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Conformation of a Single Polyacrylamide Molecule Adsorbed onto a Mica Surface Studied with Atomic Force Microscopy

Heiko Haschke*,† and Mervyn J. Miles

H. H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol BS8 1TL, UK

Vasileios Koutsos*

Institute for Materials and Processes, School of Engineering and Electronics & Centre for Materials Science and Engineering, University of Edinburgh, Edinburgh, EH9 3JL, UK

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ABSTRACT: We have studied the adsorption of a single polyacrylamide chain onto a freshly cleaved mica surface from a good solvent by force spectroscopy using a custom-built force prober. The polymer chain was covalently attached to the AFM tip at one end. The polymer loop lengths were deduced by measuring the distance between desorption events during the retraction of the tip from the surface. The loop length distribution obtained was found to obey a power law characteristic for the semidilute regime. We claim that this is due to the confinement of the polymer chain between the tip and the surface during the adsorption.

1. Introduction

The behavior of polymers at interfaces is a subject of fundamental importance for a wide range of research areas and applications. These include, for instance, adhesion, lubrication, flocculation, and colloidal stabilization. In addition, biological macromolecules play a significant role at natural and artificial interfaces, such as cell membranes and implants, respectively. Since they are water-soluble and inexpensive, polyacrylamide polymers have received the attention of many industries. In addition, polyacrylamide can be easily modified and, therefore, covers a wide range of properties. Some applications in industry can be found in oil recovery, in paper fabrics, and in water purification. In many of them, polyacrylamide adsorbs to surfaces and modifies them accordingly.

The lack of experimental techniques capable of verifying theoretical concepts on polymer conformations at interfaces has led to a limited level of understanding in that field. Neutron scattering and reflectivity, surface forces apparatus (SFA),[1,2] ellipsometry, and X-ray photoelectron spectrometry have provided some insights into the subject. However, several shortcomings, such as the need of a vacuum for some techniques or limited lateral resolution, leave many questions unanswered. With the development of scanning probe microscopy (SPM) techniques by Binnig et al., in particular, the atomic force microscope (AFM)[3] the technique used in this work, polymer molecule interactions and conformations of single polymer chains can be studied directly on the nanometer scale.

In polymer science the AFM was first used to image polymer crystals with contact mode atomic force microscopy[4] followed by the imaging of single polymer molecules with contact mode[5,6] and intermittent contact mode AFM.[7−9] One of the main advantages of using the AFM for the investigation of polymers at interfaces is that it is possible to do measurements at the solid−liquid interface by imaging in liquid. This has been of great importance especially for the investigation of biopolymers.[10]

To gain more information regarding both interactions and structure, force−distance (fd) curves were measured over one point of the sample surface, a technique called force spectroscopy.[11] Modifying the tip and the sample surface gives rise to a wide range of applications for force spectroscopy. For example, entropic elasticity of complementary strands of DNA has been studied by Lee et al.,[12] and the polysaccharide dextran has been investigated by force spectroscopy by Rief et al.[13] Protein folding and unfolding also have attracted the attention of scientists doing force spectroscopy. For instance, the unfolding of individual titin immunoglobulin domains has been investigated with AFM.[14] Because of its excellent three-dimensional capabilities, force spectroscopy based on the AFM led to the detection and localization of individual antibody−antigen recognition events.[15] In polymer science, Overney et al.[16] used a system of end-grafted polystyrene chains in toluene to study “elastohydrodynamic lubrication”. In addition, the structure of polyacrylamide monolayers has been investigated with force spectroscopy on mica by measuring the loop length distribution from molecules pulled out of a pseudobrush.[17] Single polyacrylamide molecule adsorption measurements in different liquid environments have revealed hydrogen bonding as the main mechanism of the adsorption of polyacrylamide on silicate surfaces, such as mica.[18]

In this paper, we take a novel approach to the investigation of the conformation of polyacrylamide molecules. In our experiment, a single polyacrylamide molecule was covalently attached at one end of the polymer to the AFM cantilever tip. By approaching and retracting the tip with the molecule to the surface of interest, the conformation of the individual polymer was investigated. By coating the cantilever tip with gold, the adsorption of polyacrylamide on the tip can be neglected. The covalent attachment of the chain to the tip ensures that any detachment measured in the force spectroscopy

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[†] Present address: JPK Instruments AG, Bouchéstrasse 12, 12435 Berlin, Germany.

