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Single Polymer Chain Elongation of Poly(*N*-isopropylacrylamide) and Poly(acrylamide) by Atomic Force Microscopy

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The nanomechanical properties of poly(*N*-isopropylacrylamide) (PNIPAM) and poly(acrylamide) (PAAM) in deionized water and 8 M urea aqueous solution were studied by using an atomic force microscopy based technique—single-molecule force spectroscopy (SMFS). Different force−extension traces were obtained in both systems. They could be normalized and superimposed, respectively. The force curves of PNIPAM and PAAM in 8 M urea aqueous solution could be fitted by extended freely jointed chain (FJC) model separately. These facts indicate that single polymer chain stretching was obtained. Moreover, the elasticities of PNIPAM and PAAM in pure water and in 8 M urea aqueous solution were compared. It is worthy to be pointed out that for PAAM in deionized water, a deviation from the extended FJC model at the middle regime (from ∼50 to 450 pN) in the force curve was found. When 8 M urea aqueous solution was used as buffer solution, the deviation disappeared and the force curve could be fitted with extended FJC model. This result may be related to the fact that PAAM could form certain intramolecular hydrogen bonding in water. After the PNIPAM sample was heated to 33 °C, above its LCST, more signals were observed in one stretching. This could be attributed to the fact that polymer chains form more contact points with the glass substrate after thermal treatment. In addition, another way just different from the normal one to do the SMFS experiment was also provided.

Introduction

The deformation of a polymer chain is a classical problem in polymer science. However, it is very hard to measure the nanomechanical properties of a polymer chain using conventional methods. Recently, with the appearance of many AFM-based techniques[1−5] and optical tweezers,[6] it became possible to measure the elasticity of a single-polymer chain. Among them, a new atomic force microscopy-based technique—single-molecule force spectroscopy (SMFS) with high spatial resolution and extreme force sensitivity—has allowed the study on the nanomechanical properties of biomacromolecules as well as synthetic polymers. Till now, many elegant experiments were performed on natural[7−17] and synthetic polymers.[18−23] Some nanomechanical fingerprint information was obtained on those systems, which was not accessible by conventional methods. The new information provided us with new insight into the mechanical properties of macromolecules, such as the stretching of polymer chains of normal random coil conformation,[18,21,23] whose force curves could be fitted well by using freely jointed chain model; unfolding force of Ig titin domain,[8,16] which was seen as a zigzag-like force−extension traces; the chair-to-boat conformation transition of individual glucopyronose ring,[7,9] which could be identified by a plateau in the force curves; the splitting or unwinding force of helical structure,[12,19,20] a plateau or a kink was observed on these systems. Furthermore, based on the force fingerprint of amylose, M. Grandbois et al.[24] have measured the rupture force of silicon−carbon and sulfur−gold bond that could not be obtained accurately by other methods.

Poly (*N*-isopropylacrylamide) (PNIPAM) is a thermo-sensitive, water-soluble synthetic polymer. It has been studied widely due to its fundamental research interests as well as potential applications. On one hand, as a fundamental problem in polymer physics, the coil-to-globule transition of a linear synthetic homopolymer chain, similar to the folding of a protein molecule, provides a defined model system for the study of the protein folding. On the other hand, PNIPAM has showed its great potential application in biomedical industry, such as drug delivery materials. Although lots of studies of PNIPAM have been made by laser light scattering[25−29] and calorimetry method,[30,31] no report was given on the segment elasticity and conformation transition by stretching a single polymer chain.

In this paper, we present the results of the nanomechanical properties of single PNIPAM chains by using SMFS. As a comparison, another widely used important water-soluble polymer—poly (acrylamide) (PAAM) (as shown in Scheme 1)—was also studied. From Scheme 1 we can see the differences between these two polymers: for one thing, PAAM has two hydrogen atoms on the amino group of each repeat unit, while PNIPAM only has one hydrogen atom on the amino group; second, PNIPAM has an isopropyl group on every repeat unit but PAAM did not bear it. These two different primary structures may cause different solubility and different conformation in water. In the present research we also try to study the effect of different substitutes on the elasticity and force-induced conformational transition of polymer chain. Since PNIPAM has a critical temperature at 33 °C for coil-to-globule transition, our aims also lead to a comparative study on the difference of SMFS before and after thermal treatment.

