Thermoresponsive Structural Change of a Poly(*N*-isopropylacrylamide) Graft Layer Measured with an Atomic Force Microscope

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To directly characterize the thermoresponsive structural changes of a poly(*N*-isopropylacrylamide) (PNIPAAm) graft layer at the microscopic level, the force−distance curve (f−d curve) was measured on a well-tailored end-grafted PNIPAAm surface in aqueous solution at 25 and 40 °C, using an atomic force microscope (AFM). The PNIPAAm surface was prepared by an iniferter-based photograft polymerization technique. The approach trace of the f−d curve exhibited a steric repulsion profile at 25 °C, while the range of repulsion decreased $^1/_{10}$ to $^1/_{20}$ at 40 °C, confirming the ascending-heat-induced collapse of the PNIPAAm graft layer. The change in thickness of the graft layer was complementarily measured from the scanning images of the boundary between the grafted and nongrafted regions under well-defined scanning forces. The thermoresponsive characteristics of the PNIPAAm graft layer including its interaction with proteins and the applied-load dependence of the measured graft thickness are discussed.

Introduction

Surface grafting of polymers has been employed as one of the valuable methodologies for designing material surfaces with the desired properties for specific engineering uses. The advantages of this methodology are related to the versatile options among the polymers to be grafted. Especially, external-stimuli-sensitive polymers, which exhibit marked conformational changes in response to stimuli such as temperature and pH, have been applied extensively in surface grafting to prepare surfaces with rapid switching ability for drastic changes of the surface properties.

Poly(*N*-isopropylacrylamide) (PNIPAAm), which is well-known to exhibit a lower critical solution temperature (LCST) around 32 °C in aqueous solution, is an extensively utilized polymer for this application. It is hydrated and elongated into a random coiled conformation in water below the LCST but becomes dehydrated and collapses into a globular conformation above the LCST.[1] This thermoresponsive property has been applied in the preparation of functional hydrogels,[2] tissue glues,[3] membranes,[4,5] microspheres,[6,7] etc. In those engineering studies and other surface characterization studies,[8−11] the thermoresponsive structural changes of the PNIPAAm layer have been evaluated by indirect methods such as surface wettability measurements, differential scanning calorimetry, and ellipsometry. Direct evaluation of structural changes at the microscopic level was first done on a modified-PNIPAAm-adsorbed surface using a surface force apparatus.[12] However, the thermoresponsive dimensional changes of a well-tailored end-grafted PNIPAAm layer and its mechanical properties have not been precisely clarified at the microscopic level.

In our previous study, we investigated the mechanical properties of graft-polymerized surfaces with vinyl monomers (*N*,*N*-dimethylacrylamide and acrylic acid) by analysis of the force−distance curve (f−d curve) measured by use of an atomic force microscope (AFM).[13] The surface graft polymerization was performed on an iniferter[14]-based substrate surface. The formation of end-grafted polymer surfaces with a well-defined chain length was confirmed and its conformational characteristics were investigated. The iniferter-based graft-polymerization method employed in that study was found to generate a highly dense end-grafted polymer surface, i.e., the polymer brush. As an extension of that work, we directly characterized the thermoresponsive structural changes of an NIPAAm graft-polymerized layer prepared by the same method (i.e., the PNIPAAm brush layer) by f−d curve analysis in this study. The thickness change of the graft layer was complemen-

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Table 1. Chemical Compositions and Water Contact Angles of NIPAAm Graft-Polymerized Surfaces

| | XPS elemental ratio | | | | | water contact angle/deg | | | |
| | | | | | | 25 °C | | 40 °C | |
NIPAAm concn/M	C/Si	O/Si	N/Si	O/C	N/C	advancing	receding	advancing	receding
0 (DC-glass)	8.32	1.86	0.28	0.22	0.03	75.7 ± 2.2	65.9 ± 2.2		
1.0	14.61	2.74	1.76	0.19	0.12	59.5 ± 1.1	32.4 ± 2.6	62.4 ± 4.9	31.6 ± 3.7
2.0	17.12	3.70	2.16	0.22	0.13	63.7 ± 1.0	26.5 ± 1.9	60.1 ± 5.0	34.7 ± 2.5
5.0	48.85	8.20	6.47	0.17	0.13	64.3 ± 1.0	17.8 ± 1.7	64.1 ± 2.5	33.8 ± 2.2

tarily measured from the scanning images of the boundary between the grafted and nongrafted region under well-defined scanning forces. The thermoresponsive characteristics of the PNIPAAm graft layer including the interaction with proteins and the applied-load dependence of the measured graft thickness are discussed.

