesized by free radical copolymerization according to a procedure reported previously (SP/CR monomer unit ratio = 1, $M_n = 1.6 \times 10^5$, PDI = 1.32).[3] Dodecanethiol for AFM tip modification was commercially available (Tokyo Kasei) and used as received. Other chemicals were of reagent grade and used as received.

Preparation of AFM Tip and Substrate. Commercially available V-shaped Si_3N_4 cantilevers (probe tips) coated with or without Au on the tip (Olympus, Tokyo, Japan) were used for polymer stretching experiments. Au-coated AFM cantilevers (spring constant: $k = 0.09\,N\,m^{-1}$) were cleaned by immersing into a piranha solution (concentrated H_2SO_4/28% H_2O_2, 7/3, v/v) for 10 min. Caution: Piranha solutions react violently with organic compounds and should be handled with care. After being washed with deionized water, the pretreated tips were immersed into an ethanol solution containing 1 mM 1-dodecanethiol overnight. Finally, the tips were adequately rinsed with ethanol and then dried in air. Bare Si_3N_4 cantilevers without Au coating ($k = 0.08\,N\,m^{-1}$) were cleaned with a piranha solution and then rinsed thoroughly with deionized water.

Natural mica sheets (Nilaco, Tokyo, Japan), freshly cleaved just prior to use, were rinsed with deionized water and ethanol. The mica sheets (12 mm × 12 mm) were immersed into a chloroform solution containing SP/CR copolymer (6.0×10^{-5} $g\,L^{-1}$) for 3 h. Finally, the mica sheets were withdrawn from the solution and then rinsed with chloroform and dried in air.

AFM Measurements. AFM force measurements were carried out by atomic force microscope (SPI 3800, Seiko Instruments, Tokyo, Japan) for SP/CR copolymer adsorbed physically on mica substrate. Photoisomerization of the spirobenzopyran side chain of SP/CR copolymer adsorbed onto the substrate was accomplished by UV light irradiation from outside of the liquid cell as described in our previous report.[12] Photoirradiation was made by using UV light (300–400 nm), which was obtained by passing light from a Xe lamp (L8388-04, Hamamatsu Photonics) equipped with a quartz waveguide through a color filter (UV-D36B, Asahi Techno Glass). The obtained force–distance curves were converted to force–extension curves.[6] Then, a theoretical model for polymer extension, freely jointed chain (FJC) model, was used to perform curve fitting of the force–extension curves to obtain the characteristic fitting parameters for elucidating the effect of the external stimuli on the polymer chain elasticity.

Results and Discussion

Photochromism of SP/CR Copolymer Adsorbed onto Mica. SP/CR copolymer shows dual functionality resulting from the two different side chains of spirobenzopyran and crown ether as drawn in Scheme 2. First, photochromism of SP/CR copolymer with approximately 1:1 composition of spirobenzopyran and crown ether side-chains was evaluated by UV–vis spectroscopy. Figure 1 shows absorption spectra of SP/CR copolymer adsorbed physically onto the mica substrate. After UV irradiation, a distinct absorption band appeared around 570 nm, which can be assigned to the photogenerated merocyanine form (Figure 1b). The absorption peak disappeared almost completely by following visible-light irradiation (Figure 1c). This observation indicates clearly that SP/CR copolymer attached on a mica substrate can undergo photoisomerization of its spiropyran side chains. It is well known that the wavelength for merocyanine absorption can be used to elucidate the microenvironment around the merocyanine. Generally, more polar solvent affords the shorter absorbance wavelength of the merocyanine form. Thus, the observed peak wavelength in Figure 1 may suggest that the merocyanine side chains are in less polar microenvironments. This can be explained in terms of the difference in the microenvironments of the merocyanine form in the solution state and in the surface-attached state and the change in electric charge caused by the possible protonation of the phenolate anion of the merocyanine form. At the spiropyran side chain of SP/CR copolymer in its merocyanine form, its phenolate anion might interact with metal ions complexed by the neighboring crown