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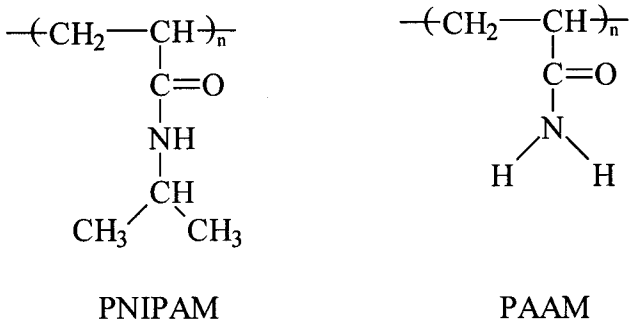
SCHEME 1



Experimental Section

Preparation of Sample for SMFS. The PNIPAM sample was provided by Prof. Chi Wu (Department of Chemistry, The Chinese University of Hong Kong, P. R. China). Its weight-average molecular weight, M_w, is 2.4×10^6 g/mol, and its polydispersity index, M_w/M_n, is 1.3.

A PNIPAM sample was dissolved in deionized water to a concentration of 6.4×10^{-7} g/mL. The solution was allowed to stand in a refrigerator (about 0 °C) for one week to ensure that the sample was dissolved completely. About 400 μL of PNIPAM solution, which had been filtered using a 1.2 μm Millipore filter, was deposited onto a clean glass substrate (18×18 mm) and incubated for about 4 h in a container isolated from the air. Then most of the solution on the glass was removed and the sample was immediately used for experiments. For the other situation, in which 8 M urea aqueous solution was used as buffer, a more concentrated solution of PNIPAM, 4×10^{-5} g/mL, was used. In the experiment of PAAM, the concentration of the solution was about 4.54×10^{-4} g/mL. As for the heating experiment, after being brought out from the refrigerator, PNIPAM solution, which was filtered by using 1.2 μm Millipore filter, was put into a water bath whose temperature was kept at 33 °C. Then the solution was kept at this temperature for 20 min**.** After that, about 400 μL of the solution was added onto a clean glass substrate and incubated for 4 h. The following steps were just the same as those not being heated.

SMFS Experiments. All of the force measurements were carried out in deionized water or 8 M urea aqueous solution on a custom-built AFM setup. Silicon nitride cantilevers from Digital Instruments (DI, Santa Barbara, CA) and Park Scientific Instruments (PARK, Sunnyvale, CA) were used. The spring constants of the cantilevers were calibrated by measuring their thermal excitation.[32,33] Measured values were around 0.03−0.08 N/m. The experimental details of single-molecule force spectroscopy by AFM have been described elsewhere.[7,12,18] In brief, PNIPAM was immobilized onto the glass substrate by physical adsorption. To do the force experiments, a drop of water (or urea aqueous solution) was put onto the substrate and mounted between the cantilever holder and the sample as buffer, and then the cantilever was immersed into the buffer. By the movement of piezo, the sample was brought into contact with an AFM tip and some molecules would adsorb onto the tip due to nonspecific interactions between the polymer and the tip, producing a connective bridge in-between. During the separation of the tip and the sample, the polymer chain was stretched and the cantilever would deflect. At the same time, a deflection−extension curve was recorded and converted into a force−extension curve.

Results and Discussions

Single-Molecule Force Spectrum of PNIPAM in Deionized Water. Figure 1 shows a superposition of several typical force−extension curves of PNIPAM in aqueous solution using different cantilevers in different experiments. Since the segment (loops or tails) length of the polymer chain on the substrate is dispersed and we cannot control the point at which the polymer chain is stretched, the contour lengths of the polymer segment being stretched vary. As shown in Figure 1, the force−extension curves show similar characteristics: the force rises monotonically with extension until a rupture force is reached. To compare the segment elasticity of polymer chains of different contour lengths, the force curves were normalized at the same given force value. The normalization process was done as follows, for each force curve we selected a force value, (such as 300 pN) then we could get a length value correspondingly, and after that the extension of the force curve was divided by that length. At last, the force curves processed as mentioned above were plotted and superimposed in Figure 2. The good superposition clearly shows that the elastic properties of PNIPAM chain scale linearly with the contour lengths. This finding corroborates the supposition that the elasticity of a single PNIPAM chain is measured.

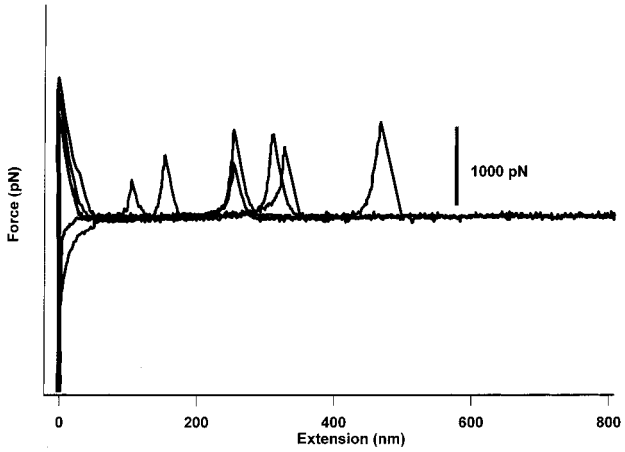


Figure 1. A superposition of some typical force curves of PNIPAM in aqueous solution obtained on different samples using different cantilevers.