Experimental Section

Preparation of NIPAAm-Graft-Polymerized Surfaces. Surface photo graft-polymerization of NIPAAm was carried out on dithiocarbamate (iniferter[14])-derivatized glass substrates (DC-glass) which were prepared by chloromethylation of a glass substrate and subsequent dithiocarbamylation with sodium *N*,*N*-diethyldithocarbamate, according to the method previously reported by us.[13,15] After nitrogen bubbling of a methanolic solution of NIPAAm (1.0, 2.0, 5.0 M), 100 μL of the solution was dropped on a DC-glass and covered with a sapphire glass ($\sim$100 μm solution thickness). UV light (5 mW/cm^2 intensity, 200-W Hg$-$Xe lamp, L2859-01, Hamamatsu Photonics Ltd., Shizuoka, Japan) was irradiated onto the DC-glass for 20 min at room temperature under a nitrogen atmosphere. After the UV irradiation, the polymerized surface was rinsed with methanol and distilled water repeatedly and then nitrogen-dried.

Surface Characterization. The chemical compositions of the outermost layer of the NIPAAm-graft-polymerized surfaces were determined by X-ray photoelectron spectroscopy (XPS; ESCA-3400, Shimadzu Corporation, Kyoto, Japan) using a magnesium anode (Mg Kα radiation) at room temperature under 5×10^{-6} Torr (10 kV, 20 mA) at an escape angle of 15°. The surface wettability was evaluated by measuring the static contact angles (advancing and receding) toward pure water using the sessile drop method with a contact angle meter (CA-D, Kyowa Kaimen Kagaku Co., Ltd., Tokyo, Japan) at 25 and 40 °C.

Force-versus-Distance Curve Measurement. The f$-$d curve on the NIPAAm-graft-polymerized surface was measured with an AFM, using a Si$_3$N$_4$ probe tip (200 μm long, wide-leg, spring constant, 0.12 N/m, Digital Instruments, Santa Barbara, CA). The probe tip was equipped with a commercial fluid cantilever holder (Digital Instruments), and immediately immersed into PBS (phosphate-buffered saline, 137 mM NaCl, 2.68 mM KCl, 8.10 mM Na$_2$HPO$_4$, 1.47 mM KH$_2$PO$_4$; pH 7.4) in a custom-designed sample cell which was fixed on a microwarmplate (DC-MP10DM, Kitazato Co. Ltd., Shizuoka, Japan) installed on the AFM stage. Measurements were performed in PBS. The temperature of the sample cell (25 or 40 °C) was controlled on the warmplate and monitored by a thermocouple digital thermometer. Force was obtained by multiplying the catilever deflection by the spring constant of cantilever and plotted against the *z*-displacement of the catilever without further calibration of the spring constant (manufacturer's value, 0.12 N/m). Here, it is noted that the forces measured with a different catilever cannot be directly compared, since the manufacturer's value of the spring constant is not accurate enough. Thus in this study, to quickly compare the f$-$d curves with avoiding such uncertainty of force determination, the same cantilevers were used through a series of the measurements on a PNIPAAm surface under different temperature and on the graft-polymerized surfaces with different concentration of NIPAAm. Typically, more than 30 f$-$d curves were obtained at one location through repeated tip approach/retract cycles, and the measurements were repeated at five locations on each sample. The frequency of the approach/

retract cycle was chosen to be 3.2 Hz so as to minimize the noise fluctuation in a single f$-$d curve, and sequential f$-$d curves were collected by computer software at 1-s intervals.

