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Single-Molecule Force Spectroscopy on Poly(acrylic acid) by AFM

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The mechanical properties of poly(acrylic acid) (PAA) were investigated on the molecular scale by using AFM. PAA was adsorbed onto a glass substrate and then picked up at one point of the chain with the AFM tip and stretched. The deformation of single PAA chains under tension was measured and modeled by a modified freely-joint-chain model. The length distribution of loops and tails of adsorbed PAA on the glass substrate was also studied by analyzing the extension of the filaments.

Introduction

The development of atomic force microscopy (AFM) has opened the floodgate for measuring the local physical and chemical properties of materials with molecular and even atomic resolution.[1] The cantilever of the atomic force microscope is a nanoscopic force sensor which is so sensitive that it can detect a force around 10 pN. Combining the high lateral resolution with extreme force sensitivity, AFM has evolved into a versatile platform for experiments with single molecules.[2−7] AFM has been used to quantitatively measure the specific interaction between ligand and receptor, such as avidin/biotin and conjugated DNA strands. Much new information provided us with new insights into inter- and intramolecular forces and even the underlying molecular mechanism. Recently, a new technique based on AFM−single-molecule force spectroscopy has been implemented and opened opportunities to study the mechanical properties of single polymer filaments varying from coiled polymers to polymers bearing superstructures.[8−10] Until now, dextran, xanthan, poly(ethylene glycol) and poly(vinyl alcohol) have been stretched, and detailed nanomechanical properties have been obtained. Also, this technique has been used to measure the unfolding forces of individual Ig domains of the protein titin.[11] Their force spectra provided us with
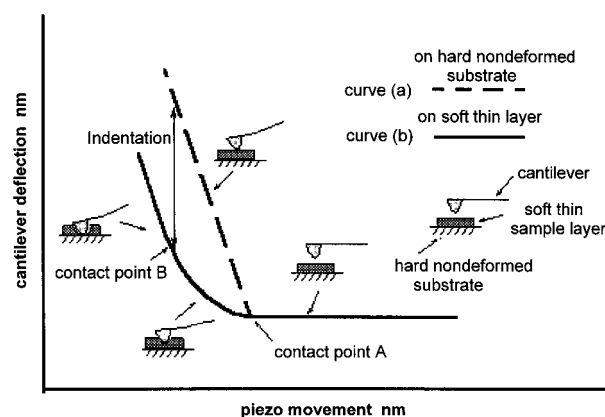

Figure 1. Approaching traces of the force curves: (a) Force curve on a hard nondeformed substrate; at point A the tip touches the hard substrate. (b) Force curve on a soft thin sample. Point A: the tip touches the soft sample. Point B: at this point the thin, soft layer is fully compressed by the tip and the tip touches the hard substrate beneath the soft sample; the region between points A and B is the contact area, where the cantilever deflection and piezomovement shows a nonliearlity.

much valuable nanomechanical fingerprint information of these polymers which may deepen our understanding of the mechanical properties of polymers. In this paper, we will report the results of single molecular force spectroscopy on poly(acrylic acid).

Poly(acrylic acid) (PAA) is an important polymer for a variety of industrial applications.[12] PAA has been widely used to stabilize the colloids, which is closely related to the adsorption. Adsorption of flexible neutral polymers at surfaces has been studied extensively and is now a well understood phenomenon.[13] The steric and bridging forces between surfaces bearing adsorbed PAA have been studied recently by using AFM.[13] Here, PAA was adsorbed onto a hydrophilic glass substrate, and an AFM tip was used to stretch PAA polymer chains and measure the deformation of a single PAA chain under tension.

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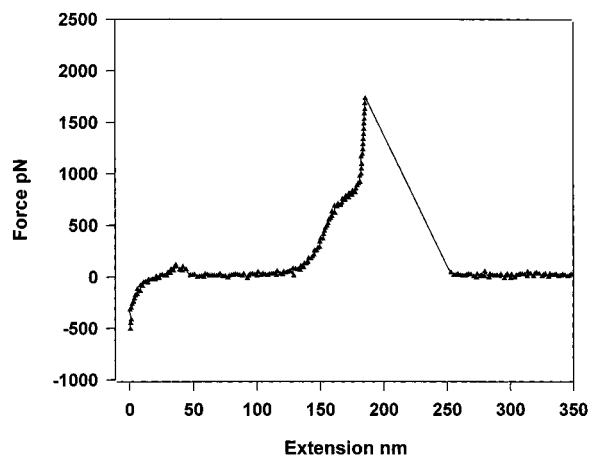
Figure 2. Force spectrum of dextran which shows a force plateau at 750 ± 50 pN, which corresponds to a force-induced conformation transition of the glucose ring in the dextran polymeric backbone.[8]