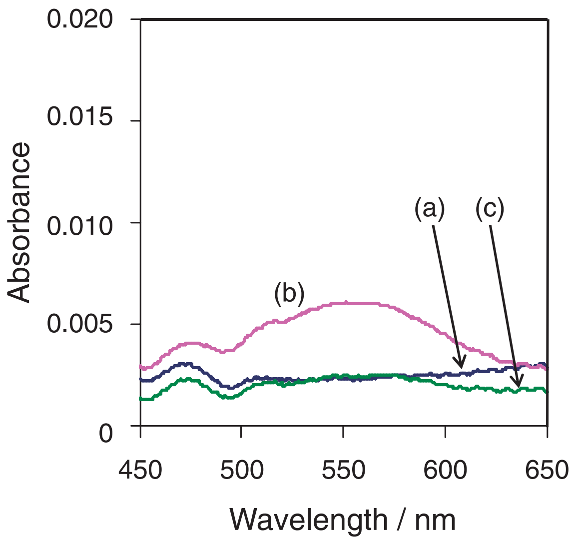

Figure 1. UV–vis spectra of SP/CR copolymer adsorbed onto mica substrate: (a) before and (b) after UV irradiation, and (c) after visible-light irradiation.

ether moiety.[2] In order to examine the effect of metal ions added on the photochromism of SP/CR copolymer, we measured UV–vis spectra of SP/CR copolymer adsorbed on the substrate in the presence of Li^+. As a result, no remarkable changes in the UV–vis spectra were observed in the presence of both of the metal ions. This suggests simply that the spirobenzopyran side chains did not undergo isomerization from spiropyran to merocyanine in the presence of the metal ions. Thus, it clearly indicated that the metal ions did not affect the spirobenzopyran side chains.

Stretching Behavior of Polymer under Different Photoirradiation Conditions. AFM stretching of SP/CR copolymer adsorbed on mica substrate was carried out by using a 1-dodecanethiol-modified AFM probe tip in deionized water. It is expected that the use of the hydrophobic tip allows the hydrophobic SP/CR copolymer on the substrate to be picked up efficiently owing to the hydrophobic interaction between the tip and the polymer. The force curves were measured under different light conditions to examine the effect of the external stimulus of photoirradiation. Figure 2 shows the observed force curves for the SP/CR copolymer stretching experiments in water. Under dark conditions, the force curves showed multiple steps at long distance, as shown in Figure 2a. Such stepwise curves could be assigned to the polymer stretching by AFM tip. Unlike SP/CR-copolymer-adsorbed substrate, no stepwise force curve was observed on the bare mica substrates. On the other hand, UV irradiation caused a decrease in the possibility of the occurrence of the multiple steps in the force curves. This suggests that the polymer adhesion by the AFM tip was suppressed probably due to UV-induced isomerization of the spirobenzopyran side chain from electrically neutral spiropyran to zwitterionic merocyanine. The photoionized side chains could cause a decrease in the interaction force between 1-dodecanethiol-modified tip and SP/CR copolymer adsorbed to the substrate. Moreover, after the following irradiation of visible light, which would induce the reverse isomerization of the side chain from merocyanine to spiropyran, the stepwise force curves similar to those observed under dark conditions were observed again. This implies that the reverse photoisomerization could recover the polymer extension by AFM tip.

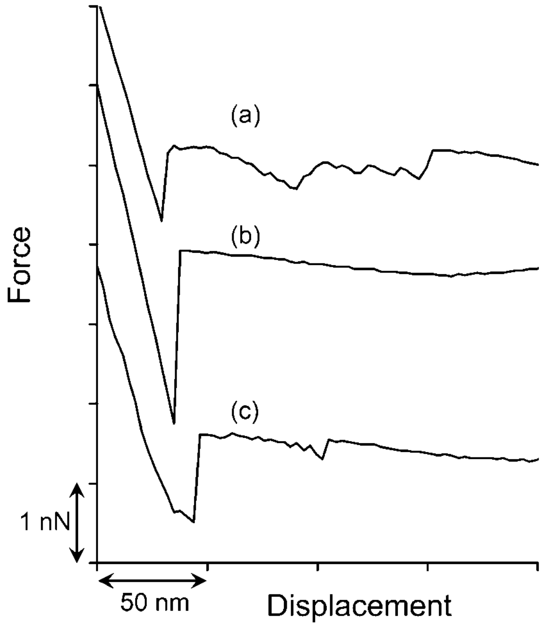

Figure 2. Force curves observed in water on the mica substrate, on which SP/CR copolymer was attached physically, under (a) dark, (b) UV irradiation, and (c) visible-light irradiation conditions.