Measurement of Thickness of the NIPAAm-Graft-Polymerized Layer. The thickness of the NIPAAm-graft-polymerized layer was measured from the AFM scanning images of the boundary between the grafted and nongrafted region, the latter formed by laser ablation of the graft layer (KrF excimer laser, L4500, Hamamatsu Photonics). The laser energy ($\sim$160 mJ/cm^2) was adjusted so as to ablate only the graft layer without ablation of the glass substrate. The images were obtained by constant-force contact-mode AFM (Nanoscope IIIa, Dimension 3000, Digital Instruments) using a commercial Si$_3$N$_4$ probe tip, through both strong force scanning (for the measurement of "compression thickness") and minimum force scanning (for the measurement of "swollen thickness") for the same scan area (deflection voltage for engaging, -5.0 V; setpoint voltage, 0 and -4.5 V, respectively). The definitions of the "compression" and "swollen" thickness are described in the third part of Results and Discussion.

Preparation of Protein-Fixed AFM Probe Tips. Proteins (albumin (Alb) and fibronectin (FN)) were covalently fixed on the Si$_3$N$_4$ AFM probe tip through the following procedure previously reported by us:[16] (1) gold deposition on the tip (500 Å); (2) formation of a self-assembled monolayer of 10-carboxy-1-dodecanethiol on the gold-deposited tip; (3) condensation reaction between the carboxyl groups on the tip and the amino acid residues on the protein surface mediated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and *N*-hydroxylsuccinimide. After the reaction, the tips were thoroughly rinsed with PBS in order to avoid multilayer adsorption of the protein.

Concerning the characterization of the protein-immobilized AFM tip, we performed two kinds of analysis. One is to investigate the adsorption of the proteins on the carboxylated SAM surface with enough wider area under the similar condition of condensation reaction. We have carried out this analysis by measuring the surface plasmon resonance (SPR670, Nippon Laser & Electronics Lab, Nagoya, Japan). As the result, we have confirmed that under the reaction condition described above, the proteins adsorbed onto the carboxylated SAM surface and the kinetics showed saturation curve (data not shown). Another method is to compare the tip treated by the protein-immobilization reaction with nontreated tip. We have systematically performed the measurement in our previous work. As the result, the treated tip showed the characteristic and reproducible adhesion profile which is clearly different from the profile obtained with the nontreated tip.[16] The surface coverage of the proteins on the AFM tip was not determined.

Results and Discussion

Characterization of the NIPAAm-Graft-Polymerized Surface. The NIPAAm-graft-polymerized surface prepared was characterized by XPS and surface wettability measurements (Table 1). After UV irradiation of the DC-glass for 20 min in methanolic NIPAAm solution, the XPS elemental ratios of C/Si, O/Si, and N/Si increased with increasing NIPAAm concentration. In addition, irrespective of the NIPAAm concentration, the elemental ratios of O/C and N/C were close to the theoretical value for PNIPAAm (0.17 for both ratios). On the other hand, the advancing water contact angle of the DC-glass decreased

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slightly after the irradiation and the receding angle decreased markedly with increasing NIPAAm concentration, indicating the presence of hydrated PNIPAAm chains grafted on the DC-glass surface. The XPS and wettability studies indicated that NIPAAm was graft polymerized on the DC-glass surface and that the extent of polymerization gradually increased with increasing NIPAAm concentration, implying that PNIPAAm surfaces with different chain lengths were formed.

In the wettability measurements, the receding contact angle of the PNIPAAm surface at 40 °C was rather higher than the value at 25 °C but lower than that at 35 °C (Table 1), which indicates that the outermost surface of the collapsed PNIPAAm layer does not necessarily exhibit high hydrophobicity. With regard to the wettability of a collapsed PNIPAAm surface, some groups have reported discordant results, as receding contact angle: 60° [8] (by the terminal grafting method using a telomerized PNIPAAm), 30° [12] (by the multiple site adsorption method using PNIPAAm slightly copolymerized with a hydrophobic monomer). Our result was the same as the latter value. Such difference is attributable to the use of different methods for the PNIPAAm surface preparation. Especially, the hydrophobic silanized surface and the hydrophilic mica surface were employed as the base substrate in the former and the latter studies, respectively. Since the collapse of the PNIPAAm chains may induce the exposure of a part of surface of the base substrate, total surface wettability may be affected by exposed area. In this sense, wettability is significantly affected by the graft density and graft chain length, i.e, the surface coverage with graft chains. In our previous study, the present photograft polymerization method was confirmed to produce the surfaces with a high graft density (~0.04 chains/nm[2]) and sufficiently long graft chains, i.e., the polymer brush surface.[13] Briefly, we measured the force between AFM probe tip and N,N-dimethylacrylamide graft-polymerized surfaces while changing the photoirradiation time, which were formed on the DC-glass surface prepared by the same method with the present study. As a result, we could detect the change of the force profile before and after the characteristic period of the irradiation time when the nearest-neighbor propagating graft chains start to overlap (20−30 s). From the vertical extent of graft chain that was measured from the f−d curve at the starting time for the overlapping, we roughly estimated the horizontal extent of a single graft chain just at the overlapping time and deduced the graft density. Besides, it was concluded from the data on the dependence of graft thickness (l) on the degree of polymerization ($l-N$) and the data on the elastic response of the graft layer (Hooke's elasticity) that the graft-polymerized surface forms a polymer brush. Since the present PNIPAAm surface was prepared with a photoirradiation time of 20 min, which is much longer than the typical overlapping time, the outermost surface of the present PNIPAAm layer was considered to form such polymer brush and to have closely packed collapsed PNIPAAm chains at 40 °C, with minimal exposure of the hydrophobic substrate surface. It is noted that the collapsed PNIPAAm brush surface exhibits low hydrophobicity, which indicates that the outermost surface of the collapsed PNIAAm layer undergoes weak hydration.