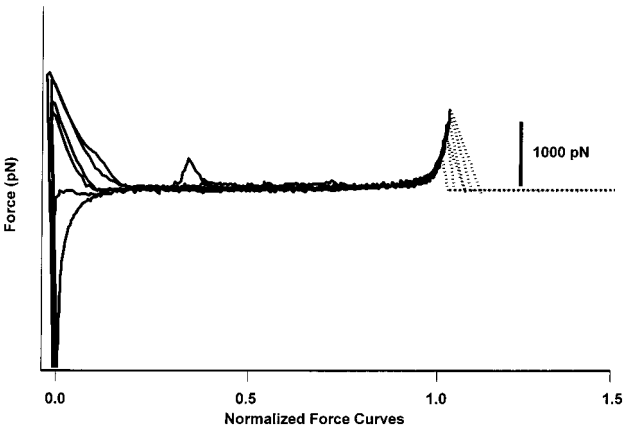


Figure 2. Normalized force curves of PNIPAM shown in Figure 1.

The force law of polymer chains under tension can be derived from the freely jointed chain (FJC) model,[34] which was given by Langevin function:

$$x(F) = \left[coth\left(\frac{FI_k}{k_BT}\right) - \frac{k_BT}{FI_k}\right] \cdot L_{contour}$$

Here, x is the extension of the polymer chain (end-to-end distance,) I_k (Kuhn length) is the length of the statistically independent segment, k_B is Boltzmann's constant, T is the

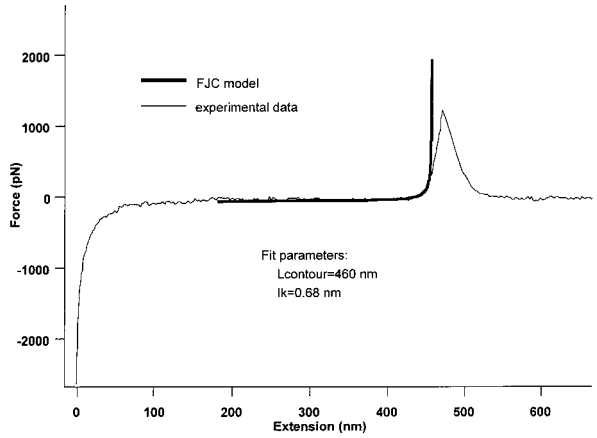


Figure 3. Measured force curve of a PNIPAM chain in water superimposed by a fit with a FJC model.

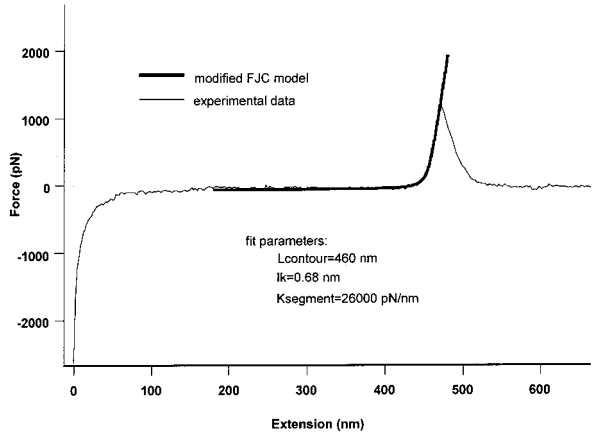
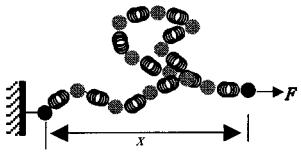


Figure 4. Elastic properties of PNIPAM in deionized water simulated by modified FJC.

temperature. The elasticity of a freely jointed chain purely comes from the entropic contribution. The FJC model assumes that the segments of a polymer are inextensible. When we used this model to fit the result of PNIPAM in water we found that the model can only fit the force curve at low forces, but cannot fit the experimental result in the high force region as shown in Figure 3. It has been shown that the elasticity of a single polymer

$$x(F)=\left[\coth\left(\frac{FI_k}{k_BT}\right)-\frac{k_BT}{FI_k}\right]\left(L_{contour}+\frac{nF}{K_{segment}}\right)$$



coil can be well described by an extended Langevin function derived from a modified freely jointed chain model (FJC model),[7,18] as shown above, where n is the number of Kuhn segments, equals to $L_{contour}/I_k$. The modified FJC model treats a polymer as a chain of statistically independent segments of lengths I_k (Kuhn length) and the segment can be deformed under stress. The deformability of segment is characterized by a specific parameter, segment elasticity, $K_{segment}$. The elasticity of a modified FJC chain is dominated by the entropic contribution at low force regime; however, at high force regime the elasticity is governed by enthalpy as well as entropy. The force−extension curves were fitted well with modified FJC model, and gave $K_{segment}$ of 25000 ± 2000 pN/nm and I_k of 0.70 ± 0.05 nm, see Figure 4.

