Experimental Studies of the Dynamic Mechanical Response of a Single Polymer Chain

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ABSTRACT: The high-frequency and low-amplitude dynamic mechanical response from a single poly(vinyl alcohol) chain was investigated. Modification of a commercial atomic force microscope enabled high-frequency and low-amplitude periodic deformations of polymer chains during extension to be performed. Such an experiment has previously been considered untenable due to hydrodynamic and viscous effects, but we introduce here a method to isolate the response of the polymer from the hydrodynamic response. For periodic deformations with frequencies of 400−2000 Hz and amplitudes of 0.55−16.9 nm, we find that the dynamic mechanical response from poly(vinyl alcohol) does not differ from its static response. This result is not unexpected as poly(vinyl alcohol) is a highly flexible polymer with intramolecular relaxation processes taking place on a short time scale. The choice of a polymer with a fast relaxation allows its static properties to be recovered from the dynamic measurements and enables the method suggested in this paper for decoupling the polymer response from the hydrodynamic response to be validated.

Introduction

During the past decade, the atomic force microscope (AFM) has been used in a number of studies of single-molecule interactions and single-molecule mechanics. Among these have been investigations of the strength of molecular bonds,[1,2] the elasticity of synthetic polymers,[3−6] the force-induced unfolding of proteins,[7,8] and force-induced structural transitions in DNA.[9,10] Most of these studies have been performed by attaching one part of the molecule to a substrate and another part of the molecule to the tip of an AFM cantilever and then separating the two parts at a constant velocity. In this way, a so-called *force-extension* curve can be obtained for the system in question. The force needed to break a molecular bond or to unfold a domain in a protein strongly depends on this pulling velocity, and therefore, this method of studying these events has been termed *dynamic force spectroscopy*. In other cases, such as investigations into the elasticity of synthetic polymers, the backbone of proteins, and of single- and double-stranded DNA, the molecular relaxation processes are much faster than the experimental time scale and the mechanical response is therefore independent of the pulling velocity. These experiments are termed *quasistatic experiments*.

Studies of synthetic polymers by single-molecule techniques are important both to address fundamental questions of polymer behavior but also because these polymers function as model systems for more complicated biological systems. On the basis of the above-mentioned experiments, it has so far been possible to test and extend the theory of polymer elasticity for both very flexible polymers and for polymers with a bending rigidity to study the effect of salt on polyelectrolytes and to investigate the effect of solvent on polymer elasticity. Addressing these issues is important, but if we want to extend our knowledge of single-polymer properties beyond quasistatic elastic measurements, we need a new approach. An alternative to the quasistatic measurements is true dynamic or so-called nanorheological measurements. In such experiments, the polymer is exposed to a periodic deformation while the response of the cantilever is recorded. There have been some preliminary attempts to perform such an experiment both on a synthetic polymer[11] and on proteins.[12−14] All of these experiments have been carried out by oscillating either the sample stage or cantilever at low frequency (0.1−60 Hz) for different degrees of polymer or protein extension. Low frequencies were used to avoid introducing hydrodynamic or other viscous contributions to the measurement. On the other hand, the disadvantage of a low frequency is that the deformation velocity will not be higher than the pulling velocity in the quasistatic experiment, and therefore, little new knowledge is obtained. In this paper, we present high-frequency dynamic measurements on a single poly(vinyl alcohol) chain and introduce a method to deal with hydrodynamic and viscous contributions to the response of the cantilever. In the above-mentioned low-frequency experiments, rather large deformation amplitudes were used (>20 nm, except in ref 13). The use of large deformation amplitudes makes it difficult to address the measured response to a clearly defined extension of the polymer, as a large range of different extensions will be probed at each period of deformation. In the present study, we have been able to use amplitudes as low as 0.55 nm. Further, we have managed to perform the dynamic measurement continuously during the extension of the polymer, allowing the dynamic response vs extension to be directly compared to the quasistatic force-extension curve. This new method enables the viscoelasticity of single polymer chains to be studied by nanorheology. The choice of poly(vinyl alcohol) as the polymer under investigation is deliberate. Poly(vinyl alcohol) is a highly flexible polymer with a fast relaxation time. In the range of frequencies and amplitudes used here, its dynamic response is not expected to differ significantly from its static response. This makes poly(vinyl alcohol) suitable for testing this new method,