Thermoresponsive Changes of the Force-versus-Distance Curve of a PNIPAAm Surface. Figure 1 shows the approaching trace of f−d curves measured between a raw Si₃N₄ tip and PNIPAAm surfaces with different chain lengths. Here, the zero position of the tip in the Z-direction is defined as the starting position of the linear part in the tip−surface contact region. At this
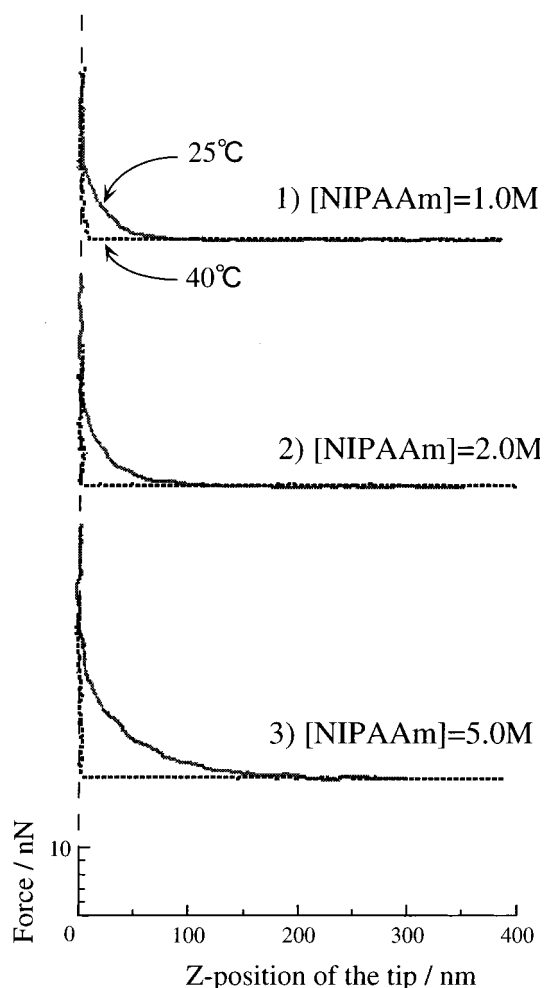

Figure 1. Representative force-versus-distance curves (approach trace) measured on NIPAAm graft-polymerized surfaces: solid curves, 25 °C; dashed curves, 40 °C. The zero position of the tip is defined as the starting point of linear response of the cantilever in the tip−sample contact region. Detailed explanation of such a definition of the zero position is given in the text.

position, the graft layer just under the tip is fully compressed by the tip so that further compression deformation is not induced by additional increase of the applied load. Therefore, the linear response of the cantilever deflection to its vertical movement is attained in the contact region.

In all the f−d curves measured at 25 °C, a repulsive force derived from the steric interactions of PNIPAAm chains was observed. The repulsive interaction distance was observed to almost disappear by ascending heat from 25 to 40 °C, irrespective of the NIPAAm concentration, indicating thermoreponsive collapse of the graft PNIPAAm chains.

