Intermolecular Interactions of Polymer Molecules Determined by Single-Molecule Force Spectroscopy

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ABSTRACT: Force spectroscopy on the single-molecule level allows the investigation of intramolecular as well as interdomain and intermolecular interactions of both synthetic and natural polymers. In these experiments, a single molecule is usually stretched between two strong attachment points. The force then increases with increasing extension. Under certain conditions, however, force−extension curves result in force plateaus; an increase in extension is observed under constant force. These plateaus do not depend on pulling speed and therefore indicate transitions at equilibrium. Here we present single-molecule data where carboxymethylcellulose (CMC) molecules are pulled out of a polymer film into a poor solvent. The resulting force−extension curves show not only one but up to seven force plateaus. The step height from one force plateau to the next is nonuniform but follows a characteristic spacing. This is the first time that nonuniform force plateaus are reported for polymer molecules. A simple model for the polymer−polymer and polymer−solvent interactions explains the measured data. In this model the new solvent−polymer interface gain during the pull is minimized by the formation of a tightly packed polymer bundle. By introducing a parameter for the product of effective radius of the polymer multiplied by surface tension, as well as a second parameter describing the interaction between polymer strands in the bundle, one finds excellent agreement between predicted and measured plateau heights. This model, therefore, provides a basis to investigate the interactions of polymer chains and the influence of solvent in well-defined geometries using single-molecule force spectroscopy.

Introduction

Single-molecule force spectroscopy has proven to be an important method for studying the flexibility of single polymer molecules[1] and their adhesion to surfaces[2] as well as their intramolecular[3] and intermolecular[4] interactions. Commonly, a polymer is stretched between a substrate surface and a cantilever. The measured force then increases with polymer extension. Force−extension curves of this type reveal the entropic and the enthalpic response of the polymer to the applied force. Their analysis yields microscopic parameters for the polymer elasticity such as the Kuhn length[5] or persistence length.[6] The experimental findings have been summarized in several reviews.[7−10]

Recently, plateaus in the force−extension curves of many different polymers have been observed, which is a new phenomenon.[11−15] In contrast to the force−extension curves described above, the force remains constant upon retraction of the cantilever. To understand the appearance of force plateaus, it is important to note that the formation and breakage of individual bonds happens on time scales much faster than those accessible in the experiment. Thus, one can consider the experiments to be performed under quasi-equilibrium conditions. Many different physical reasons can lead to the appearance of force plateaus, namely conformational changes of the molecule,[11] the desorption of a polymer from a surface,[15] the Rayleigh instability of extending a collapsed molecule,[16] and the bending of tubular molecules.[17] Various theoretical studies exist which explain the nature of force plateaus. These can arise

due to adhesion[18,19] or, under poor solvent conditions, due to the phase transition of a collapsed layer (or globule) to a stretched chain.[20−22] Celestini et al. describe the transition between force plateaus due to solvation and due to adsorption, by varying the substrate interaction energy.[23]

In this work, we demonstrate that multiple force plateaus arise when polymer molecules are pulled out of a thick polymer film into a poor solvent. As an example, we chose carboxymethylcellulose (CMC) which we pulled from a film into water. In contrast to cellulose, which is not water-soluble, carboxylation of the polymer weakens the intermolecular hydrogen bond formation and makes CMC water-soluble. However, water is still a rather poor solvent for CMC, and high concentrations of CMC molecules lead to the formation of a gellike structure, where water molecules are embedded in a tightly packed CMC network.[24] Under our experimental conditions, a ladder of force plateaus with a distinct spacing is observed. This presents the first time that a nonuniform spacing pattern is reported for polymer molecules. We present a simple geometric model that describes the spacing of the plateaus. The model gives detailed information about the interaction of single polymer strands and allows us to derive the solvation energy for a single CMC molecule. Further applications of this approach to probe intermolecular interactions in polymer films at the molecular level are discussed.