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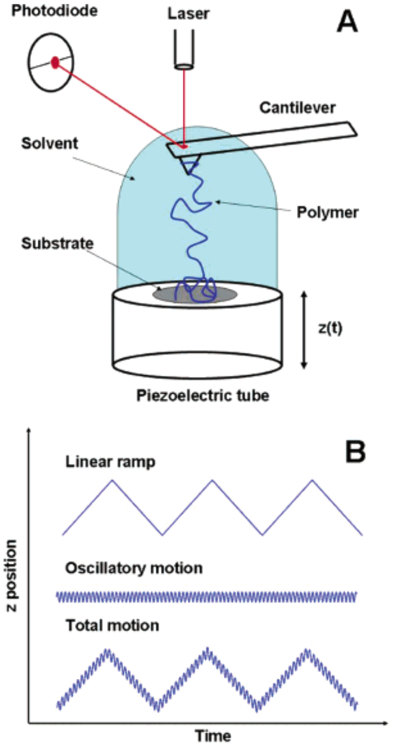

Figure 1. (A) Schematic illustration of the experimental arrangement with a polymer bridging the substrate and the tip of a cantilever. (B) A depiction of the change in z position of the substrate as a function of time when a linear ramp is coupled to an oscillatory motion.

since the hydrodynamic response changes with amplitude and frequency, while the polymer response does not.

Method

The experiments were performed on a modified Nanoscope III multimode AFM (Digital Instruments). The high-voltage z ramp from the Nanoscope controller was coupled to a high-frequency, small-amplitude oscillatory signal, which was provided by a dual-phase lock-in amplifier (SR830, Stanford Instruments). In this arrangement, a flat substrate was oscillated relative to a stationary cantilever (see Figure 1). The response of the cantilever due to both static forces and the oscillatory motion of the substrate was measured by the split photodiode of the AFM. The component of the signal with the same oscillating frequency as the drive was isolated by feeding the signal back to the lock-in amplifier. Both the in-phase and out-of-phase components of the signal were detected by the lock-in amplifier and stored on a computer for further data analysis. The two outputs from the lock-in amplifier, X and Y, are given as:

$$X = A \cos \theta \tag{1}$$

and

$$Y = A \sin \theta \tag{2}$$

where A is the amplitude of the signal, θ is the phase of the signal relative to the driven oscillatory motion of the substrate, X is the in-phase or elastic response and Y is the out-of-phase or viscous response. From eqs 1 and 2, it is seen that A and θ can be expressed as:

$$A = (X^2 + Y^2)^{1/2} \tag{3}$$

and

$$\theta = \arctan\left(\frac{Y}{X}\right) \tag{4}$$

The amplitude was measured as a voltage but can be translated into an amplitude of the cantilever motion, A_c, measured in meters by dividing A by the sensitivity of the optical lever system (measured in V/m). A more detailed description of the instrumental arrangement has been published previously.[15,16]

Sample Preparation and Force Measurements. A silicon wafer was cleaned using water and ethanol followed by treatment with radio-frequency-generated low-temperature water plasma. The silicon wafer was subsequently incubated in a 1 g/mL solution of poly(vinyl alcohol) (Sigma-Aldrich, $M_w \approx 125\,000$ g/mol) in 0.1 M NaCl. Before use, the wafer was gently rinsed in 0.1 M NaCl solution, which was also used as a solvent during measurements. This procedure for sample preparation and the subsequent force measurements with a polymer bridging the substrate and the tip on the cantilever relies on strong adsorption of the polymer to both the substrate and the tip. Hence the range of the force-extension region investigated is dependent on the magnitude of this adhesion force. By use of the water plasma treatment, the possibility for the polymer to form hydrogen bonds with the wafer and the tip is enhanced, and therefore, the adhesion is increased.[17]

Several different, both silicon and silicon nitride, cantilevers were used in the experiments. Before use, all cantilevers were water plasma treated and the spring constants were determined by both the Sader and the Hutter & Bechhoefer methods.[18,19]

Dynamic measurements were performed using a constant piezovelocity of 60 nm/s coupled to an oscillatory motion with frequencies of 400−2000 Hz and amplitudes of 0.55−16.9 nm (peak to peak). The slow linear component of the velocity was important to obtain the best resolution in the dynamic parameters as a function of polymer extension. To ensure the reliability of the data, a minimum of five successful single-polymer stretching events were recorded for each set of amplitudes and frequencies. The measured phase response was corrected for phase lags in the detection electronics of the AFM and in the piezoresponse.