Measurement of Thickness of the NIPAAm-Graft-Polymerized Layer. The thickness of the graft layer was measured by two approaches: (A) cross-sectional analysis from AFM scanning images of the boundary area between the grafted and nongrafted regions and (B) f−d curve analysis. It should be noted that the thickness of the PNIPAAm graft layer in aqueous media measured by method A depends on the applied scanning force, because of structural deformation of the graft PNIPAAm chains caused by the applied load. Thus, in this study, two different scanning forces were imposed on the graft layer to obtain the scanning images, i.e., a sufficiently strong
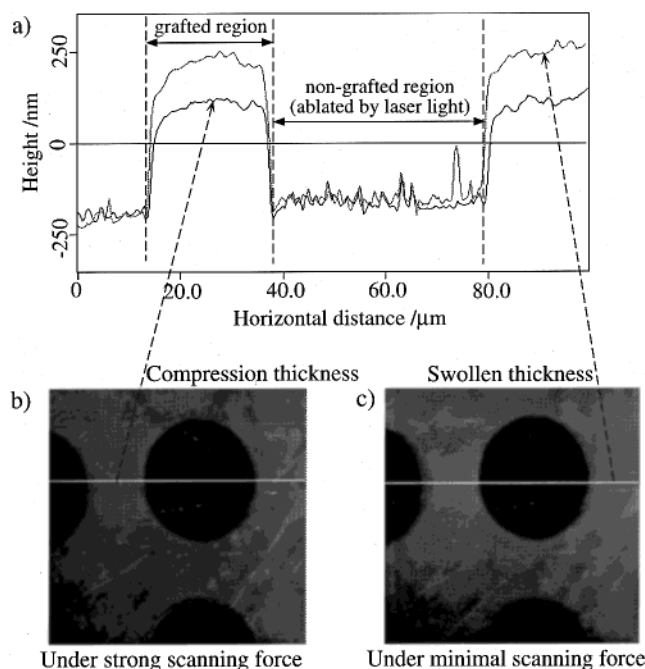
Figure 2. Scanning-force-dependent change of the apparent thickness of the PNIPAAm graft layer prepared in [NIPAAm] = 5.0 M observed under 25 °C: (a) cross section of the graft layer partially ablated by laser light; (b) AFM image of the partially ablated graft layer obtained under strong scanning force; (c) AFM image of the partially ablated graft layer obtained under minimum scanning force. The sample used for this observation was prepared in different experimental runs from the sample used for measurement of thickness in Table 2. The run involves some change of preparation procedure of DC-glass, which is described in ref 17.

force to fully compress the graft layer and a minimal force to appropriately engage the probe tip for generation of the topographical images. In the former measurement, the applied load was set so as to attain a condition in which the graft thickness measured did not vary with change of load due to full compression of the graft layer. In the latter measurement, the load was set to the minimum value below which the probe tip cannot keep contact with the sample surface, i.e., false engaging. Under the minimum scanning force, the graft layer becomes markedly swollen and the apparent graft thickness was observed to be larger than that obtained with the application of a strong scanning force. Figure 2 exemplifies such scanning-force-dependent changes of the apparent graft thickness observed under 25 °C.[17] We expediently define the thickness measured under the application of a strong scanning force and minimal scanning force as "compression thickness" and "swollen thickness", respectively, in this study (as for the actual condition of the applied load, see Experimental Section).

On the other hand, by method B, the repulsive interaction distance in the approaching trace of the f−d curve was measured. According to the definitions of the tip zero position in the Z direction, this distance reflects the thickness of a completely swollen graft layer in the absence of any applied force, which is measured from the outermost surface position of a fully compressed graft layer. The distance is defined as "repulsive thickness". In the repulsive interaction region, there should be contributions from force other than steric interaction, such as van der Waals, electrostatic, solvation, and hydrophobic interaction between the tip and grafted PNIPAAm chains. Within these interactions, contributions from electrostatic and hydrophobic interactions are considered to be much

smaller than the steric one, as the PNIPAAm is a noncharged and hydrophilic polymer. The solvation interaction is also considered to be much smaller, since the interaction is a short-ranged one. With regard to the van der Waals interaction, this force should contribute both to the long-ranged attractive interaction and to the short-ranged repulsive interaction between the tip and the segments of PNIPAAm chains. The significant attractive van der Waals force could not be detected in the tip−sample noncontact region of the approach trace of the f−d curve (Figure 1), which may be attributable to the measurement limit of the cantilever used in this study. The repulsive van der Waals force should appear only in the narrow space where the tip and the segment of PNIPAAm chain closely approach, which does not necessarily provide significant contribution to the total repulsive interaction distance in the f−d curve. Thus, main contribution of repulsive character in the f−d curves can be considered to be provided by the steric interaction, and the repulsive interaction distance (i.e., "repulsive thickness") can be considered to reflect the vertical extent of the graft layer.