[*] To whom correspondence should be addressed: e-mail haschke@jpk.com, vasileios.koutsos@ed.ac.uk.

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experiment is between the molecule and the sample surface. In contrast, a nonspecific adsorption between the tip and the molecule, i.e., the standard technique of performing force spectroscopy on single molecules, could always lead to a detachment on either the tip or the sample. In addition, because of the strength of the covalent bond, the experiment can be repeated thousands of times with the same molecule. The statistics of the chain conformation was, therefore, obtained by the different conformations measured in different force−distance curve cycles. It has to be pointed out that the time scale in this experiment is different from that in the paper from Senden et al.[17] Instead of letting a layer of polyacrylamide molecules adsorb over hours on mica surfaces, in our experiment the molecule was forced to adsorb only in the time scale on the order of seconds onto the mica surface.

Single molecule force spectroscopy with AFM has a few shortcomings. First, the large cantilever tip (tip radius of the order tens of nanometers) in comparison to the width of the molecule disturbs the adsorption of the single molecule. Furthermore, care has to be taken when fitting the force−distance curves with polymer unraveling models, such as the wormlike chain (WLC) or the freely jointed chain (FJC) model. These models tend to produce fitting parameters for the persistence length far smaller than the values obtained by other methods.[9,19] Thus, an assessment of solvent quality based on these models is questionable. Second, strong intramolecular interactions are not detected because the molecule desorbs from the surface first. Weak intramolecular interactions result in weak force events in the force−distance curve, which were not taken into account in our analysis.

The loop distribution appears to be an important tool for the theoretical description of the conformation of adsorbed polymer chains on surfaces.[20,21] The experimental verification of the predicted exponents is of paramount importance. Recent experiments by Senden et al.[17,22] showed that the scaling framework is a solid basis for the discussion of the results. For adsorption from the melt, they have verified the exponent predicted by the scaling theory. In contrast, for adsorption from a good solvent they have found the same exponent as for the melt, while scaling predicts a significantly different one. Their experimental procedure involved preparation of a polymer monolayer after several hours of equilibration and subsequent force−distance curve investigation. They estimated the loop size by physical adsorption and desorption events during the measurement of fd curves. The desorption events could happen at both the AFM tip and the substrate. In our system, the chain was covalently bonded to the tip. Thus, we know that all desorption events happened on the mica surface.

2. Experimental Section

Polyacrylamide, $-[CH_2CH(CONH_2)]_n-$, was synthesized by Ciba Speciality Chemicals using radical polymerization. This synthesis resulted in polyacrylamide with $-SCH_2COOH$ as one end group and $-CH_3$ on the other end. In that way, covalent immobilization on to gold surfaces via the gold−sulfur bond can be achieved. The molecular weight of this polymer given by the manufacturer is 5.71×10^5 g/mol and the polydispersity $M_w/M_n = 17.3$. (Since we are isolating individual polymer chains, the high polydispersity does not create problems in this particular study.) Park Scientific silicon nitride cantilevers have been used in these experiments after coating them with a 4 nm chromium layer and a 15 nm layer of gold. The nominal spring constant of these cantilevers is 30 pN nm^{-1}. The true spring constant was determined after each individual experiment with the reference cantilever method with an accuracy of approximately 10%. For the two data sets reported in this study the spring constants were found to be 39 and 35 pN nm^{-1}.