Results and Discussion

Before presenting experimental data, it is useful to describe the form of the data that is expected from a nanorheological examination of a polymer in a solvent. Upon first approach, the polymer is assumed to be adsorbed to the substrate, with no polymer bridging the substrate and the tip. Therefore, any dynamic interaction is due to the solvent. In the absence of any bridging polymer, the phase and amplitude on retraction will be equivalent to that on approach once the surfaces have separated from hard contact. If, during contact, a single-polymer chain adheres to the tip of the cantilever, the polymer will be extended during retraction. This will contribute an elastic and a viscous component to the dynamic interaction that is superimposed on the hydrodynamics. The aims of our experiment are therefore to observe how the polymer alters the amplitude and phase and from this extract information on the polymer's dynamic mechanical response.

An example of the outcome of a cycle where the cantilever and the substrate first approach each other and then are separated is shown in Figure 2. A polymer chain bridges the surfaces during contact and is extended when the surfaces are separated. The substrate was oscillating with a frequency of 800 Hz and an amplitude of 16.9 nm. The direct measured quantities were the force (or the cantilever deflection) and the intensity of the signal in the X and Y channel as a function of separation (Figure 2A, B, and C); the cantilever amplitude, A_c, and phase, θ, were determined by use of eqs 3 and 4 (Figure 2D and E).

The force-extension curve in Figure 2A looks qualitatively similar to previously presented force-extension curves for poly(vinyl alcohol) and other flexible polymers in a good solvent.[4,6,20,21] Force curves for different stretching events nicely superimpose if normalized to the same contour length, which