Stretching Behavior of Polymer with Metal Ion. We carried out polymer-chain extension experiments in the presence of a metal ion in order to investigate the effect of metal ion complexation of the crown ether side-chain on SP/CR copolymer stretching. Figure 3 shows the typical force curves obtained in acetonitrile, which is favorable to the cation binding of the crown ethers, by using bare Si_3N_4 tips. In the absence of any metal ion, the force curve had multiple steps in addition to the first due to nonspecific adhesion (Figure 3a) whereas a few steps were observed in the presence of Li^+ (Figure 3b). This indicates that complexation of crown ether moieties at the side chain with Li^+ caused the distinct decrease in the probability of adhesion of SP/CR copolymer due to the decrease in the interaction between the tip and the polymer.

Effect of External Stimuli on Polymer Chain Elasticity. As has been reviewed,[7] AFM spectroscopic studies on single-molecule polymer stretching have been reported for various artificial and biological polymers. Polymer stretching can be described by several theoretical models based on polymer chain elasticity. We used a theoretical model, freely jointed chain (FJC), to analyze the force–extension curves of SP/CR copolymer in order to evaluate its polymer chain elasticity. In the FJC model, the polymer chain extension (x) is described by

$$x(F) = L\left[\coth\left(\frac{Fl_K}{k_B T}\right) - \frac{k_B T}{Fl_K}\right] \qquad (1)$$

where F is the force applied externally on the polymer chain, L is contour length, l_K is the Kuhn length of the segment, k_B is the Bolzmann constant, and T is absolute temperature. Based

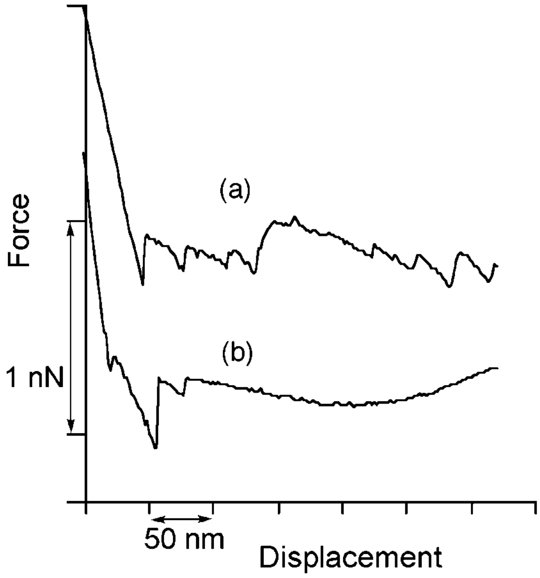

Figure 3. Force curves observed in acetonitrile on the mica substrate, on which SP/CR copolymer was attached physically, (a) in the absence of any metal ion and (b) in the presence of Li^+.

on eq 1, curve fitting was conducted for the experimentally obtained force–extension curves to obtain the characteristic fitting parameters of l_K and L. Figure 4 shows a plot of the typical force–extension curve for SP/CR copolymer under dark conditions and its fitting curve based on the FJC model. Force–extension curves showed multiple polymer extensions, which would be attributable to plural polymer chains being picked up and stretched at the same time. For the multiple curves, the well-fitted theoretical curves were selected manually for evaluating the fitting parameters.

Before the external stimuli were applied, almost all of the L values obtained from curve fitting from the FJC model were estimated to be 100–150 nm, which is roughly in agreement with that calculated from the molecular weight of SP/CR copolymer. This indicates that SP/CR copolymer main chain was stretched by the AFM tip, although the polymer chains were not always extended at its end points in our experimental setup. The deviation in the L values may be also ascribed to the dispersion of the molecular weight of the polymer. The estimated value of the contour length provides evidence that SP/CR copolymer chain was stretched between the AFM tip and substrate.