The chemisorption of one individual molecule to the gold-coated tips was ensured by using a low enough concentration of the polyacrylamide solution and the correct exposure time. These parameters were attained by varying systematically the incubation time and polymer solution concentration, and performing and analyzing fd curves in order to exclude the occurrence of a polymer brush or structures of multiple chains. When the concentration of the polymer solution was far too high or the incubation time was far too long, a stable force spectroscopy experiment was impossible probably because of the presence of polymer multilayers and/or mesoscopic polymer structures on the tip. In the concentration range of tens of micrograms per milliliter and incubation times of several minutes, stable force spectroscopy experiments were obtained, but usually there was a polymer brush covalently attached to the tip (grafting density was too high): The approach curve was characterized by a slope starting at approximately 5 μm, indicating a weak, long-range, repulsive interaction caused by the polymer brush coming into contact with the surface. In addition, the retraction curve showed tens and sometimes hundreds of desorption events at a range of force values. By further reducing the polymer concentration and incubation time, the brush behavior disappeared, and only a few desorption events were observed in each force curve at the mica−water interface. When the concentration and/or incubation times were reduced even further, no desorption effects were observed. This systematic study led to the optimal parameters for obtaining a single attachment of an individual polymer on the tip for our system: polymer concentration of 5 μg/mL and an incubation time of 60 s.[9] All experiments were performed on freshly cleaved mica surfaces. The mica surface is hydrophilic, and our previous studies have shown that polyacrylamide forms hydrogen bonds with the oxygen sites on the mica surface.[18]

The fd curves were acquired with a custom-built force probing machine. Antivibration construction, stainless steel components, and preloading of mechanical devices led to a stability allowing the performance of experiments on an individual polyacrylamide molecule for several hours without further manual mechanical adjustment. The *z*-movement is carried out by a capacitatively controlled piezo crystal with a *z* range of 12 μm and a servo resolution of 0.05 nm (Physik Instrumente, Waldbronn, Germany). The cantilever motion was detected with a Veeco Instruments optical lever head from the MultiMode AFM. All force measurements were performed in liquid with a liquid cell containing approximately 50 μL of liquid sealed with an O-ring. A National Instruments data acquisition system is used together with in-house developed software in LabView to give maximum control over the force−distance cycles.

Each set of data was acquired with a freshly cleaved mica surface and was taken with exactly the same molecule at precisely the same sample position. The piezo *z*-movement has been set usually to higher than 3 μm because the average molecular weight corresponds to a contour length of approximately 2.5 μm. Thus, the molecule breaks free after each force−distance cycle, and no desorption events are missed. The approach rate was set to approximately 2 μm/s, whereas the retract rate was significantly higher at approximately 10 μm/s. In that way, readsorption to the mica surface during the retraction is minimized.

Furthermore, separate control experiments showed that the number of desorption events on gold surfaces is more than an order of magnitude less than on mica. The extent of the decrease in the adsorption was investigated with a polymer brush on the gold-coated cantilever and on a template-stripped gold surface. We deduce that the polymer prefers to be in contact with the water (good solvent) than gold probably due

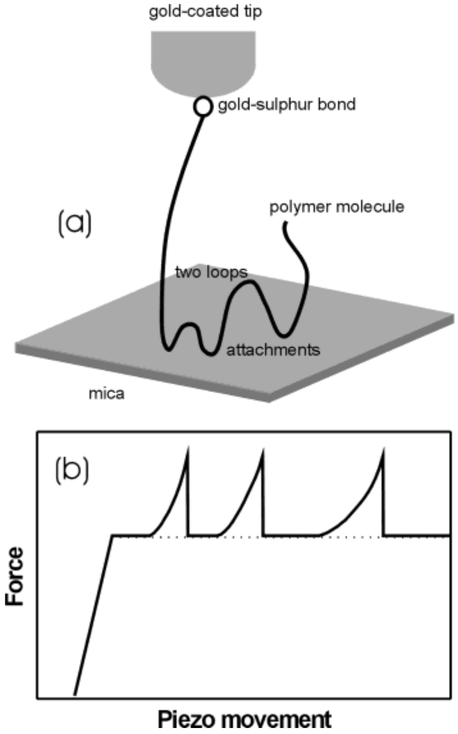

Figure 1. (a) Schematic of the force spectroscopy experiment in water with a covalently end-grafted polyacrylamide molecule on the gold-coated cantilever tip (drawing not to scale). (b) The resulting force–distance curve shows three force events for the desorption of the molecule. Three force events correspond to two loops on the surface.

to the absence of any specific interactions (e.g., hydrogen bonding is not possible) with the gold. Therefore, the adsorption of the polymer chain onto the gold tip can be neglected.