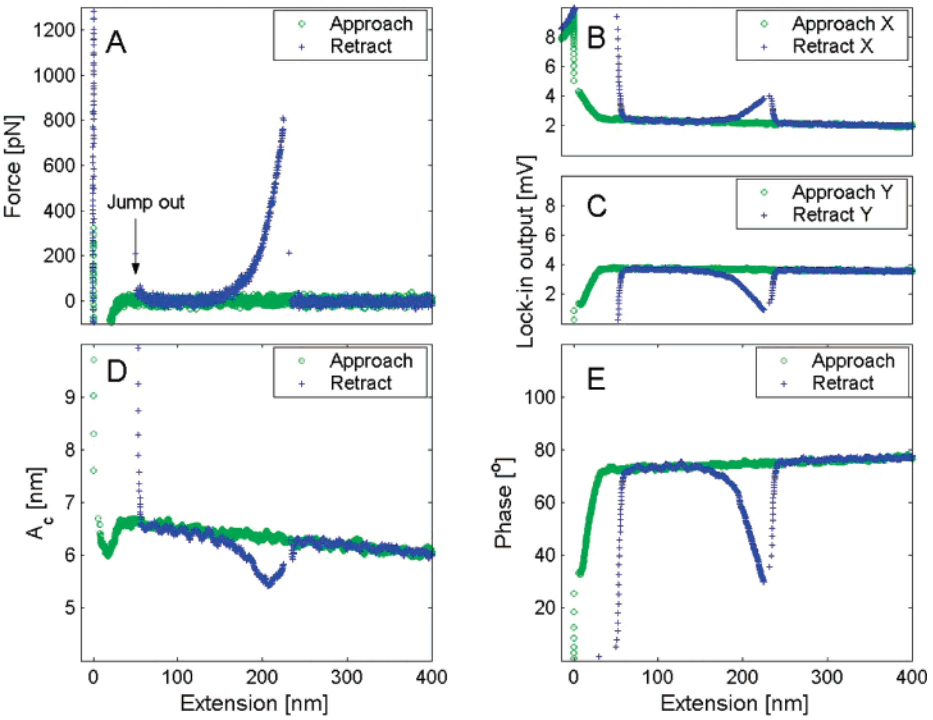

Figure 2. Example of data obtained during a stretching event while the substrate was oscillating with an amplitude of 16.9 nm and a frequency of 800 Hz. (A) Force-extension curve. On approach, no significant force is detected before a separation of approximately 25 nm where the tip jumps into contact. On retraction, a strong adhesion between the tip and the substrate leads to a jump out to a separation of approximately 50 nm. After the jump out, a polymer with a contour length of approximately 240 nm is being stretched. (B,C) The intensity of the signals measured in the *X*- and the *Y*-channels of the lock-in amplifier. (D) The amplitude of the cantilever oscillations as a function of separation. (E) The phase of the cantilever oscillations as a function of separation.

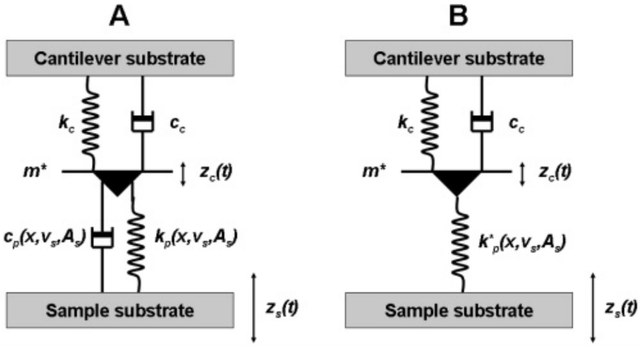

Figure 3. (A) Schematic illustration of the system where a cantilever with a spring constant, k_c, a damping coefficient, c_c, and an effective mass, m^*, are driven by a substrate oscillating with an amplitude, A_s, and a frequency, ν_s, through a polymer with a dynamic spring constant, k_p, and damping coefficient, c_p. $z_c(t)$ and $z_s(t)$ describe the motion in the z direction of the cantilever and the substrate, respectively, and x gives the tip−substrate separation or the polymer extension averaged over the oscillatory motion. (B) Schematic illustration of the simplified system where the polymer is described by an effective dynamic spring constant, k_p^*.

indicates that they correspond to single-molecule events (data not shown). A previous discussion of whether the mismatch between the force curve in the crossover between the low- and the high-force regime, and a fit with the freely jointed chain or the wormlike chain model is due to some kind of suprastructure of the polymer,[4,20] is not within the scope of this paper and will not be discussed here.

In Figure 2B and C where the intensity of the *X* and the *Y* signal is plotted, two important observations can be made. First, it is noticeable that, in the approach curve where no polymer is bridging the tip and the substrate, a significant background signal is observed in both *X* and *Y*. Second, it is evident in the retraction curve that, when the polymer is stretched, it leads to a decrease in the *Y* signal and an increase in the *X* signal. In Figure 2D and E, it is seen that the cantilever oscillates with an amplitude of several nanometers and a phase shift of ∼75°, even when no polymer is bridging the tip and the substrate. Further, it is observed that, as the separation distance decreases, the amplitude slightly increases, while the phase shift slightly decreases. As the polymer is stretched, it is seen that the amplitude decreases rapidly, while the phase shift at the same time drops to ∼30° before the polymer pulls loose from the tip and the signals return to the values observed during approach.

The background signal (the signal observed when no polymer is stretched) is due to hydrodynamic coupling between the substrate and the cantilever. Hydrodynamic coupling between an oscillating substrate and a colloidal probe attached to a cantilever has previously been investigated.[16] They observed a strong coupling equivalent to a low-phase value and a high amplitude at small separations, while at large separations, the coupling disappeared, that is, the amplitude approached zero and the phase approached 90°. At intermediate separations, a rapid decay in the signals is observed. For separations larger than ∼400 nm, the tail of the decay in the signals is approximated by a slightly tilted plateau (for clarity, see Figure 3A in ref 16). The hydrodynamic drag decreases with the inverse of separation and both the tip and the cantilever are involved. At very small separations, the drag is dominated by the tip, but at larger separations, the drag is dominated by the cantilever because it has a much larger surface area. However, as the cantilever−surface separation is the sum of the tip−surface separation and the tip height, the magnitude of the signal changes only gradually with separation. Further, as the polymer is stretched, it is observed that the amplitude decreases at the same time as the phase drops toward 0°. The polymer introduces an additional elastic coupling with the substrate, and a new friction term is introduced. When no polymer is bridging the tip and