In order to investigate the changes in the mechanical properties of SP/CR copolymer induced by photoirradiation, we attempted to evaluate the polymer elasticity as a change in Kuhn length l_K by curve fitting of the force–extension curves obtained under dark conditions and UV irradiation, respectively. The resulting values of Kuhn length were 0.22 ± 0.10 (sample number $n = 29$) and 0.29 ± 0.27 nm ($n = 20$) for dark and UV-irradiation conditions, respectively. The corresponding L values were calculated to be 108 ± 27 and 78 ± 40 nm for dark and UV-irradiation conditions, respectively. The difference of the estimated contour lengths for different light conditions can be explained by the fact that the copolymer chain was picked up at a different position. The evaluated l_K values revealed that the increase in the Kuhn length was caused by UV irradiation and that the value was recovered by the following visible-light irradiation. This indicates clearly that the photoisomerization of the spiropyran side chains affected the polymer chain elasticity. Theoretically, the increase in the Kuhn length shows that the polymer chain can be easily stretched. Namely, polymer chains with larger Kuhn length are in the more extended states. Thus, from the comparison of the values of Kuhn length, it is revealed that the elasticity of SP/CR copolymer chain was decreased after UV irradiation. The changes in the mechanical properties of the polymer can be explained by the photogenerated merocyanine side-chain, whose electrical charges would cause electrostatic repulsion between them, resulting in the elongation of the polymer chain.

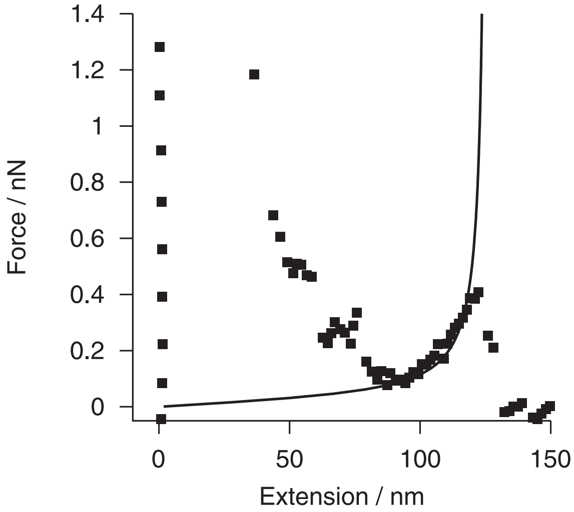

Figure 4. Force–extension curve for SP/CR copolymer under dark conditions (closed squares) and theoretical curve fitted based on a FJC model (solid line).

In addition, the curve fitting was also carried out for the force–extension curves obtained in acetonitrile in the absence and presence of a metal ion. As a result, the calculated values of Kuhn length l_K were 0.27 ± 0.22 and 0.10 ± 0.13 nm for the force–extension curves obtained in the absence and presence of Li^+, respectively. The value of l_K estimated in the presence Li^+ is likely to be too small to explain by general polymer chain stretching. This might indicate that a more suitable model of the polymer chain should be used to obtain acceptable fitting parameters for the real polymer chain. For such modification of the polymer model, the extended FJC model[5] can be introduced, where the polymer is modeled as consisting of elastic segments with a spring constant. However, under our experimental conditions, the extended FJC model is not suitable for the present system, since the extension of the SP/CR copolymer results from its physical adsorption onto the tip and the substrate, leading to difficulty in extending the polymer chain at higher applied force. Thus, we used the values of l_K estimated by the FJC model as a measure of the relative difference in the mechanical properties of the SP/CR copolymer under different conditions. The significant decrease in the Kuhn length caused by Li^+ indicated that the SP/CR copolymer chain could be more easily stretched in the absence of Li^+

than in the presence of Li^+. It was reported that the monoaza-12-crown-4 ether ring at the side chain of SP/CR copolymer shows a high binding affinity with Li^+.[1,2] Thus, SP/CR copolymer would complex with Li^+ with its counter ion at the crown ether moieties, resulting in structural changes and a significant increase in the polarity of the side chains. Therefore, the changes in the Kuhn length by the addition of Li^+ can be attributed to the changes in the state of SP/CR copolymer induced by Li^+ complexation at the crown-ether side chain.

Conclusion

We have carried out AFM-based polymer stretching experiments of stimuli-responsive polymer bearing spiropyran and crown-ether moieties at the side chains. The bifunctional polymer attached physically onto mica substrate was successfully extended by AFM tips and its polymer chain stretching was observed. The observed polymer stretching curves (force–extension curves) were analyzed based on a theoretical model of the polymer chain elasticity. The resulting fitting parameter of contour length for the polymer was in good agreement with the calculated value from the molecular weight. Furthermore, the effect of the external stimuli (photoirradiation and metal ion addition) on the polymer stretching was investigated. Such changes in the copolymer stretching behavior upon applying external stimuli indicate that the stimuli-induced reactions of the functional groups at the side chain could affect the mechanical property of the copolymer chain.

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