Experimental Section

The experimental details of single-molecule force spectroscopy by AFM have been described elsewhere.[8,9] In general, the polymer was chemically or physically adsorbed onto a substrate. When the AFM tip was brought into contact with the sample, some molecules adsorbed onto the tip due to the nonspecific interaction and made a connective bridge between the sample and the tip. While separating the tip and the sample, these molecules were stretched. The deflection−extension curves were recorded and converted into force−extension curves.

Sample Preparation. Commercially available poly(acrylic acid) was used for this study (M_v of 4.5×10^5 from Adrich). PAA was dissolved in Millipore water to a concentration of 1 mg/mL; the pH value was between 3 and 4. An appropriate amount of PAA solution was deposited onto a clean glass cover slip and dried in air, and then PAA formed a thin layer. Afterward, the substrate was rinsed with Millipore water to remove the loosely adsorbed PAA and immediately used for force measurements.

Force measurements were carried out with a Nanoscope IIIa instrument (Digital Instruments, Santa Babara, CA) in a 10^{-3} M KNO$_3$ buffer solution. A silicon nitride cantilever (180 μm long and 0.6 μm thick) from Park Scientific Instruments (Park, Sunnyvale, CA) was used.

The thickness of the adsorbed PAA layer on the glass was estimated from the approaching trace of the force curve. As shown in Figure 1, when the probe tip is brought into contact with a hard nondeformed surface, the deflection of the cantilever will be proportional to the piezomovement (curve a); however, if the sample is soft, the soft sample deforms with increasing cantilever deflection, resulting in a nonlinear contact region of the force curve (curve b, the region between points A and B). If the soft layer is very thin, the layer may be fully compressed and the tip may touch the hard substrate beneath the soft layer at one point (point B of curve b); the linearity of the cantilever deflection and piezomovement will appear again. The distance between contact point A and "contact point" B (the point where the tip touches the hard substrate) is approximately equal to the thickness of the soft sample layer. The difficulty in determining the real contact points A and B may result in the error of estimating the thickness. Here, we neglect the contribution of the electrostatics to the thickness; thus, we can only qualitatively estimate the thickness of the soft layer from the approaching trace of the force curve. In this study, the thickness of the PAA adsorbed layer was around 50−90 nm.

Calibration of the Spring Constant of the Cantilever. Calibration of the spring constant of the cantilever is very important for force measurements. The most widely accepted techniques are the spring resonance method introduced by Cleveland[15] and the thermal noise method introduced by Butt[16] and Florin.[17] Here, we adopt a new method for cantilever
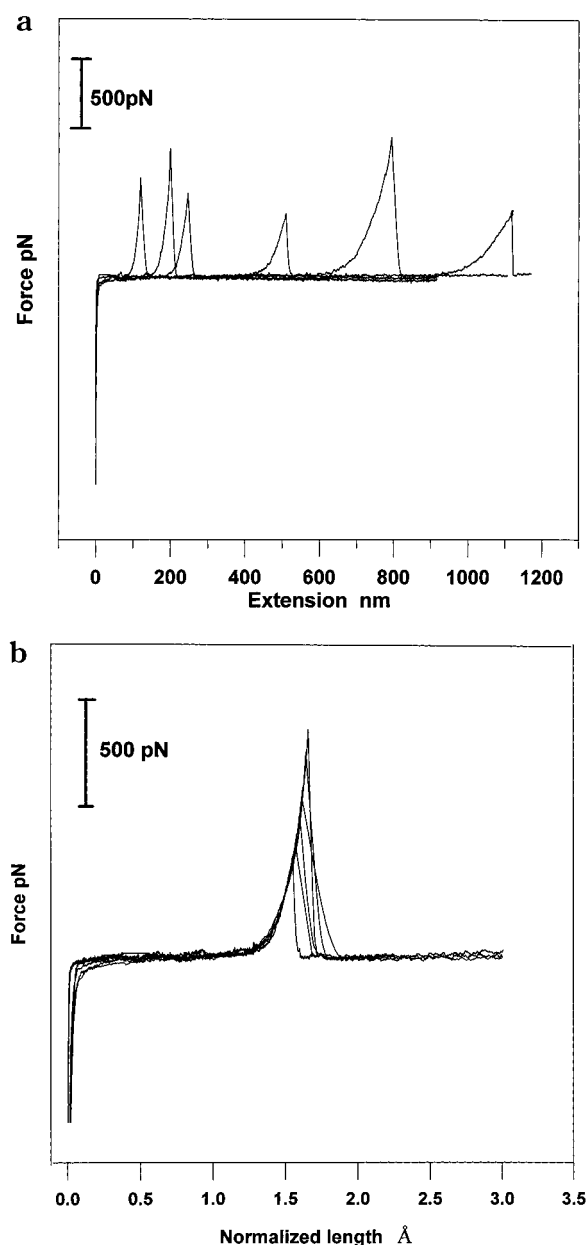

