Elasticity of Single Polymer Chains

M. Maaloum* and A. Courvoisier

Institut Charles Sadron-Université Louis Pasteur, 6 rue Boussingault,
F-67083 Strasbourg, Cedex, France

Received June 30, 1998; Revised Manuscript Received April 26, 1999

ABSTRACT: Telechelic polymer chains (hydrophobic end-capped poly(ethylene oxide)) linked to a hydrophobic surface were probed with the atomic force microscope tip by vertical stretching. Under conditions that allowed only a limited number of molecular chains to interact with the hydrophobic atomic force microscope tip, the gradient of the forces was found to be quantized in integer multiples of 540 ± 30 and 330 ± 25 μN/m for polymers of mass 20 000 and 35 000, respectively. The measured force gradient quanta is interpreted as the stiffness of a single polymer chain. This work may shed new light on macromolecules at interfaces involving chain extensions and opens the route for elasticity studies of single macromolecules.

Introduction

Interest in the mechanics of biopolymer chains has grown in recent years, due to the possibility of working with individual molecules.[1−4] To gain an understanding of the behavior of the macromolecules at interfaces, a direct measurement between single molecules and surface is required. The atomic force microscope has recently been applied to the study of single intermolecular interactions of the biotin−streptavidin system and to the stretching of individual molecular springs.[5−8] The force microscope is an ideal instrument for investigating the force required to extend polymer chains. This paper demonstrates that the force microscope can be used to measure the elasticity of single polymer chains under stress.

Experimental Section

Technique. All surface force measurements were obtained using a commercial atomic force microscope (AFM), Nanoscope III (Digital Instruments, Santa Barbara, CA). For surface force measurements, it is necessary to determine the stiffness of the cantilever *k*. We have used the method described by Senden et al.[9] In fact, the cantilever deflection was measured after addition of the tungsten particles (Goodfellow, Cambridge, UK) of known size (controlled by an optical microscope) and then again after the cantilever was rotated 180° in a vertical plane (placed upside down the AFM head). The difference between the measurements is twice the deflection due to gravity. We found that the spring constant is equal to 0.12 ± 0.02 N/m. To measure HPOE−hydrophobic surface interaction forces, we coated the flat surface (oxidized silicon wafer) with a polystyrene layer, by spin coating from toluene solution, to make it hydrophobic.

The AFM tip was coated with a thin film of polystyrene. In fact, a very small drop of polystyrene in toluene was deposited on the tip using a micropipet under the control of an optical microscope. The cantilever was not coated with polystyrene (only the AFM tip) because this would cause the spring constant of the cantilever to be changed. We have included a scanning electron microscopy image (Figure 1A) showing the comparison between the coated and uncoated AFM tips. It can be clearly seen that the pyramidal shape of the coated tip has not changed in a significant manner. In contrast, we can see that the base of the pyramid of the coated tip is very smooth while the uncoated tip is very distinct. It is very difficult to determine with precision the radius of the coated tip, but from the SEM image I would say that the radius may be 2−3 times larger than the uncoated tip, (i.e., 60−100 nm). The cantilever was annealed at 100 °C to firmly attach the polystyrene to the probe. The adhesion forces with coated tip, measured with AFM, is 10 times higher than that uncoated. The measured