Sometimes we obtain force curves that have smaller segment elasticity than those of Figure 1. They cannot be normalized and superimposed with those shown in Figure 2. We would attribute this result to the fact that there may exist different local segment elasticities in the aqueous solution of PINPAM. The different local segment elasticities may arise from the intra- or intermolecular hydrophobic interactions between the isopropyl groups or hydrogen bonding between hydrogen atoms of the amino groups and the oxygen atoms of the carboxyl groups, especially from the former one. The argument is also supported by the observation that when we first attempted to do the experiment by using PNIPAM aqueous solution of 1 mg/mL as usual,[17,18] we could not obtain clean force−extension traces. In other words, under this concentration many large force signals were observed before the last peak appeared in one stretching, see Figure 5. Then we tried to dilute the solution step by step

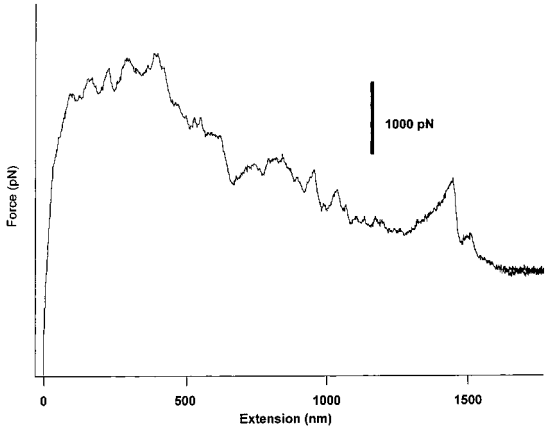


Figure 5. Force curves obtained on PNIPAM in high concentration (2.5×10^{-4} g/mL).

to 5×10^{-4}, 2.5×10^{-4}, 5×10^{-5}, 1.5×10^{-5}, 4×10^{-6}, 2.5×10^{-6}, and 6.4×10^{-7} g/mL. At last, we chose 6.4×10^{-7} g/mL aqueous PNIPAM solutions. Although the probability that we could pick up a polymer chain in this case was not large, we could obtain clean single-polymer chain stretching.

Single-Molecule Force Spectrum of PNIPAM in 8 M Urea Aqueous Solution. It is well-known that urea is an effective breaker of hydrogen bonding and hydrophobic interactions and had been used widely in the denaturation of proteins.[35] Similarly, we performed single polymer chain elongation of PNIPAM using 8 M urea aqueous solution as buffer instead of water and prepared the sample using solution of PNIPAM with higher concentration, 4×10^{-5} g/mL. If the concentration was below this, it became very hard to pick up polymer chains. The experimental results showed that only one type of force−extension curves was obtained. This may indicate that 8 M urea aqueous solution is a better solvent for PNIPAM than pure deionized water. At the same time, when 8 M urea aqueous solution was used as buffer, the intramolecular hydrogen bonding and hydrophobic interactions can be diminished and the polymer chains exist as extended conformations in solution. This result may support our speculation of different local segment elasticity in water further. These force curves can also be normalized and superimposed well, as shown in Figure 6. Extended FJC model can describe the elasticity of PNIPAM in urea aqueous buffer well, see Figure 7. The corresponding fit parameters of the force curves are almost the same, $K_{segment} = 40000 \pm 5000$ pN/nm and $I_k = 0.78 \pm 0.06$ nm, although their contour lengths are different. From the fit parameters, we can

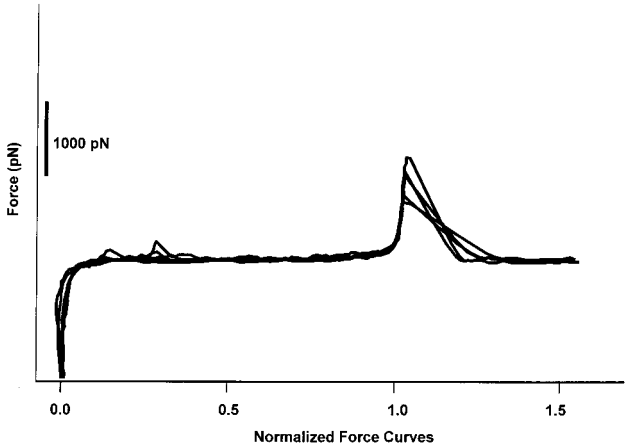


Figure 6. Normalized force curves of PNIPAM in 8 M urea aqueous solution.