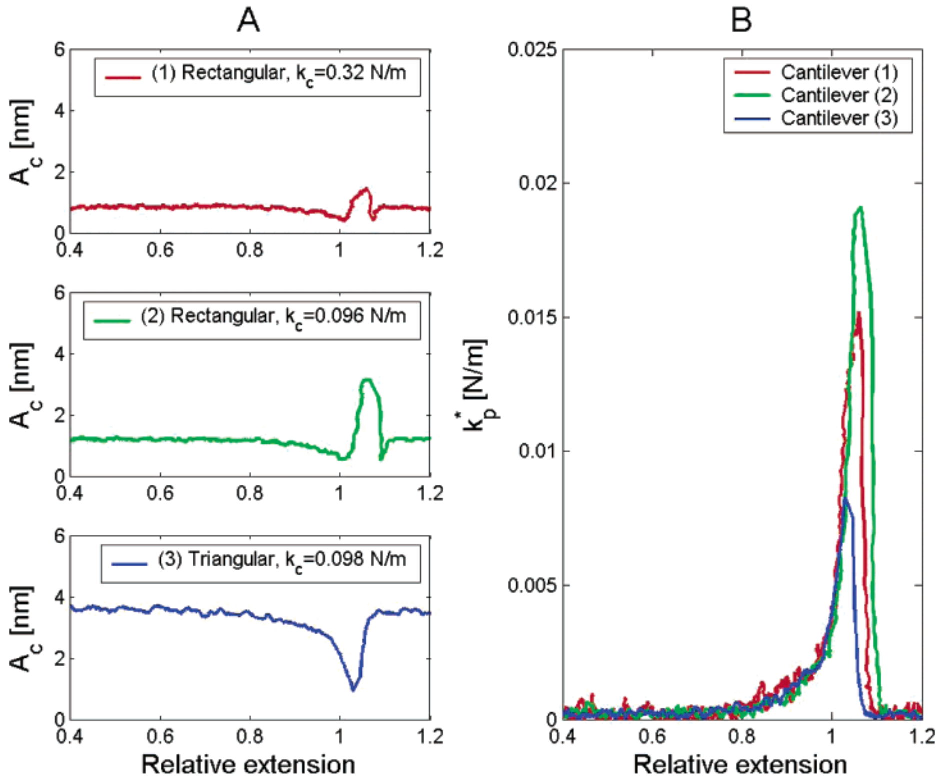

Figure 4. (A) Shows how the cantilever amplitude varies for three stretching events in experiments with three different cantilevers, all with comparable tip heights. In all three experiments, the substrate was oscillating with $\nu_s = 800$ Hz and $A_s = 8.5$ nm. Even though the magnitude of the signals cannot be directly compared, some trends can be deduced. Cantilever (1) and (2) had the same geometry but different spring constants. This geometry has given rise to a low degree of hydrodynamic coupling. When the polymer was stretched, it therefore only resulted in a short decrease in the amplitude followed by an increase. Further, as expected, the weaker cantilever oscillates with larger amplitude than the stiffer cantilever. Cantilever (2) and (3) have a different geometry but a similar spring constant. The triangular cantilever has a much higher degree of hydrodynamic coupling. Therefore, for this cantilever, only a decrease in the amplitude when the polymer was stretched was observed. Even though the response looks qualitative differently for the two types of cantilevers, the magnitude of the responses were similar due to the similarity in spring constants. (B) Shows how the data from the three experiments nicely superimpose when transformed from A_c to k_p^*.

the substrate, the cantilever moves in phase with the surrounding water flow. However, when the polymer starts to drive the motion of the cantilever, this is no longer the case, and the motion will therefore be strongly damped, leading to a decrease in the amplitude.