Figure 3. Force−extension curves of poly(acrylic acid): (a) force curves of PAA of different lengths; (b) superposition of normalized force curves of part a.

calibration, which was first suggested by Gaub.[8] By using this method, dextran was used as an internal standard sample for calibration. It had been found that there existed a force-induced conformation transition of the glucose ring in the polymer backbone for dextran at 750 ± 50 pN, which corresponds to a force plateau in the force spectrum, as shown in Figure 2. To calibrate the cantilever we used here, we did force measurements on dextran in phosphonate buffered saline (from Sigma). We devided 750 pN by the value of the cantilever deflection at the plateau obtained from the force curve and thus obtained the spring constant. The value we obtained here was 0.069 ± 0.003 N/m (the force constant supplied by the manufacture is 0.05 N/m). Compared with other calibration methods, this one is much easier and more convenient for Nanoscope users.

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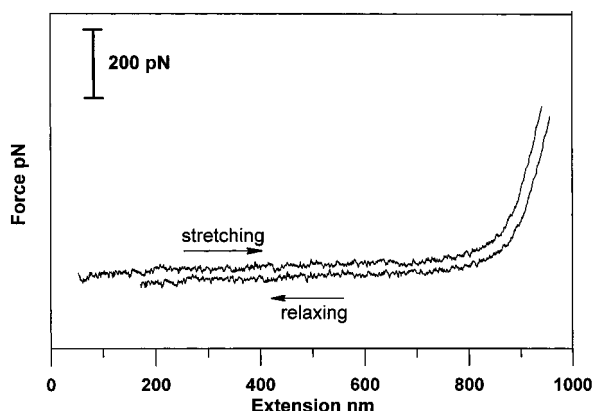(17) Florin, E. L.; Ludwig, M.; Rief, M.; Gaub, H. E. *Biosens. Bioelectron.* **1995**, *10*, 895.

Figure 4. Stretching and relaxing force curves of a PAA molecule; for clarity the traces are offset.

Results and Discussions

Typical force−extension curves of PAA are shown in Figure 3a. Since the polymer is polydisperse and we cannot control the point at which the polymer chain is picked up with the tip, the contour length of the polymer which is stretched between the tip and sample varies. Despite the different contour lengths of polymers chains, all of the force curves show similar characteristics: the force rises monotonically with the extension of the polymer chain until a rapture point is reached, and then the force drops to zero suddenly. The rapture force can be as high as 1.8 nN.

All of the force curves are normalized according to their contour lengths, superimposed, and plotted in Figure 3b. The superposition of these curves clearly shows that the elastic properties of PAA chains scale linearly with their contour lengths. Thus, we can deduce that interchain interaction does not contribute strongly to the elastic properties of PAA, because interchain interactions would scale nonlinearly with the length.

The curves shown in Figure 4 are the subsequent deformation curves of a single PAA chain. It clearly shows that the deformation of a single PAA chain is fully reversible, indicating that the experiment is carried out at an equilibrium state.