Experimental Section

All experiments were performed on a commercial AFM microscope (Asylum research, MFP). Cantilevers were purchased from Veeco (Microlevers) and used as delivered. Glass coverslips were cleaned with a 2% Hellmanex II (Hellma) solution and thoroughly rinsed with HPLC-pure water. Two

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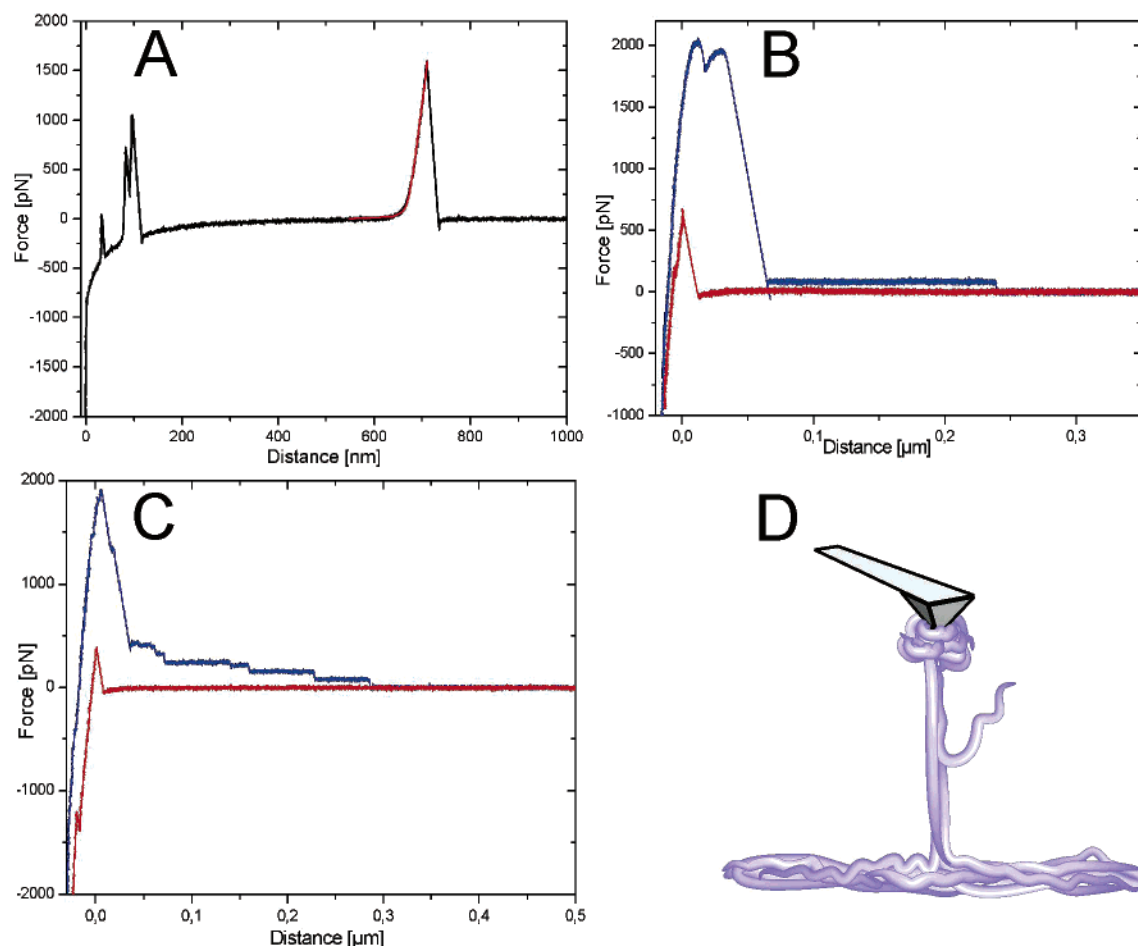
Figure 1. Comparison of characteristic single-molecule force−extension curves. (A) Force−extension curve of a single CMC molecule attached nonspecifically to a glass surface and cantilever (black). At short extensions (up to about 100 nm) a few rupture events are observed. Very short rupture events are hard to distinguish from nonspecific attachments and are therefore discarded from the analysis. At longer extensions, the force rises continuously up to ∼1500 pN where we observe a rapid drop in the force at ∼700 nm extension. The curve is fitted with an extended freely jointed chain model (red), yielding a Kuhn length of 2.2 nm and a segment elasticity of 12 000 pN/nm. (B) Force plateau of single CMC molecule. Both approach (red) and retraction (blue) data are shown. The retraction curve shows a large force peak at short distances due to the interaction of many CMC molecules with the cantilever. Such large nonspecific adhesion peaks are only observed in measurements on thick polymer films. At extensions larger than ∼70 nm, a constant nonzero force is observed. The force drops to zero when the molecule is pulled out of the polymer film, in this case at an extension of ∼250 nm. The reason for the formation of the force plateau is the solvation of a single CMC molecule. (C) Often, multiple force plateaus are observed when pulling CMC molecules out of polymer film. (D) Schematic drawing illustrating the experiment. A bundle of polymer molecules is pulled out of a film of CMC. The molecules are nonspecifically attached to the cantilever. Shorter molecules detach from the film earlier than longer molecules, leading to a distinct decrease in measured force.