The number of data points per cycle has been set to 32 768. The quality of our force–distance curves and the number of data points allowed the measurement of the event positions with an accuracy of better than 1 nm. The quality of the events was judged by the stretching curvature and the break-off jump. Only clean stretching events with sufficiently rapid break-off jumps have been taken into account. As a result, loops of less than 25 nm cannot be identified. Desorption events resulted in similar pull-off forces between 120 and 160 pN, which corresponds to one or two individual hydrogen bonds.[23]

During the approach of the cantilever to the surface, the force exerted on the tip was limited to 200 pN to avoid damaging the molecule. When the cantilever retracted from the surface, the force exerted on the cantilever was measured. A settling time for the molecule at the surface to explore the mica of 1 s was set. In that case, desorption events can be found in approximately 95% of the force curves. If a polyacrylamide molecule adsorbs on to the mica surface, the polymer chain is stretched, and at a certain force it desorbs (due to the applied force and stretching). Only loops that can clearly be identified by two clean induced desorption events have been taken into account for the data set. Figure 1a shows a schematic of the force–distance experiment, and in Figure 1b the resulting force–distance curve can be seen. In Figure 1, three force events are depicted in the force–distance curve, which correspond to a molecule conformation of three adsorption attachments and two loops on the surface according to the situation in Figure 1a.

One experimental force curve is shown in Figure 2. Here, as in the schematic drawing in Figure 1, the force–distance curve shows three force events, which corresponds to two loops. It has to be pointed out, however, that three events were by no means the rule. The number of events varied significantly but was usually between 1 and 10. For clarity reasons, a force curve with relatively large loops has been chosen.

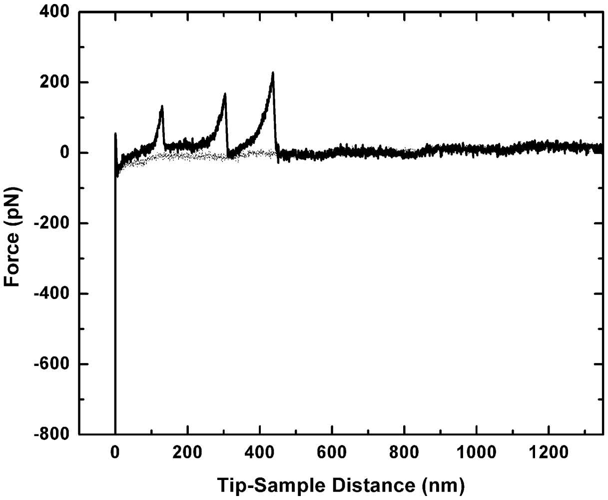

Figure 2. Force–distance curve with a polyacrylamide molecule end-grafted to a gold-coated cantilever tip obtained on mica in water. The force curve shows three desorption events which correspond to two loops adsorbed to the surface.

To cover a decade in the exponential law for the loop length distribution with an error of approximately 10% for the exponent, several hundred loop events had to be analyzed. In that way, bins for short loop lengths could be trusted, and bins for long loops could be taken into account because of respectable occupation. Each loop length distribution data set consisted of several hundred loop events in several hundred force curves.

Our experimental setup is more appropriate for long polymer molecules, such as our polyacrylamide. Therefore, the bins in the loop length distribution for larger loops are more reliable giving the opportunity to cover more than 1 decade for the exponent fit. An uncertainty in our experiments is the position of the adsorption site. If it is not directly underneath the retracting cantilever tip, an angle component will influence the distance measurements in the force curve.

3. Theoretical Background

When polymer chains are brought into contact with an attractive wall, some of their monomers adsorb, forming a continuous succession of loops and tails. The size distribution of loops was calculated first by de Gennes[24] using scaling arguments and has been revisited and extended by Aubouy et al.[20,21] and Guiselin.[25] It strongly depends on the conditions during adsorption. Here, we present a short and simplified version of the scaling theory results.

$S(n)$ is the distribution function and is defined as the number of loops per surface unit having more than n monomers. The probability density function, $f(n) = -\mathrm{d}S/\mathrm{d}n$, is defined as the number of loops per surface unit having exactly n monomers. n is a function of the height z above the surface. (There is the implicit assumption that the nth monomer of each loop is at the same height z from the surface.) It follows that at height z the polymer conformation is identical to a polymer brush with a surface grafting density $S(n(z))$.