The force law of polymer chains under tension can be derived from the freely-joint-chain (FJC) model.[18] The FJC model treats a polymer as a chain of statistically independent segments of length I_k (Kuhn length). The force law of the FJC model is given by Langevin function:

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

The elasticity of a freely-joint-chain purely comes from the entropic contribution. The curve fit with the FJC model is plotted in Figure 5a together with the measured force−extension curve. The fit gives a Kuhn length of 0.64 nm. We can see that the freely-joint-chain model is able to describe the elastic behaviors of PAA at low forces, but a great discrepancy between data predicted by the FJC model and the experimental data can be found at high forces.

The failure of the FJC model in the high-force regime may be due to the coarse description of the polymer in terms of discrete segments. The FJC model assumes that the segments of a polymer are inextensible. Following Bustamante[19] and Gaub[8], a modified FJC model is used

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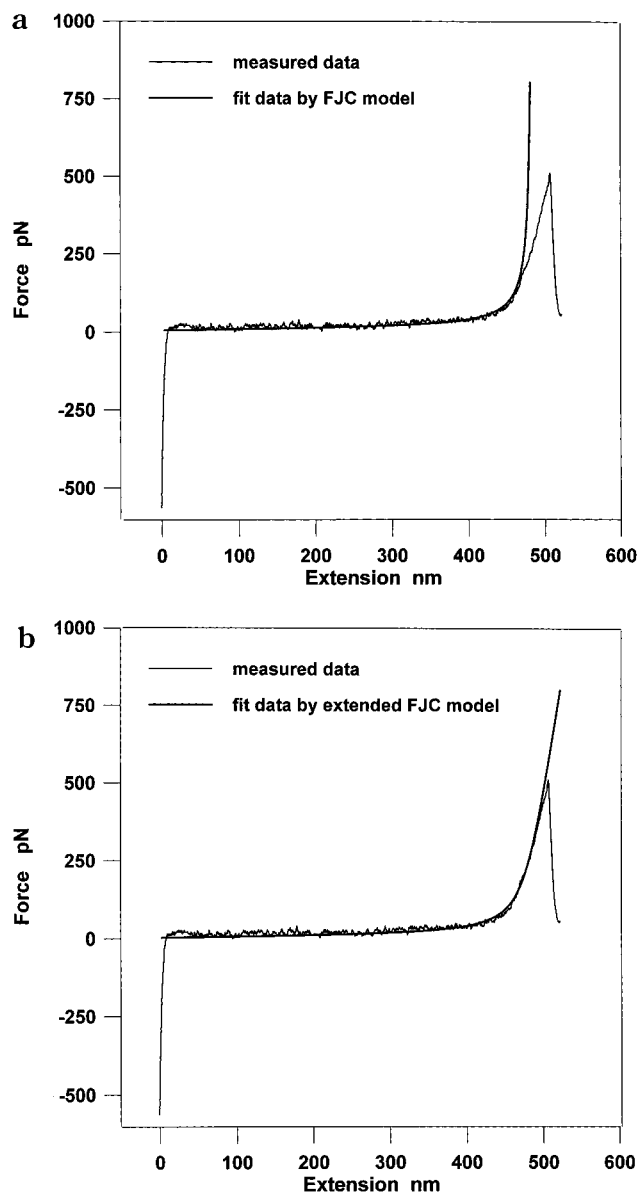(19) Smith, S. B.; Cui, Y.; Bustamante, C. *Science* **1996**, *271*, 795.

Figure 5. (a) Measured force curves of a PAA chain superimposed by a fit with a FJC model; the fit parameter $I_k = 0.64$ nm. (b) Measured force curves of a PAA chain superimposed by a fit with a modified FJC model; the fit parameters $I_k = 0.64$ nm and $K_{segment} = 1300$ pN/Å.

to fit the data. This modified FJC model is constructed from a fixed number of segments whose lengths increase upon stress.

Thus, the Langevin function is extended by a new parameter $k_{segment}$ (segment elasticity) and given as follows:

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

Here n is the number of Kuhn segments, which is equal to $L_{contour}/I_k$.

From this extended Langevin function, we can see that at low forces the elasticity of the polymer is still entropy dominated, which means that most of the work done on the chain would orient these segments' end−end vectors (decreasing entropy), whereas at higher forces additional work would be required to straighten the segments (increasing enthalpy).