The following is a summary of the above definitions of the three kinds of graft thickness (see Figure 3): The "repulsive thickness" is the thickness of the compressible part in the completely swollen graft layer without any applied load, which is measured from the f−d curves. The "compression thickness" is the thickness of the fully compressed graft layer by the tip, which is measured from the cross-sectional observation under maximum scanning force. The "swollen thickness" is the thickness of the weakly compressed layer, which is measured under the minimum scanning force.

The NIPAAm-concentration dependence and thermoresponsiveness of the three aforementioned kinds of thicknesses are summarized in Table 2. The following three trends were found: (1) the compression, swollen, and repulsive thicknesses increased with increasing NIPAAm concentration, irrespective of the temperature, except for the repulsive thickness at 40 °C which remained almost constant with increasing NIPAAm concentration. An increase of the graft chain length was confirmed. (2) At 25 °C, the swollen thickness and repulsive thickness were approximately 1.8-fold and 1.2- to 2.8-fold larger than the compression thickness, respectively, irrespective of the NIPAAm concentration, reflecting the differences of the applied load. At 40 °C, the swollen thickness was similar to the compression thickness and, in addition, the

(17) The DC-glass substrates prepared in the usual method described in the Experimental Section inevitably produced the graft-polymerized layer with some scars, wheel tracklike defects. In the tracklike region, the graft layer was markedly lost. The defect is considered to be produced by the detachment of a dithiocarbamate group, which is induced by scratching and shaving of the outermost surface of the substrate through the collision among the glass substrates in the stirring process of the substrates in the preparation solution. The measurement in Table 2 has been carried out for the sample with such a defect. The usual procedure to simultaneously prepare many DC-glasses (~100 pieces) is suitable in this case because so many DC-glass samples are needed in order to check reproducibility of the measurement of graft thickness. On the other hand, to show a clear image of the graft layer without a defect, the DC-glass substrate was prepared under special care to avoid such collision of substrate, and the graft-polymerization in Figure 2 was performed on the newly prepared DC-glass. Since only a few pieces of DC-glass can be prepared in an experimental run by the latter procedure, it was rather difficult to prepare enough DC-glasses of the same lot to systematically check the reproducibility of the thickness of the graft layer. Thus, the data in Table 2 were taken through the former procedure, and the clear image in Figure 2 was observed through the latter procedure. The difference of the graft thickness of the PNIPAAm layer ([NIPAAm] = 5 M) between the results obtained in Figure 2 and those obtained in Table 2 is due to such difference of experimental condition.
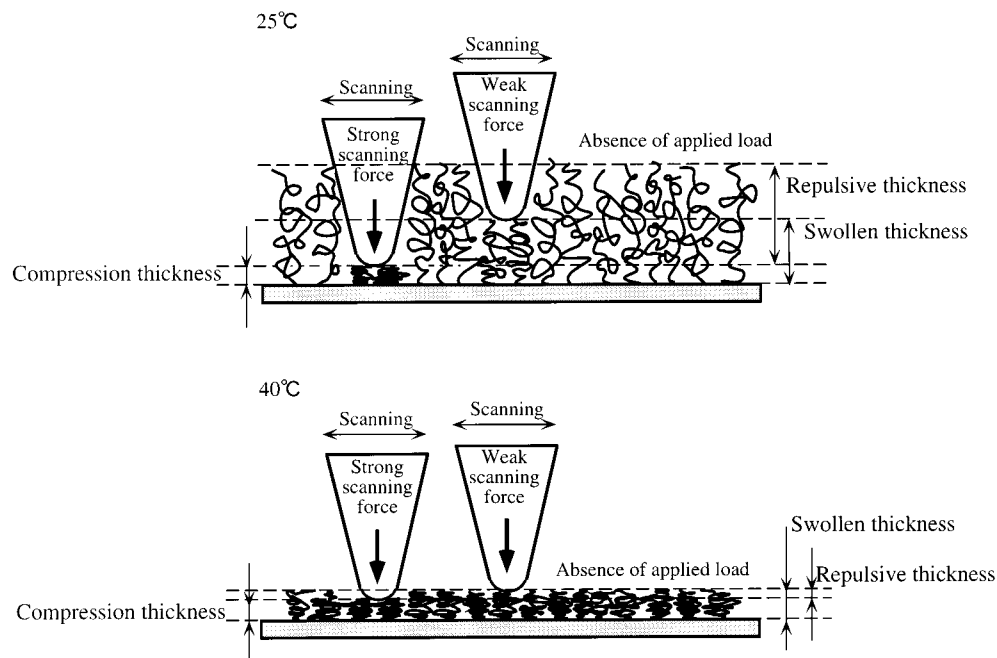
Figure 3. Schematic representation of the compression, swollen, and repulsive thicknesses under 25 and 40 °C.