Isolation of the Polymer Response. As described above, the measured response of the cantilever includes a range of factors that are not related to the polymer properties but can vary due to the experimental arrangement. First, the hydrodynamic coupling is strongly dependent on the tip height and the geometry of the cantilever. Even though this effect can be minimized by choosing a cantilever with a tall tip and appropriate geometry, it can never be completely removed. However, experimentally removing the hydrodynamic coupling is not mandated, as we can easily measure the magnitude of the coupling in the absence of a polymer bridging the tip and the substrate (i.e., the approach curve). Subtracting the signals measured in the X and Y channel in the approach motion from the signals measured in the retract motion allows the component of the response that is due to the polymer driving the cantilever to be isolated:

$$X_{\text{polymer}} = X_{\text{total}} - X_{\text{hydrodynamics}} \tag{5}$$

$$Y_{\text{polymer}} = Y_{\text{total}} - Y_{\text{hydrodynamics}} \tag{6}$$

The calculated response of the cantilever after the subtraction of the hydrodynamic coupling, found by substituting X_{polymer} and Y_{polymer} into eqs 3 and 4, is then a function of the properties of the polymer, the properties of the cantilever such as the spring constant, k_c, and resonance frequency, ν_0, as well as damping of the cantilever motion due to the surrounding media, and the substrate amplitude, A_s, and substrate frequency, ν_s. To obtain the quantities related to the polymer, we follow the formalism previously described by Burnham et al.[22] The model describing the driven and damped cantilever motion is shown in Figure 3A. Solving the equation of motion for this system, the cantilever amplitude, A_c, and phase, θ, are found to be

$$A_c = \frac{A_s\sqrt{{k_p}^2 + {c_p}^2(2\pi\nu_s)^2}}{\sqrt{\left(k_c + k_p - k_c\dfrac{{\nu_s}^2}{{\nu_0}^2}\right)^2 + \left[\left(\dfrac{k_c}{2\pi\nu_0 Q} + c_p\right)2\pi\nu_s\right]^2}} \tag{7}$$

and

$$\tan(\theta) = \frac{c_p 2\pi\nu_s(k_c{\nu_s}^2 - k_c{\nu_0}^2) + \dfrac{\nu_0\nu_s k_p k_c}{Q}}{k_p{\nu_0}^2\left(k_p + k_c - k_c\dfrac{{\nu_s}^2}{{\nu_0}^2}\right) + {\nu_s}^2 c_p\left(c_p(2\pi\nu_0)^2 + \dfrac{2\pi\nu_0 k_c}{Q}\right)} \tag{8}$$

where Q is the quality factor of the cantilever, which describes the degree of damping. The Q value can be found by fitting the simple harmonic oscillator equation to the thermal power spectrum of the cantilever obtained in the relevant media. k_p and c_p are the dynamic spring constant and damping coefficient