For chains adsorbed from a good solvent in the dilute regime, the number of loops per surface unit having more than n monomers is $S(n) \cong 1/\alpha^2 n^{6/5}$, where α is the size of the monomer unit. It follows that the number of loops per surface unit having exactly n monomers is

$$f(n) = -\frac{\mathrm{d}S}{\mathrm{d}n} \cong \frac{1}{\alpha^2 n^{11/5}} \qquad (1)$$

For adsorption from a melt, we have $S(n) \cong 1/\alpha^2 n^{1/2}$ and subsequently

$$f(n) \cong \frac{1}{\alpha^2 n^{3/2}} \qquad (2)$$

Furthermore, for adsorption from good solvent conditions in the semidilute regime, a more refined treatment by Guiselin[25] has shown that small and large loops follow a different distribution

$$f(n) \cong \frac{1}{\alpha^2 n^{11/5}} \quad \text{for } 1 < n < \phi_0^{-5/4} \qquad (3)$$

$$f(n) \cong \frac{\phi_0^{7/8}}{\alpha^2 n^{3/2}} \quad \text{for } \phi_0^{-5/4} < n < N \qquad (4)$$

where N is the polymerization index and ϕ_0 is the monomer volume fraction during adsorption. If ϕ_0 is close to its minimum value $\phi^* \cong N^{-4/5}$ (overlap volume fraction or volume fraction within a polymer chain in good solvent conditions), we recover eq 1 of the dilute regime, whereas for high values of ϕ_0 (close to 1), we recover eq 2 of the melt regime.

An adsorbed single chain resembles *locally* a polydisperse brush with a grafting density of $S(n)$ (at height $z(n)$). In the dilute regime, we expect the single-chain loop statistics to be identical to the statistics of a collection of chains. If the chain is made to change its unperturbed dimensions by other means (e.g., confinement) locally (i.e., for lengths smaller than the chain dimensions), the chain segments experience higher volume fractions, and they could effectively be in the semidilute regime. In the limiting case of complete exclusion of the solvent molecules, the chain parts are in the melt regime taking Gaussian conformations within the volume of the single chain.[26]

4. Results and Discussion

The loop length was measured in the fd curves as the distance between the desorption events. The loop length measured in that way gives the length of the part of the polymer chain between two adsorption sites. No conclusions can be drawn on the actual location of the sites and the distance on the surface between them.

Two typical sets of loop length probability densities obtained with that procedure are shown in Figure 3. Data set (a) possesses an ensemble size of 463 loop events, and in data set (b) the ensemble size is 219. In Figure 3a, the bin size of the loop length histogram has been set to 100 nm in order to avoid the effects of the uncertainty of small loop lengths and to have reasonably occupied bins for larger loops. In Figure 3b, a smaller bin size of 50 nm has been chosen. However, the first bin and bins for larger loop lengths were ignored to reduce the effect of small loop length uncertainty and unsatisfactory occupation of bins, respectively. Generally, bins of less than two counts for the corresponding loop length have been ignored. The data set depicted in Figure 3a results in an exponent for the loop length probability density of −1.49 ± 0.18, and the data set in Figure 3b gives an exponent of −1.48 ± 0.16. The values differ by only 0.01, which is well within the given error range. The two sets of data were acquired in different experiments using different cantilevers and, thus, ultimately different individual molecules at different positions on a freshly cleaved mica sheet. Therefore, our experiment with a single polyacrylamide molecule in water on a mica surface agrees with the exponent of −3/2 given in the theory for loop formation from adsorption in melt (eq 2) or for long loop formation from adsorption in the semidilute regime (eq 4).