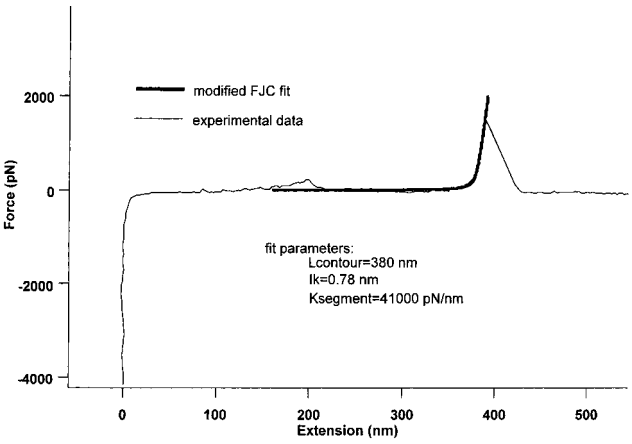


Figure 7. Extended FJC fit on force curve obtained on PNIPAM in 8 M urea aqueous solution.

see that the segment elasticity and Kuhn length of PNIPAM in 8 M urea is larger than those in pure water. In other words, in the environment of urea aqueous solution, the segment elasticity of PNIPAM increased. We would like to attribute this result to the fact that the urea molecules in 8 M urea aqueous solution might attach to the side groups of PNIPAM since the concentration of urea is high enough, and then they would increase the effect of side groups on the stiffness of polymer chain.

SMFS Study on PAAM in Deionized Water and in 8 M Urea Aqueous Solution. In contrast, we have done SMFS study on poly(acrylamide) (PAAM) in pure water and in 8 M urea aqueous solution. In the case of pure water as buffer, only one type of force−extension curves was obtained (see Figure 8), and those force curves could also be normalized and superimposed well (see Figure 9). The concentration ($\sim 5.45 \times 10^{-4}$g/mL) of PAAM for film preparation was larger than that of PNIPAM. It is understandable since PAAM is much easier to dissolve in water than PNIPAM; as a result the amount of polymer chain adsorbed onto the substrate decreases. It is worth pointing out that for PAAM in deionized water, a deviation from the extended FJC model at the middle regime (from ~50 to 450 pN) in the force curve was found (see Figure 11). This situation is similar to what Oesterhelt et al. met on poly(ethylene glycol) (PEG)−water system[20] and our previous results in poly(vinyl alcohol) (PVA) system,[19] but the kink in our present system is not so obvious as in those two systems. Extended FJC model cannot describe the elasticity of the whole regime in the force curve of PAAM though the fit parameters had ever been adjusted. It should be related to the intramolecular

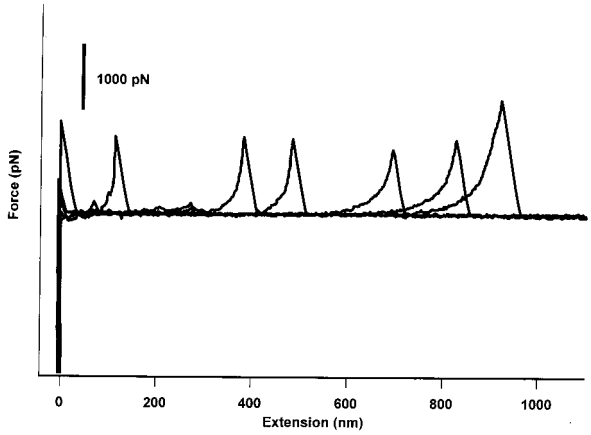


Figure 8. A superposition of some typical force−extension traces of PAAM in water, obtained on different samples using different cantilevers.

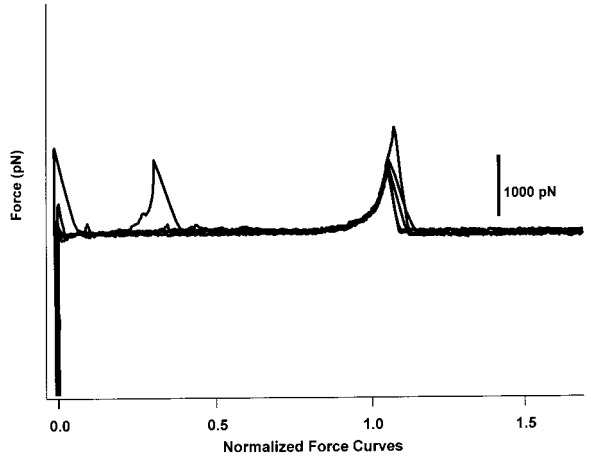


Figure 9. Normalized force curves of PAAM in water.