Table 2. Thickness of NIPAAm Graft-polymerized Layers

| NIPAAm concen/M	thickness at 25 °C/ nm			thickness at 40 °C/nm		
	compression[a]	swollen[a]	repulsive[b]	compression[a]	swollen[a]	repulsive[b]
1.0	27.9 ± 3.3	50.9 ± 3.7	80 ± 15	25.7 ± 2.2	27.8 ± 1.7	<10
2.0	42.1 ± 5.7	72.4 ± 4.9	120 ± 15	48.2 ± 6.4	49.4 ± 5.8	<10
5.0	152.2 ± 8.7	291.4 ± 17.6	180 ± 15	157.0 ± 10.6	156.3 ± 16.8	<10

[a] Determined from the AFM micrographs. [b]Determined from f−d curves, which correspond to the distance between the top of the graft layer swollen under no applied load and the surface of the completely compressed graft layer. The precise definitions of each thickness are given in the text.

repulsive thickness was negligible (<10 nm). These findings indicate that the PNIPAAm graft layer at 40 °C was tightly collapsed without long-ranged diffuse layer. Considering the weakly hydrated state of the outermost surface of the collapsed-PNIPAAm layer, which was characterized by the contact angel measurement, the presence of a quite short-ranged diffuse layer (<10 nm) on the outermost surface may be expected. Absence of the long-ranged diffuse layer is considered to be induced by the high degree of dehydration throughout the collapsed PNIPAAm layer. (3) The compression thickness was determined to be approximately constant under ascending heat from 25 to 40 °C, regardless of the NIPAAm concentration, suggesting that complete compression of the graft layer was achieved with the application of a strong scanning force under the present conditions. On the other hand, the swollen thickness and repulsive thickness decreased markedly with the ascending heat, reflecting the collapse behavior of the graft PNIPAAm chains.

Concerning the evaluation of the real thickness, recall that the repulsive thickness indicates the distance of compression deformation of the grafted polymer chains from an extended conformation to a forcibly compressed conformation under the present definition of the $Z = 0$ position in the f−d curves (i.e., the outermost surface position of the fully compressed graft layer). Thus, the real thickness of the graft layer can be determined as the sum of the compression thickness and the repulsive thickness, though this does not mean that the graft layer consists of two parts. From the calculated values of the real thickness, the thickness of the PNIPAAm graft layer

at 40 °C was found to be approximately one-half to one-fourth of the value at 25 °C. Besides, since the real thickness should always be larger than the swollen thickness due to the absence of applied load, the following relationship is valid among the three kinds of thicknesses: (compression) + (repulsive) ≥ (swollen), as schematically shown in Figure 3. These relationships were certainly observed both for 25 and 40 °C, irrespective of the NIPAAm concentration (Table 2).