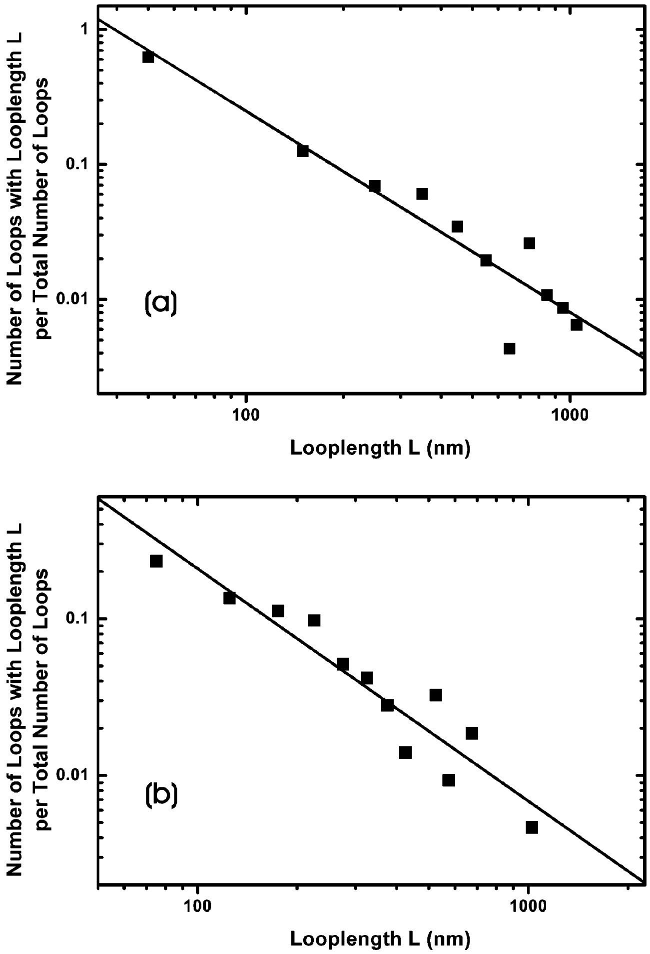

Figure 3. Two sets of loop length distributions for the adsorption of a single polyacrylamide molecule onto a mica surface in water. The loop length is measured from AFM force−distance curves. In (a) the bin size is 100 nm with a number of loop events of 463, and in (b) 219 loop events have been put in bins with a width of 50 nm. The data sets are obtained with different cantilevers and with different individual molecules. The data set (a) gives a slope of −1.49 ± 0.18, and the data set (b) gives a slope of −1.48 ± 0.16.

Despite the significant differences in the experimental procedures, our experiment also agrees with the exponent obtained in the experiment by Senden et al.[17] In their experimental procedure, it is not certain that (a) all desorption effects are happening from the tip side and (b) they have access to all loop sizes. (It could be that the large ones screen the small ones.) In our system, the chain is covalently bonded to the tip; thus, we know that all desorption effects are happening on the mica surface, and accessibility to all loop sizes is enhanced.

Our experimental procedure is very different from that in ref 17 but also includes some subtle points and uncertainties. It is still difficult to account for very short loops since the relevant events could be also attributed to weak intramolecular interactions. An important question is the following: has the adsorption process of the polymer chain reached an equilibrium? The relaxation time of the chain is expected to be much shorter than the typical experimental time (ca. 1.5 s). However, the kinetics of the adsorption of monomers (hydrogen bonding) is largely unknown. *Nevertheless, it is quite remarkable that two very different experimental protocols for adsorption from a good solvent (Senden et al.'s and our experiments) result in the same exponent which is actually characteristic for a different regime (!).* It seems reasonable to assume that there is an underlying common reason for the observed behavior. This reason could be the confinement effect between tip and the surface at the end of the approach path: a percentage of the solvent is taken out of the gap, and the polymer volume fraction increases locally, $\phi^* \ll \phi < 1$. If the adsorption happens under this condition, eqs 3 and 4 predict two effects: (a) the short loops are composed of very few monomers, and (b) the long loops dominate the loop distribution. Thus, the correct exponent for the accessible loop probability density function is $-3/2$.

Our experiments demonstrate the dramatic influence of confinement on the polymer conformation during a fd curve. This subtle but strong effect, which is usually ignored, should be taken into account for the correct interpretation of AFM images and fd curves of single biomolecules and polymer monolayers.

5. Conclusions

We have measured for the first time the loop length distribution of a single polymer chain adsorbed to a flat surface in good solvent conditions using force spectroscopy. The exponent obtained for the loop-length probability density function is close to $-3/2$. This exponent can be explained if the polymer adsorption happens during the induced confinement of the polymer between the tip and the surface.

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