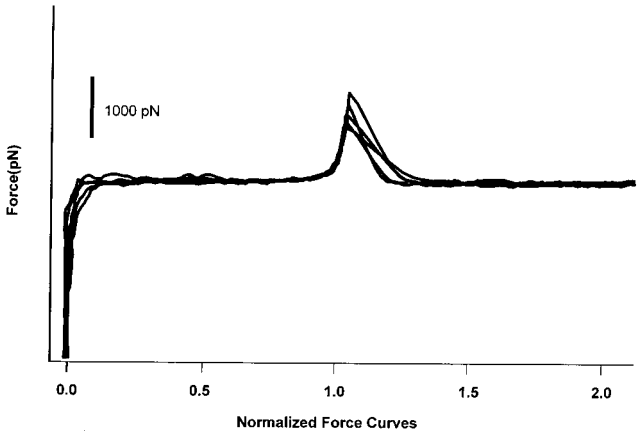


Figure 10. Normalized force curves of PAAM in 8 M urea aqueous solution.

hydrogen bonding in the PAAM chain. In the system of PEG in water, Oesterhelt et al. ascribed the kink in the force curve to the force-induced destruction of nonplanar suprastructure stabilized by water bridges. In our system of PAAM, it would be more likely that the polymer chain forms intramolecular hydrogen bonding via H atoms of the amino groups and the oxygen atoms in carboxyl groups. Tanaka et al.[36] revealed a hydrogen-bonding-stabilized conformation of PAAM in water was not the regular helical structure but the normal one. To prove our speculation (intramolecular hydrogen bonding) further, 8 M urea aqueous solution was used to destroy the hydrogen

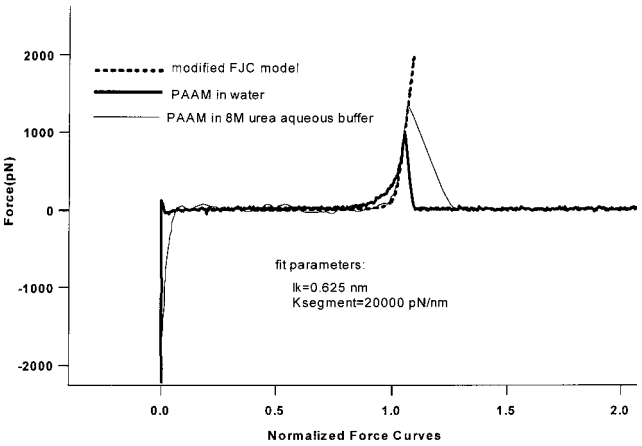


Figure 11. Comparison of an extended FJC fit and normalized force curves of PAAM in water and in 8 M urea aqueous solution.

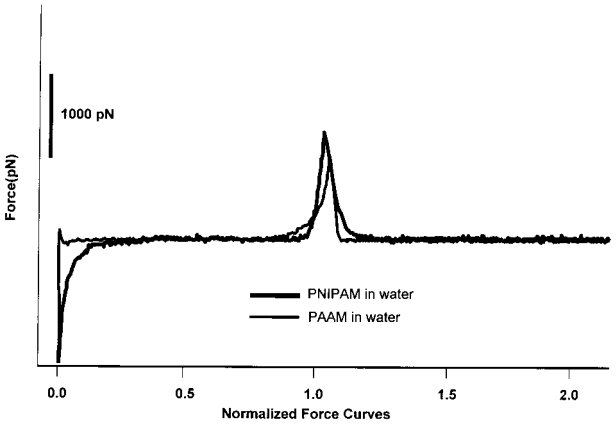


Figure 12. Comparison of the normalized force curves of PNIPAM and PAAM in water.

bonding in the polymer chain. It was found that the force−extension curves of PAAM could also be normalized and superimposed as shown in Figure 10. However, in 8 M urea aqueous solution, the stretched PAAM chain behaves as an ideal entropy spring and can be well-described by modified FJC model producing fit parameters: $I_k = 0.60 \pm 0.05$, $K_{segment} = 20000 \pm 3000$ pN/nm. Figure 11 shows a comparison of normalized force curves of PAAM obtained in deionized water and in 8 M urea aqueous solution and the fit result of FJC model on PAAM chain stretching in urea buffer solution. The difference among them was demonstrated very clearly. Although the force curves of PAAM in water and in 8 M urea are different in the middle regime of the force curve, they exhibit exactly the same elasticity in the high force regime which is mainly controlled by enthalpy corresponding to the deformation of the bond angle.