Thermoresponsive Changes of the Adhesion Force of the Protein/Graft Layer Interface. It is of paramount interest that protein adsorption and desorption will take place in response to the phase transition of a thermally reversible graft layer. To this end, a preliminary study on mechanically enforced adhesion/detachment of protein on a PNIPAM-grafted surface was conducted by AFM as follows. Using a protein-modified probe onto which a protein is covalently bound according to the method described in detail in our previous paper,[16] the adhesion force of a plasma protein (albumin or fibronectin) was determined on the NIPAAm graft-polymerized surface ([NIPAAm] = 5.0 M) in aqueous solution at 25 and 40 °C. As expected, a large repulsion force in the approach trace of the f−d curve was observed at 25 °C, regardless of the protein species (Figure 4). This is in good agreement with our previously reported result that, when an albuminated probe approached a water-soluble nonionic polymer-grafted surface, a graft-thickness-dependent repulsion force was observed.[13] On the contrary, at 40 °C, little repulsion force was observed. No significant adhesion force in the retract trace, determined as the force required for detachment of a protein after enforced adhesion, was
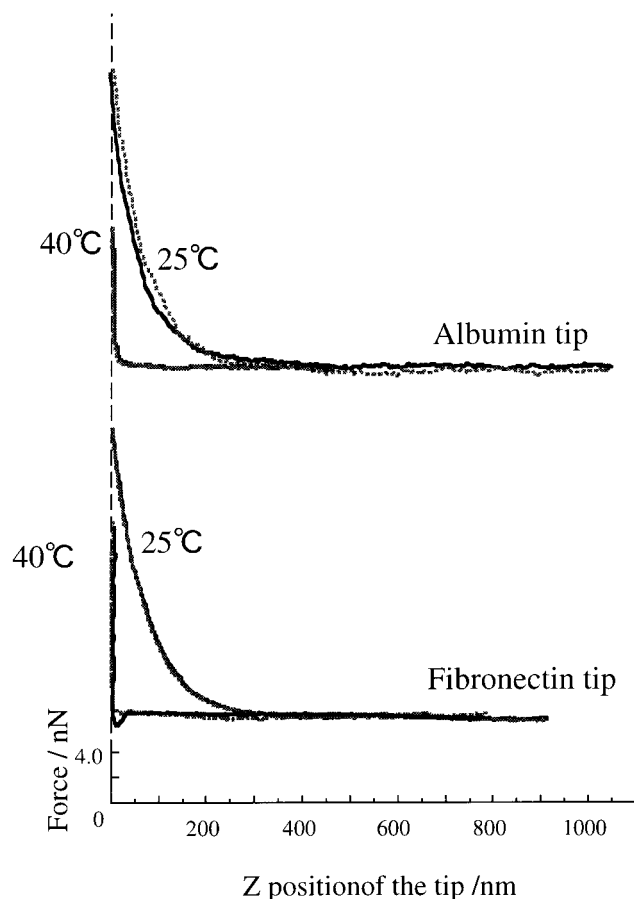
Figure 4. Representative force-versus-distance curves measured between protein-fixed probe tips and an NIPAAm graft-polymerized surface: solid curves, retract trace; dashed curves, approach trace. The zero position of the tip is defined as the starting point of linear response of the cantilever in the tip–sample contact region. Detailed explanation of such a definition of the zero position is given in the text.

observed for albumin under the measurement limit of the cantilever used in this study, whereas appreciable adhe-

sion force (approximately 0.6 ± 0.2 nN) was observed for fibronectin. It should be mentioned that the absolute value of such an adhesion force contains rather large experimental errors, because the precise calibration of spring constant of each protein-modified cantilever was not performed in these force measurements. However, such a trend itself in the adhesion force profile between albumin–tip and fibronectin–tip was reproducible in further experimental runs. Albumin (a non-cell-adhesive protein) is more hydrophilic than fibronectin (a potent cell-adhesive protein) which should be classified as a hydrophobic protein (this has been estimated from their solubility in buffer solution). Our result suggests that a dehydrated, collapsed PNIPAM-grafted surface at 40 °C exhibits a slightly hydrophobic characteristic, which may cause adhesion of hydrophobic fibronectin via a hydrophobic interaction. Our study on a thermally reversed adhesion force will be reported in detail soon.

Conclusion

In the present study, the thermoresponsive change of thickness of a NIPAAm graft-polymerized layer was precisely characterized at the microscopic level and under well-defined applied loads. Especially, the degree of collapse of the PNIPAAm graft layer and the applied-load dependence of the measured graft thickness were determined. Such microscopic information on the structural changes of stimuli-sensitive polymer graft layers could be useful for appropriately designing surfaces for specific functions. Further application of the present method for various surface-grafting systems using stimuli-sensitive polymers is required.