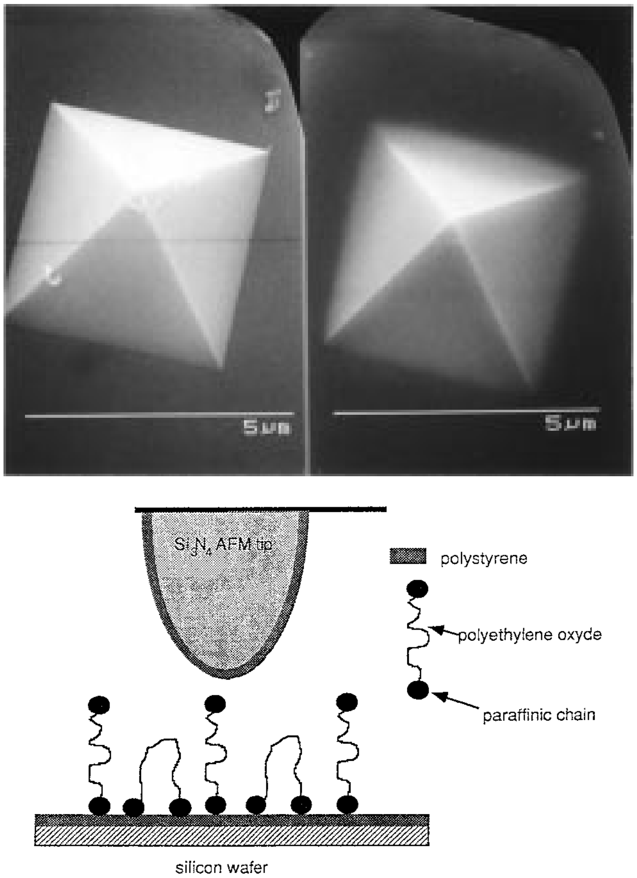

Figure 1. (A, top) Scanning electron microscopy image of the AFM tip. The image shows the comparison between the coated (right) and uncoated (left) AFM tips. It can be clearly seen that the pyramidal shape of the coated tip has not changed in a significant manner. In contrast, we can see that the base of the pyramid of the coated tip is very smooth while the uncoated tip is very distinct. (B, bottom) Schematic of the polystyrene-coated AFM tip and the diluted adsorbed HPOE on a polystyrene-coated silicon wafer surface.

---

* Author for correspondence.

10.1021/ma981023p CCC: $18.00

roughness, using the atomic force microscope, of the flat surface coated with polystyrene thin film is of the order of 1 nm.

The force curves recorded with the Nanoscope III AFM was written into a buffer which could then be saved on computer disk. Force data collected by the AFM were captured as a screen file of photodiode (volts) as a function of the sample displacement. This file was converted to force versus distance using a program especially written for this purpose. Constant compliance between the probe and the flat surface occurs when the two surfaces are "in contact"; here the signal difference across the photodiode detector is linearly proportional to the piezotube expansion. This defines the zero separation distance and also serves to calibrate the photodiode reading with spring displacement.

Polymers. The polymers, hydrophobic end-capped poly(ethylene oxide) (HPOE), exhibit the simple following chemical structure

where the jagged line represents a poly(ethylene oxide) chain (POE) with molecular weights 20 000 and 35 000, and the black box represents a paraffinic chain consisting of 12 carbon atoms.

The HPOE were prepared in two stages: (i) by preparation of the dodecyl *p*-toluenesulfonate (DPS) obtained by action of *p*-toluenesulfonyle chloride on 1-dodecanol in the presence of pyridine, according to a classical procedure;[10] (ii) by reaction between DPS and a α,ω-dihydroxylated poly(ethylene oxide) (PEO), previously deprotonated by potassium diphenylmethane. The samples are monodisperse ($I = 1.01$ for mass 20 000 and $I = 1.03$ for 35 000) and have been well characterized by size exclusion chromatography in water.[11]

Results and Discussion

The force was measured between the tip and the diluted adsorbed HPOE layer (from concentration solution of 10^{-5} g/g $\ll$ critical association concentration CAC $= 3 \times 10^{-3}$ g/g) on a flat surface (Figure 1B) in aqueous solution. The cantilever tip was carefully moved toward the substrate surface, and a series of approach-and-retract cycles were collected. The typical separation forces originating from telechelic HPOE experiments are shown (Figure 2A). On retraction the lever detected an attractive force at a distance from 0 to approximately 50 nm above the surface. This phenomenon was also observed within a few nanometers of the surface without touching it.

In contrast, a typical approach-and-retract cycle taken between two surfaces without HPOE (Figure 2B) showed that adhesion took place directly at the surface; this observation indicates the presence of short-range forces, and the slope of the adhesion curve shows that there was no elasticity in the sample itself. For this experiment, no interaction force was detected until the surfaces were about 15 nm apart. At this distance the inward motion increased rapidly, and the surfaces jumped into an adhesive contact. The jump is due to mechanical instability and is expected whenever $\partial F/\partial D > k$ (here k is the spring constant of the cantilever). These forces are consistent with hydrophobic surfaces. If only the van der Waals attractive forces were present, we would expect the surfaces to jump together from a much closer separation of less than 5 nm.[12]

The compatibility between POE and PS is known to be very weak, but in the test experiment we performed using only POE (without the paraffinic chains), no jumps were observed. We detected very small adhesion forces, indicating that the compatibility between the PS and POE is low. Of course, this does not mean that the PS/HPOE/PS interface will phase separately into a laminar structure. There is also a significant number of adsorbed chains on the PS surface. But this latter does not change our result, because of the weak adsorption of POE on the polystyrene surface. The HPOE can be opened into tail structures through the repeated effects of attractive interactions with the polystyrene tip. These results were demonstrated in our paper published.[13,14]