Effect of Substitute on the Elasticity of Polymer Chain. When we review the fit parameters of force curves of PNIPAM and that of PAAM again, we will find that PNIPAM chain has larger Kuhn length (around 0.70 nm) and segment elasticity (about 25000 pN/nm) than that of PAAM in high force regime ($I_k \sim 0.60$ nm, $K_{segment} \sim 20000$ pN/nm) in deionized water. Figure 12 shows a comparison between the normalized force curves of PNIPAM and PAAM, which make the difference more obvious. From these results, we can see the influence of different side groups on the stiffness of polymer chain. That is, polymer chain becomes stiffer when larger substituent groups exist, and the internal steric effects of polymer chain will affect the whole elasticity of polymer chain both in the low force region and high force section. Solvent quality is thought to affect the elasticity in the low force regime, which is attributed to entropic contribution. Let's take the force spectrum of PAAM in pure water and 8 M urea aqueous solution for example. In both buffer solutions, the shapes of force curve in the high force region (above 450 pN) are the same but in the low force regime, especially in the middle regime, the force profiles are different. When the solvent was changed from good to poor, in the low force regime, we observed a bumpy baseline, but at high forces the shapes of the force curves did not change.

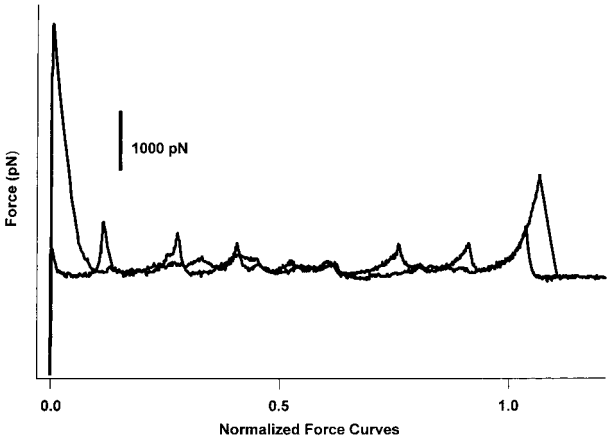


Figure 13. Normalized force curves of PNIPAM in pure water after thermal treatment.

Another difference between PNIPAM and PAAM lies in the fact that when 8 M urea aqueous solution was used as a buffer, the stiffness of the polymer chain of PNIPAM increased but for PAAM the segment elasticity in the high force regime (above 450 pN) was almost the same compared with that of in deionized water. It is understandable because PAAM has two hydrogen atoms on every repeat unit. As a result it is much easier for PAAM to dissolve and interact with water than PNIPAM. When 8 M urea aqueous solution was used, it is more likely that urea molecules may form strong interactions with PNIPAM (than water molecule). Then they increase the effect of side groups on the stiffness of polymer chain. We have ever studied the interactions between urea molecules and PNIPAM by using FT-IR method (data not shown). The experimental process was as follows: we prepared a mixed aqueous solution of PNIPAM and urea, in which the ratio between the side groups of PNIPAM and urea molecule was about 1:1, and then the solution was dried using a freeze-drying machine. At the same time, the pure urea and pure PNIPAM sample were processed by using the same method. Then we performed FI-IR study on these samples by normal ways for solid samples. By subtracting the pure spectra of urea and PNIPAM from the mixture, a few new peaks appeared (such as at 1218 cm^{-1} and 1725 cm^{-1}). This should be attributed to the interactions between urea molecules and the side groups of PNIPAM.

Single-Molecule Force Spectrum of PNIPAM after Thermal Treatment. It has been shown that PNIPAM undergoes a coil-to-globule transition in aqueous solution after being heated, and many investigations have been made on the transition by using laser light scattering.[25−29] We also studied the effect of thermal treatment on the conformational transition of PNIPAM by using SMFS. In contrast with the force curves before thermal treatment, more force curves of multi-force signals were found after thermal treatment. Although more force signals were observed, the last force peak can be superimposed well; indicating that a single polymer chain stretching was obtained as shown in Figure 13**.** There are several possible reasons for

this phenomenon. One of the reasons for this is that the polymers could form crumpled globes on the glass surface, and then this kind of structure was destroyed by stretching one end of the crumpled coil. Because there exist some strong interactions within it, as a result more peaks were observed during stretching. This seems something like the unfolding of Ig titin domain structures.[8,16] Another possible reason is multi-chain stretching. The last reason is that the polymer chain has formed multisite contacts with the substrate and formed more loops after thermal treatment. By stretching one end of the loops of the polymer chain the contact points on the substrate were destroyed one by one. Considering the fact that our sample was anchored on the substrate by physical adsorption, it is hard to stretch a crumpled coil. Because our solution is very dilute, the multi-chain stretching can also be excluded. So we would like to explain the result after thermal treatment on the basis of thermally induced multisite adsorption. We did another control experiment as follows: we first prepared a sample without thermal treatment and then investigated it and obtained some force curves. After that, the same sample was kept at 33 °C for about 10 min. Then we investigated it using SMFS method again. We found that before this kind of thermal treatment, we can obtain clean force curves, and the probability that we can obtain stretching signals was low. After heating, the probability we can stretch a polymer chain became larger, and at the same time more unclean force curves were obtained. This supported in a way our assumption of thermally induced multisite adsorption.