different sample preparations were used to prepare samples containing either isolated adsorbed molecules of CMC (CMC sodium salt, C-5013, Sigma-Aldrich, degree of carboxylation 0.75) or a thick film. Isolated, adsorbed molecules were prepared by incubating a 0.1−0.5% (w/v) CMC solution in water for 1 h, thus avoiding desiccation of the sample. For the preparation of a thick CMC film, CMC was dissolved in HPLC water to 0.01% (w/v). A 20 μL droplet was applied to the clean coverslip and dried by overnight incubation. Before usage the sample was repeatedly washed with water and inserted into the microscope. This procedure resulted in a homogeneous polymer film with a thickness on the order of 100 nm and a surface topography of about 20 nm roughness as revealed by AFM measurements (data not shown). The sensitivity of the inverse optical lever system of the AFM was calibrated for each cantilever prior to the experiment by indentation on the sample. Force−extension curves were then obtained by re-peated indentation of the cantilever into the polymer film and retraction with a velocity varying from 0.1 to 16 μm/s. The resulting single-molecule force curves were analyzed with custom-programmed analysis software (Igor Pro, Wavemetrics) to find force plateaus. All experiments were performed at temperatures of 23 ± 3 °C.

Results and Discussion

Force−extension curves of CMC were taken on the two different sets of samples, with the glass covered by isolated molecules or a by homogeneous polymer film. The profiles of the resulting force−extension curves on the two sample types exhibited very different, but characteristic, shapes. In the case of isolated molecules, the majority of tethered events showed force curves where the force increased with extension until a char-acteristic peak rupture event. The data can be fitted with an extended freely jointed chain model (see Figure 1A).[11,25,26]