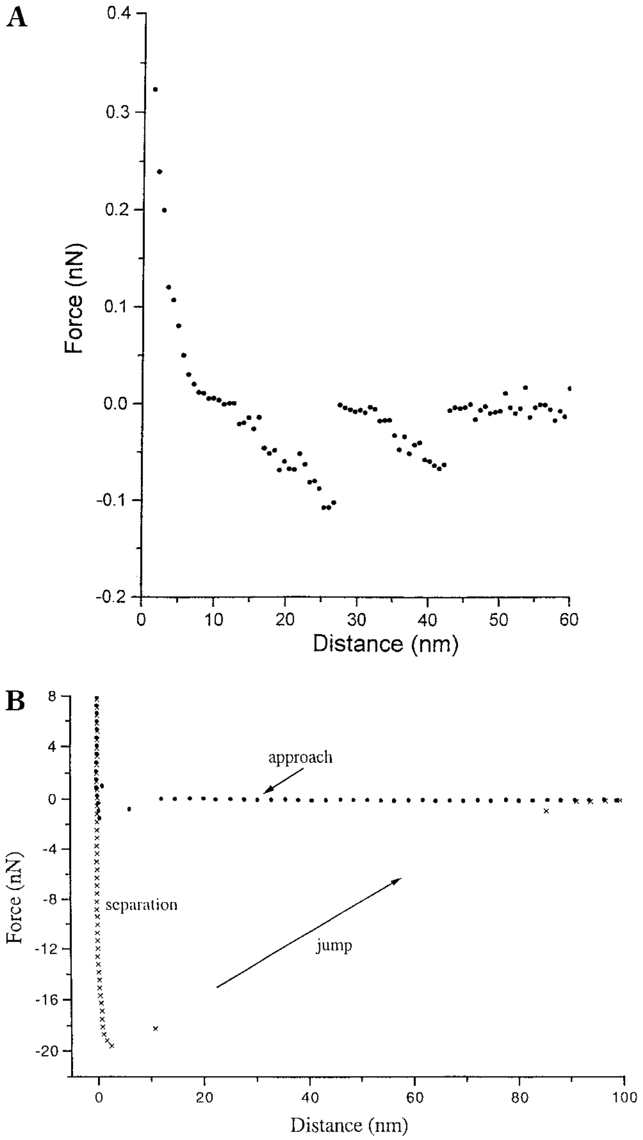

Figure 2. Typical separation force interactions between the AFM tip and adsorbed HPOE versus distance. (A) represents typical AFM tip−HPOE interactions. (B) is an example of the interaction between the tip and silicon wafer both covered with polystyrene layer without HPOE.

The shape of the separation force curves between HPOE and the tip suggests the presence of long-range interactions, interpreted as the lifting and extension of the chains, followed by further stretching until the elastic force of the cantilever equals the strength of the binding and the lever "jumps off". Multiple jump-offs were frequently observed, indicating polyvalent binding (Figure 2A). These observations are consistent with a model in which the parrafinic chains are responsible for the adhesion. Because of their hydrophilic nature, the POE did not participate in the adsorption to the AFM tip and thus remained free to irradiate into the solution.