The fit curve is plotted in Figure 5b, which gives a Kuhn length of 0.64 nm and a segment elasticity of 1300 pN/Å.
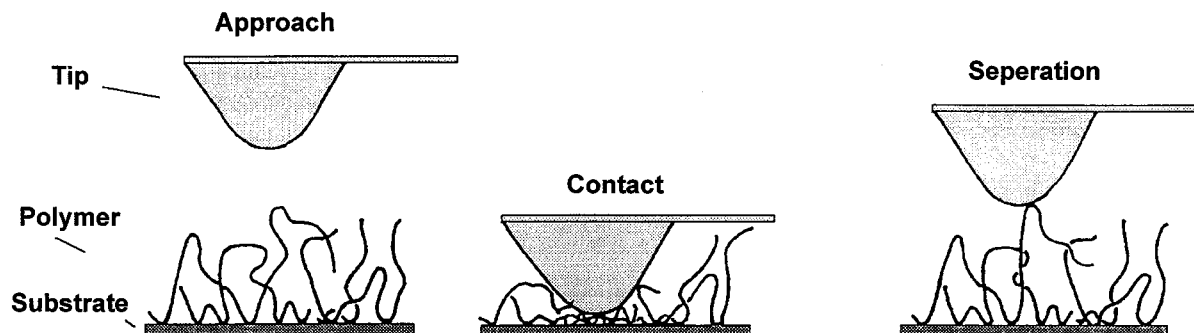
Figure 6. Schematic of the formation of a connective bridge between the tip and the sample.
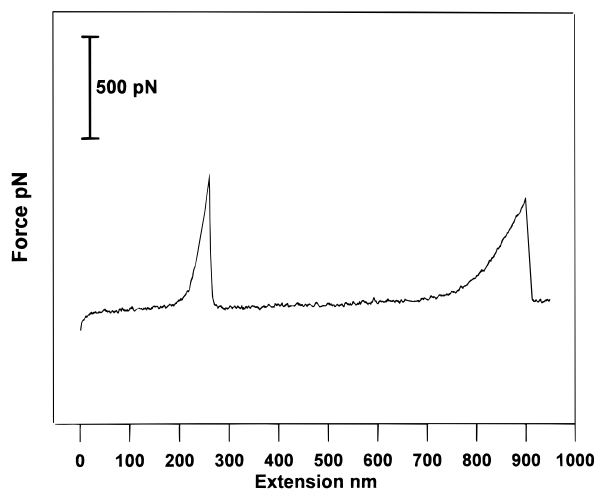

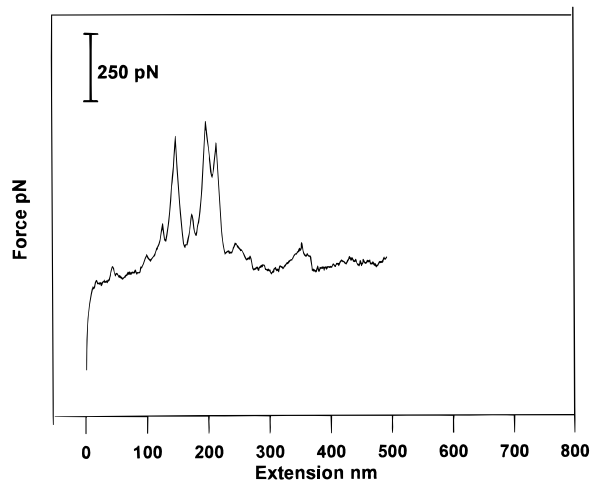**Figure 7.** Force extension curve of PAA showing two rising slopes.

Figure 8. Force extension curve of PAA, showing that a bundle of PAA chains is stretched.

Figure 9. Histogram of the extension length of PAA chains.

We can see that the extended Langevin function is able to model the elastic properties of PAA in the low- and high-force regimes. This indicates that the elasticity of PAA at low forces is dominated by the entropic contribution; in the high-force regime, the elasticity of PAA is governed by both entropy and enthalpy.