If the polymer molecule is immersed in a good solvent, it is simply stretched against entropic and enthalpic forces between two rigid attachment points. In contrast, immersion in a poor solvent, results in a collapse of the molecule. By collapsing, the polymer reduces the size of its interface with the solvent, which minimizes the overall energy through the formation of intermolecular bonds. Water is known to be a poor solvent for CMC,
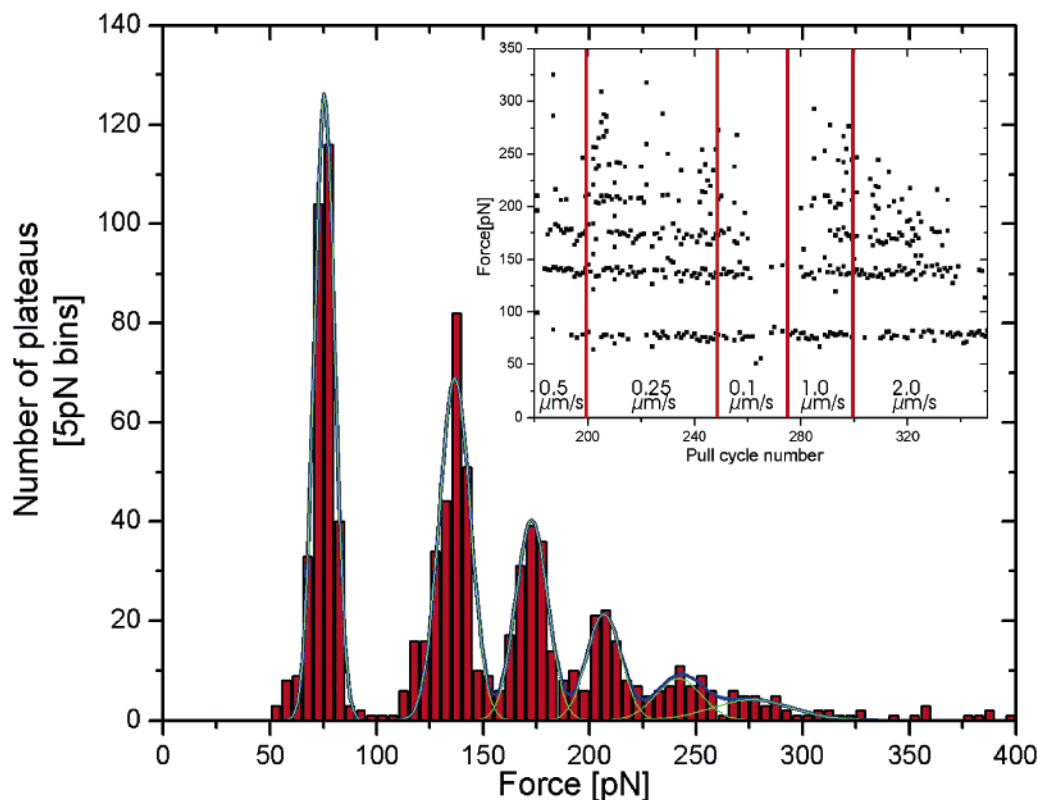
Figure 2. Histogram of plateau heights. The histogram shows the measured plateau heights from 464 successive pull events, resulting in a staircase of plateaus. The combined histogram was fitted with a function containing six Gaussians; individual peaks are shown in green, and the combined function is shown in blue. The inset shows the plateau heights of successive curves. The data spans pulls with velocities between 0.1 and 2 μm/s, as indicated. A dependence of plateau height on pulling speed was not observed.

and high concentrations of CMC in water lead to the formation of a gel.[27] We therefore increased the local concentration of the CMC molecules and pulled molecules out of a film of CMC absorbed on glass to study the solvation of single molecules.

Pulling CMC molecules out of a homogeneous film into the poor solvent (water) leads to very different force−extension curves (see Figure 1B). Now, the curves show two characteristic features: first, a very strong (on the order of 10 nN) adhesion peak at low extensions, and second, a distinct force plateau at larger extensions. Furthermore, under these conditions we oftentimes observed not only a single force plateau but also a whole ladder of up to seven plateaus. A typical example is shown in Figure 1C. The frequent occurrence of multiple plateaus allows us to analyze the spacing between plateaus in great detail. Interestingly, the spacing between the plateaus is not uniform as reported previously for poly(N-isopropylacrylamide).[16] Instead, the step height between plateaus decreases for higher plateaus. A careful analysis of several hundred of such force events yields histograms which correspond to well-defined Gaussian distributions, as shown in Figure 2. While some molecules could be identified through many successive pulls, the complete histogram is built up of several experiments on different sets of molecules. In addition, the experiments were repeated many times using different cantilevers and different samples, yielding reproducible results within our experimental errors.

To prove that the plateau heights are indeed determined by the sample−solvent interaction and not by the pulling speed, we checked for variations in the plateau height with varying pulling speeds. The inset in Figure 2 shows the data from a few hundred successive pulls with pulling speeds ranging from 0.1 to 2 μm/s as indicated. There is no visible plateau height difference for different pulling speeds. In fact, the displayed data are incorporated in the histogram of Figure 2. Since the Gaussian peaks in these histograms are very sharp, we conclude that a velocity dependence of the forces, which would lead to a broadening of the peaks, was not observed in our experiments.