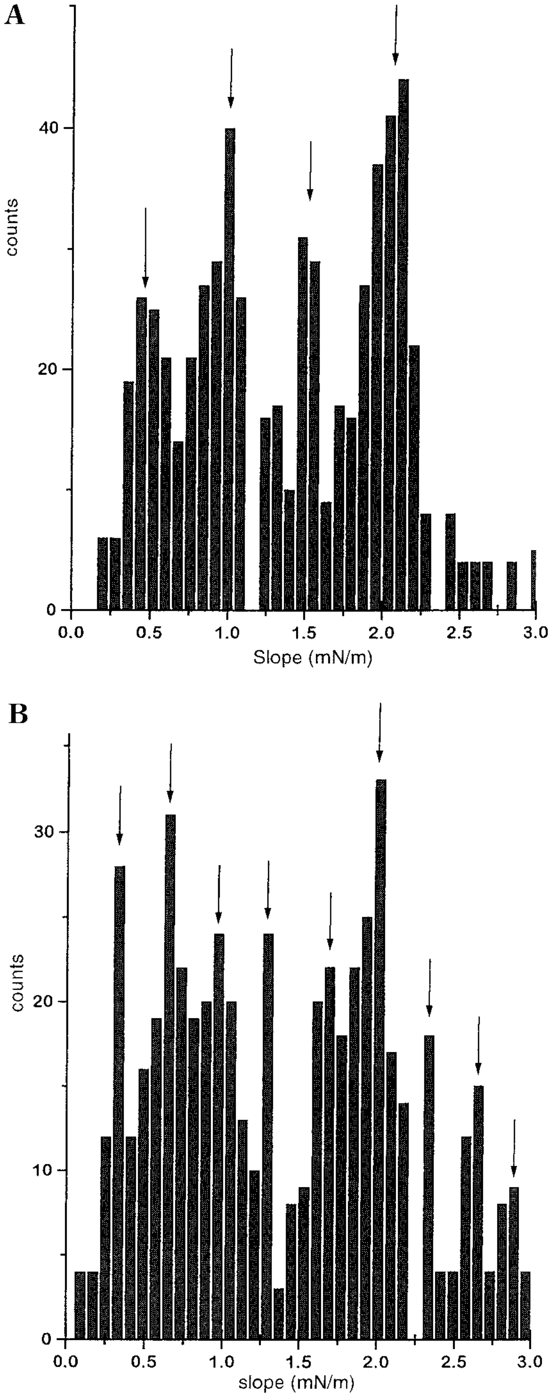

Figure 3. Histogram of the gradient forces derived from 300 force measurements between the polystyrene probe and HPOE polymer chains. (A) Polymer of mass 20 000. The histogram shows four maxima highlighted with arrows. (B) Polymer of mass 35 000. The histogram shows nine maxima highlighted with arrows.

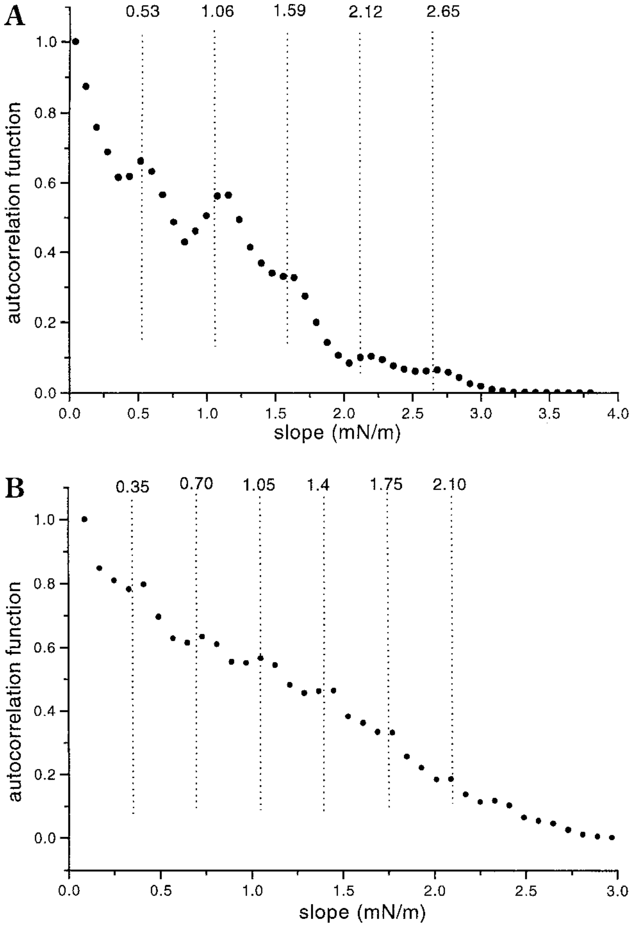

Figure 4. Autocorrelation analyses of histograms A and B for polymers of mass 20 000 and 35 000, respectively.