Another Way To Perform SMFS Experiment. Usually, we did the experiment in the way described before. The polymer film was brought to contact with AFM tip so that the polymer chain in the film could adsorb onto the tip, then the polymer chain was stretched away from the substrate (see Figure 14 a, A). During the experiment we sometimes observe the situation that the polymer chain was adsorbed onto the tip from the polymer film and it could not break off from the tip easily. Then we just brought this tip-absorbed polymer chain to contact with a new clean glass substrate. In this way, we can also stretch the polymer chain from AFM tip and get nice force−extension curves of PNIPAM as shown in Figure 14a, B. The force curves achieved in this way can also be normalized and superimposed well with those of normal way as shown in Figure 14b, which clearly show single-polymer chain stretching. This fact implies that we can prepare the experiment in another way: we just need to attach the polymer to the AFM tip and then to contact with the substrate. This method can be helpful if we have very little sample and only want to study the segment elasticity of single-polymer chain.

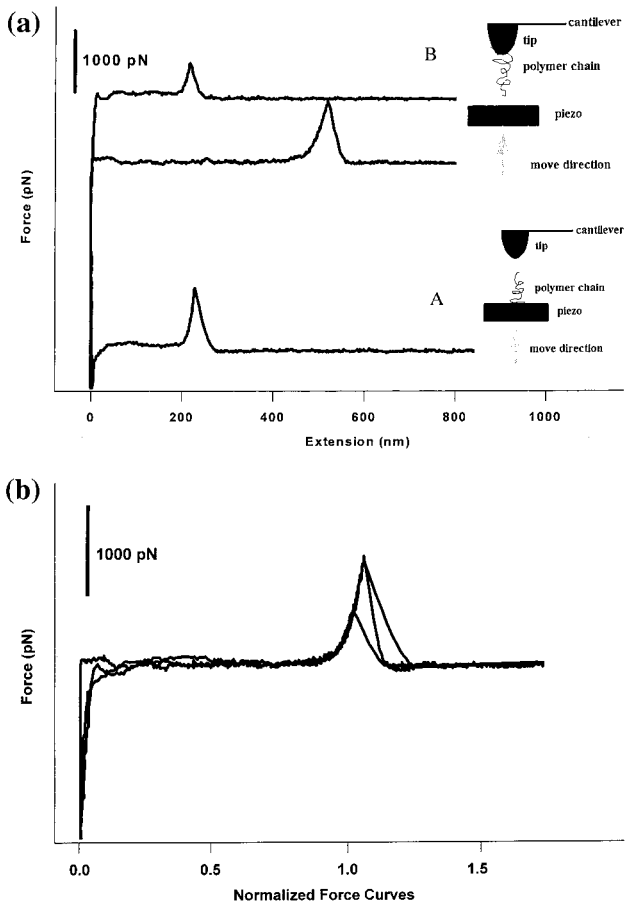


Figure 14. (a) Force−extension traces obtained by usual way and opposite one. A: normal way, B: another new way. (b) Normalized results of (a).

Conclusions

In conclusion, the nanomechanical properties of poly(*N*-isopropylacrylamide) and poly(acrylamide) in deionized water and 8 M urea aqueous solution were studied by using an AFM-based technique—single molecule force spectroscopy (SMFS). Different force−extension traces were obtained in both systems. They could be normalized and superimposed separately. The force curves of PNIPAM and PAAM in 8 M urea aqueous solution could be fitted by extended freely jointed chain (FJC) model separately. These facts indicate a single ideal random coil polymer chain stretching was obtained. At the same time, the influence of side groups on the stiffness of polymer chain was studied. Our results show that a larger side group may make the polymer chain stiffer. In deionized water PAAM may form intramolecular hydrogen bonding via its side groups, whose force curve cannot be fitted with extended freely jointed chain model. When 8 M urea aqueous solution was used as buffer, the hydrogen bonding was destroyed. As a result, that deviation disappeared and the force curve can be fitted with extended FJC model. When the PNIPAM sample was heated to 33 °C, above its LCST, more signals were observed in one stretching, this could be interpreted as thermally induced multisite adsorption. Another way just different from the normal one to do the SMFS experiment was provided.

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