To explain this unusual plateau spacing, one has to consider the precise conditions under which the experiments were performed (cf. illustration in Figure 1D). The thick film of CMC has been prepared by desiccating the polymer solution on a glass substrate. Before the measurement, the film was soaked with water, which results in a swelling of the film and gel formation at the surface.[27] When the cantilever approaches this gel matrix, a bundle of polymer molecules attaches to the cantilever. The huge number of tethers leads to a large nonspecific adsorption peak at low extensions in the force−extension curves (Figure 1C). After moving the cantilever about 50 nm away from the surface, most of the molecules snap back and the force drops to a constant nonzero level. One is left with only a handful of molecules still attached to the cantilever. These now span a bridge between the cantilever and the polymer film. Upon further retraction of the tip, shorter molecules lose contact to either the cantilever or the polymer film. This results in an apparent drop in the measured force. Interestingly, there is no increase in force before the molecules are pulled out. This is in sharp contrast to experiments in good solvent conditions, where tethers are formed through strong, non-

Table 1. Comparison of Measured and Predicted Plateau Heights[a]

	plateau 1	plateau 2	plateau 3	plateau 4	plateau 5	plateau 6
plateau height [pN]	75	137	173	207	242	278
step height [pN]		62	36	34	35	36
relative height [%]		83	48	45	47	48
calcd relative height [%]			41	41	41	41

[a] The table shows a comparison of the measured plateau heights, as determined from the center positions of the Gaussians from Figure 2 to the values expected from the interaction model. Note that the height of the first two plateaus is used to determine the two parameters, $r\sigma$ and α, of the model. With these two parameters, one can calculate the relative step height of the successive plateaus. The last lines show the measured as well as compute relative step height, which is defined as the force difference between a plateau and the next lower plateau relative to the height of the first plateau.

specific attachment at both ends of the molecules. In our experiments, however, the molecule is only firmly attached at one end, while the other end is free to slide. In repeated measurements, a well-defined plateau pattern, both in number of plateaus (see insert in Figure 2) and in plateau length (data not shown), often emerges, over tens, sometimes hundreds, of pulls. This is a strong indication that we repeatedly pull the same molecules that are firmly attached to the cantilever.

On the basis of the picture that molecules attached to the cantilever are pulled away from the surface, we propose a model that explains the characteristic spacing of the force plateaus. The existence of a single force plateau for experiments under poor solvent conditions has already been explained by Haupt et al.[16] In brief, these authors explain that the polymer–water interface is increased as the chain is pulled monomer by monomer out of the polymer film. The polymer can be thought of as a cylinder of radius r; thus, the increase in interface will be $2\pi r$, and the expected force will be

$$F = 2\pi r\sigma \qquad (1)$$

where σ is the surface tension of the polymer.

When multiple molecules are pulled at the same time, two possibilities for the arrangement exist: either the molecules are separated from each other, or they form a bundle, thus minimizing the new polymer–solvent interface. For the case of separated molecules, the plateau height would simply be the plateau height for a single molecule times the number of molecules that form the bridge between cantilever and surface. This would result in a uniform distribution of step height, which is in strong contrast to our experimental data. For the case of bundle formation, a simple geometric model can be used to describe the interface growth (Figure 3). In this model, we introduce an angle α to describe the nearest-neighbor interactions between individual polymer molecules that form the bundle. When pulling a bundle of 2, 3, ..., n molecules from the film into the solvent, the solvent–polymer interface increase results in a force plateau given by

$$F(n\text{th-plateau}) = ((n+2)\pi - n\alpha)r\sigma, \quad \text{for } n = 2, 3, ...$$
$$(2)$$