During each approach-and-retract cycle, multiple noncovalent bonds between the HPOE chains attached to the sensor tip, and the bridging chains become overstretched and eventually break free at one of their anchor groups. Because the radius of a typical coated polystyrene AFM tip is about 60−70 nm, only a few HPOE chains from the surface could participate in the measured interaction with the AFM tip. The observation that the retract curves often exhibited multiple jump-off steps indicates that binding was polyvalent. Each step of 60 ± 18 pN corresponds to the unbinding of a chain of HPOE with the deviation caused by a varying degree of mutual overlap. One feature of the scans is the quantification of the gradient of the force, k (stiffness of the chains). A histogram derived from 300 force measurements is plotted in Figure 3A. The histogram shows maxima indicated with arrows. Figure 4A presents the autocorrelation analysis of the histogram. Periodicities are clearly visible on the autocorrelation function and show that the measured slopes are composed of integer multiples ($k = \sum_i k_i = nk_0$) which are equivalent to a gradient force quantum k_0 of 540 ± 30 μN/m.[15] We attribute this measured force gradient quanta to the stiffness of individual molecular chains. Figure 3B presents the histogram derived from 250 force measurements of the polymers of mass 35 000. The histogram shows maxima indicated with arrows. Periodicities in the autocorrelation function (Figure 4B) allow us to deduce the elementary gradient of the force quantum which is equal to 350 ± 25 μN/m. Note that the stiffness of the chain decreases with increasing molecular weight. The force applied to pulling the chain is not too large, and the chains are not fully stretched. This is due to the weak adsorption energy between the parafinic chain and the surface, which is of the order of the thermal energy kT.[13] In this case, if we consider that the chains are Gaussian, its "spring constant" is equal to $3kT/Na^2$ [15,16] (where kT is thermal energy, N number of segments, and a is the monomer size of POE), which is equal to 430 and 250 μN/m for polymers of mass 20 000 and 35 000, respectively. These values are of the same order of magnitude as the value estimated from the force measurements. Our data also provide information about the behavior of molecules during the interaction between the end groups of HPOE chains and the hydrophobic surfaces. Though the rupture force of a single covalent C−C bond is between 3 and 13 nN,[17,18] the measured noncovalent parrafinic chains−hydrophobic surface binding forces are about 20 times weaker (600 pN). These findings explain that the rupture forces are due to the lifting of the HPOE chains by the end anchor groups and do not break at the level of the POE chain.

Conclusion

This experiment illustrates the function of the force microscope in performing work on the molecular scale, and as a consequence valuable observations of discrete molecules under stress may be made. This experimental technique should also be applicable to experimental investigations of bridging effects in many other systems and opens the route for mechanical studies on individual molecules.

Acknowledgment. We are indebted to J-F. Joanny and J-M. Di Meglio for critical reading of the manuscript and helpful comments about the elasticity of single polymer chains.

References and Notes

(1) Kishino, A.; Yanagida, T. *Nature* **1988**, *334*, 74.
(2) Smith, S. B.; Finzi, L.; Bustamente, C. *Science* **1992**, *258*, 1122.
(3) Bustamante, C.; Marko, J.; Siggia, E. D.; Smith, S. *Science* **1994**, *265*, 1599.
(4) Bensimon, D.; Simon, A.; Croquette, V.; Bensimon, A. *Phys. Rev. Lett.* **1995**, *74*, 4754.
(5) Florin, E.-L.; Moy, V. T.; Gaub, H. E. *Science* **1994**, *264*, 415.
(6) Florin, E.-L.; Radmacher, M.; Fleck, B.; Gaub, H. E. *Rev. Sci. Instrum.* **1994**, *65*, 639.
(7) Ludwig, M.; Moy, V. T.; Rief, M.; Florin, E.-L.; Gaub, H. E. *Mycroscopy, microanalysis, microstructures: scanning probe methods and near field microscopy;* 1994; Vol. 5, p 321.
(8) Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E. *Science* **1997**, *275*, 1295.
(9) Ducker, W. A.; Senden, T. J. *Langmuir* **1992**, *8*, 1831.
(10) Alami, E.; Rawiso, M.; François, J. *Macromolecules* **1996**, *29*, 2229.
(11) François, J.; Maitre, S.; Rawiso, M.; Sarazin, D.; Beinert, G.; Isel, F. *Colloids Surf.* **1996**, *112*, 253.
(12) Dai, L.; Toprakcioglu, C. *Macromolecules* **1992**, *25*, 6000.
(13) Courvoisier, A.; Isel, F.; François, J.; Maaloum, M. *Langmuir* **1998**, *14*, 3727.
(14) The histograms were repeated three times with different hydrophobic AFM tip and on different diluted adsorbed HPOE. The value of the chain stiffness did not vary within the given error range.
(15) de Gennes, P. G. *Scaling Concepts in polymer Physics*; Cornell Universiy Press: Ithaca, NY, 1979.
(16) Orr, W. T. *Trans. Faraday Soc.* **1947**, *43*, 12.
(17) Odell, J. A.; Keller, A. *J. Polym. Sci. B* **1986**, *24*, 1889.
(18) Ngyen, T. Q.; Kausch, H. H. *Colloid Polym. Sci.* **1991**, *269*, 1099.

MA981023P