By fitting 30 experimental force−extension curves of different contour lengths, we found that all of the PAA filaments possess an almost identical Kuhn length of 0.64 nm (standard deviation is 0.05 nm) and a segment elasticity of 1300 pN/Å (standard deviation 200 pN/Å), although the lengths of these filaments vary from 60 nm to more than 1 μm.
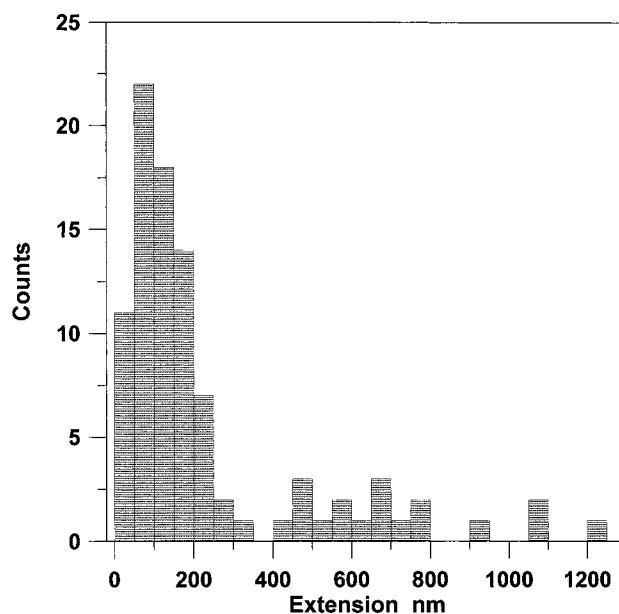
These two findings, the linear scaling of the elastic properties with the contour lengths and the identical segment elasticities and Kuhn lengths for all polymer chains, corroborate that predominately individual PAA chains are stretched and the deformation of a single chain under tension is measured.

Since PAA adsorbs strongly onto hydrophilic glass substrates, the resultant adsorbed PAA layer consists mainly of PAA loops and tails of various lengths. Thus, the deformation of single PAA chains in this study is virtually the deformation of single PAA loops or tails, as schematically shown in Figure 6.

At first sight, it seems to be unlikely that always single polymer chains are stretched. Considering the small tip radius of 20−50 nm and the gyration radius of PAA of approximately 35 nm,[14] we can imagine that interaction between the tip and many molecules is allowed (as shown in Figure 6). However, the protocol we used here to couple the molecule and the tip is based on the nonspecific interaction. Thus, the binding of molecules to the tip is a very rare event during a short contact period (In our experiments, we did not obtain any pull-ups in more than 70% of the force measurements). Only very few molecules can build a bridge between the tip and sample during this short contact period. Even if three polymer chains luckily attach themselves to the tip in a parallel manner at the same time, it is very unlikely that two of them have exactly the same lengths and are subject to the tension at the same time. They rather show up as three subsequent rising slopes in the force curve, each of which reflects the

elasticity of a single polymer chain. In Figure 7 an example of two PAA chains sticking to the tip is shown; two subsequently rising slopes in one force curve are observed. Since the adsorbed PAA layer is continuous, the entanglement of polymer chains sometimes has a great influence on the experiment. In some cases, we stretch a bundle of polymer chains, instead of a single polymer chain, as shown in Figure 8.

As mentioned above, the rapture force in the force−extension curve can be as high as 1.8 nN. This high rapture force could be caused either by the scission of a C−C bond in the polymer chain or by the desorption of polymer filaments from the tip surface or the substrate surface. Considering the C−C bond energy of 345.6 kJ/mol (70 kT),[20] the first possibility could be discounted. Thus, the rapture force reflects the interaction between PAA and the tip or the substrate. It is likely that several hydrogen bonds between the surface silanols and carboxyl groups and van der Waals interactions are responsible for this high rapture force.

Single-molecule force spectroscopy is not only powerful in studying the nanomechanical properties of a single-polymer chain, it is also useful in studying the length distribution of loops in the adsorbed polymer layer. As predicted by the FJC model, the polymer chain will be stretched to 95% of its contour length at 102 pN; thus, we could measure the length distribution of PAA loops and tails on the surface. Figure 9 presents the histogram of the polymer chain extension at 102 pN determined from 100 pull-ups. It can be clearly concluded that most of the loops are shorter than 200 nm and that some tails exhibit lengths of more than 1 μm.

In summary, the deformation of single PAA chains under tension has been measured by using AFM and modeled by a modified FJC model. The deformation behaviors of single PAA chains may represent some common deformation characteristics of polymers provided that there does not exist suprastructure (for example PEG[10]) and great force-induced conformation changes (for example dextran[8]). It also shows that single-molecule force spectroscopy is useful for studying the polymer adsorption at solid surfaces.

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LA9800304