The experimentally determined histogram of plateau heights (Figure 2) shows a nonuniform plateau spacing. The step height decreases with increasing plateau number, a clear indication of bundle formation. We use the plateau height of the first two plateaus to determine the two parameters of the model, the product of surface tension times the radius (σr), and the angle α, describing the nearest-neighbor interactions between the polymers of the bundle. With this we can compare to the heights of force plateaus $n = 3, 4, 5,$ and 6 without any
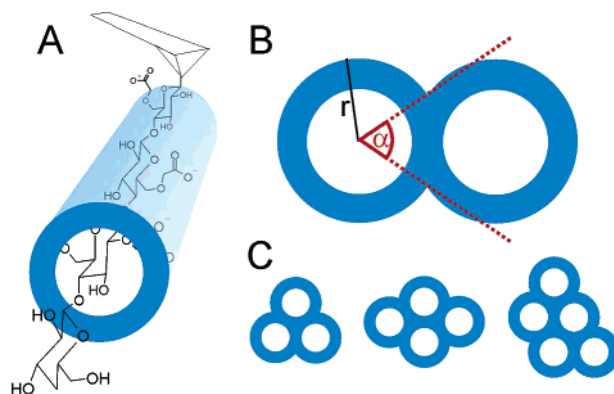
Figure 3. Schematic for interaction model. (A) A polymer molecule inside a solvent is treated as a cylinder with radius r. This radius includes both the actual size of the molecule including side groups and a nearest-neighbor interaction region (marked in blue). (B) Under poor solvent conditions, two (or more) molecules will tend to aggregate in order to minimize the surface area exposed to the solvent and form a bundle. The resulting polymer–solvent interface can be described by the number of molecules n, the radius of the individual molecule r, and an angle α, which describes the interaction of the molecules as indicated. (C) Schematic for bundle arrangement for $n = 3, 4,$ and 5.

additional free parameters. Table 1 shows a comparison of our experimental results, taken from the Gaussian fits to histogram peaks in Figure 2, to values calculated from the geometric model. The simple model provides plateau heights that are in good agreement with our experimental data. After the step height drops dramatically from the first to the third plateau, it reaches a constant level for higher plateaus. This effect is both predicted by the model and observed experimentally.

From the Gaussian fits to the histogram in Figure 2, consisting of 464 pulls and 927 analyzed plateaus, we determine $\alpha = 31^\circ$ and $r\sigma = 12$ pN. Molecular modeling[28,29] yields a radius of the CMC molecule of about 4 Å. Therefore, the surface tension for the polymer–water interface can be estimated to be ~30 mN/m, which is comparable to the surface tension of other cellulose derivatives.[30]

While water is a rather poor solvent for CMC, the question arises why a bundle that is pulled out of the polymer film does not dissolve immediately into water. Apparently, the rigid attachment of the CMC molecule to the cantilever on one end and the strong interactions with the polymer film on the other end change the quality of the solvent and make the molecules insoluble due to entropic penalties. Thus, the bundle remains tightly packed even far away from the polymer–water interface, as illustrated by the fact that the plateau height does not depend on the pulling speed. The bundle is highly stabilized by hydrogen bonds that are formed between CMC molecules and does not dissolve.

In conclusion, when performing single-molecule force spectroscopy experiments under poor solvent conditions, where polymers tend to aggregate, it is possible to study the solvation of a single molecule. Most often, one then pulls not a single molecule, but a tightly packed bundle of strongly interacting molecules. This packaging minimizes the surface of the molecules which is exposed to the solvent. The intermolecular interaction can be described by a simple geometric model introducing an angle α designating the interaction of the polymers. This parameter α depends on the nature of the solvent, as well as on the nature of the polymer, since molecules that have a high tendency to form hydrogen bonds will tend to from tight aggregates. In the limiting case of a good solvent, one would observe isolated molecules or interaction angles α ∼ 0. We therefore propose the analysis of plateau heights as a new tool for studying the quality of the solvent as well as the intermolecular interactions of polymer chains. Furthermore, for a given solvent, changes of intermolecular interactions can be probed by using modified polymer side groups or cross-linking reagents, since these will result in a closer packaging of the molecules and therefore larger angles α.

In this paper we have presented data from CMC, which is used as model system for a self-interacting polymer. Similar results should occur for different polymers under poor solvent condition. However, to observe and evaluate the nonuniform force spacing, experimental conditions have to be found, where many tethers between cantilever and surface are formed. This situation is more likely to be found for measurements on polymer films rather than for isolated adsorbed molecules.

This technique could provide a better understanding of intermolecular interactions caused by a particular molecular architecture of polymer networks and has a high potential for the optimization of various applications.

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