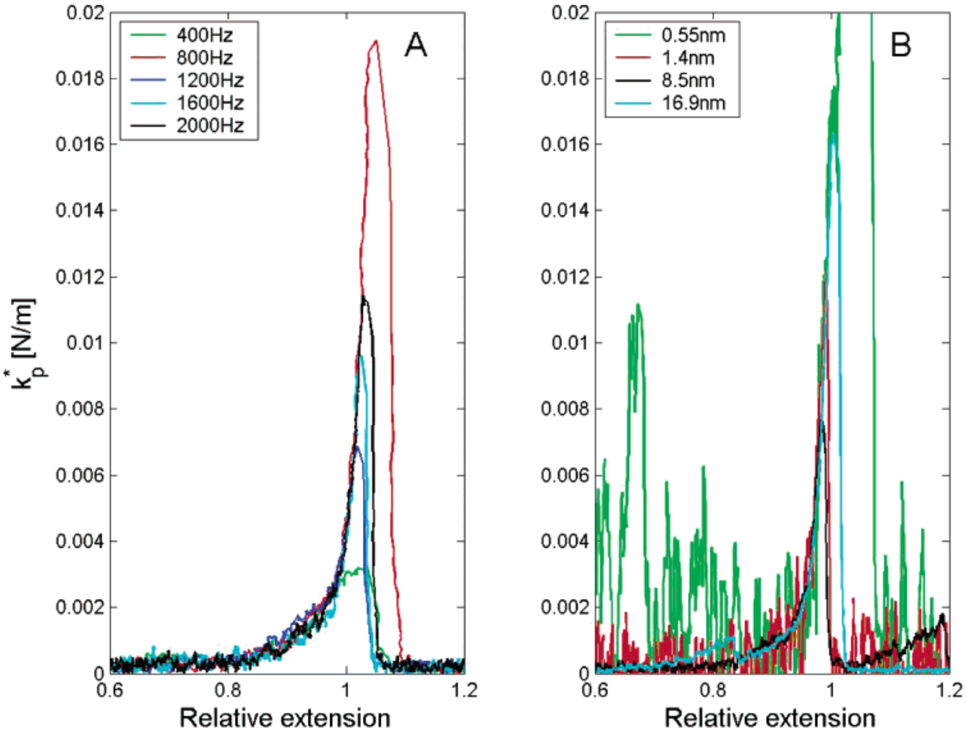

Figure 5. (A) k_p^* for five different frequencies at $A_s = 8.5$ nm. It is observed that k_p^* does not change within this frequency range. (B) k_p^* for four different amplitudes at $\nu_s = 1500$ Hz. Even though the signal-to-noise ratio decreases significantly at low amplitudes, no effect of changing the oscillatory amplitude is observed. The magnitude of each of the responses are not to be compared because it depends on the degree of extension for each stretching event.

of the polymer, respectively. The terms spring *constant* and damping *coefficient* are a little misleading because both of these quantities are functions of the polymer extension. Further, the quantities are termed *dynamic* because they might differ from the static quantities. The reason for this is that the nature of a polymer spring is different from a macroscopic mechanical spring. For example; the restoring force in the low force stretching regime is entropic in nature, meaning that the response does not take place instantaneously but on a time scale that is related to the relaxation of the chain.

From eqs 7 and 8, k_p and c_p can then in principle be identified. However, in practice, we find that the amplitude data is considerably more stable than the phase data. This is not attributable to the noise in X_{polymer} and Y_{polymer}, which should influence the amplitude and phase similarly. Rather it appears as though the phase oscillates due to inherent feedback in the system between the phase of the oscillation and the damping. As the response of the polymer can be assumed to be mostly elastic, we have therefore chosen to reduce the model to including only one parameter describing the polymer (see Figure 3B). The effective dynamic spring constant, k_p^*, will differ from the dynamic spring constant, k_p, defined in relation to eqs 7 and 8, if any dissipative contributions are present. For the simplified model, eq 7 reduces to:

$$A_c = \frac{A_s k_p^*}{\sqrt{\left(k_c + k_p^* - k_c \frac{\nu_s^2}{\nu_0^2}\right)^2 + \left(\frac{k_c \nu_s}{Q \nu_0}\right)^2}} \qquad (9)$$

By solving eq 9 for k_p^*, one can obtain a quantity describing the polymer's dynamic response that is independent of the cantilever characteristics. Figure 4A shows how the measured cantilever amplitude significantly varies for three different stretching events from three experiments carried out with different cantilevers, and Figure 4B shows how the same data superimposes when transformed to k_p^* as described above. This proves that this quantity is only related to the polymer properties.

Having established a procedure for isolating the response of the polymer, this can now be used to investigate if the polymer response depends on the oscillating frequency and amplitude. In Figure 5, k_p^* is presented for five different frequencies at a fixed amplitude and for four different amplitudes at a fixed frequency. It is here observed that the response of the polymer within the investigated range of amplitudes and frequencies are both length and time scale independent. Since poly(vinyl alcohol) is a highly flexible polymer, with a Kuhn length of 5.1 Å (found by a fit with the freely jointed chain model to the force-extension curves; the value is comparable to a previously reported value of 6.2 Å[20]), it is not surprising that the response does not change for the investigated amplitudes. Further, the independence of k_p^* over the frequency range investigated shows that the relaxation processes in the polymer take place on a time scale shorter than 0.5 ms, corresponding to the fastest deformation rate of 2 kHz.

In Figure 6, k_p^* is compared with $k_p = \mathrm{d}F/\mathrm{d}x$, where F is found by a fit with the freely jointed chain model to the quasistatic force extension curve. It is observed that these curves nearly superimpose, which is proof that k_p^* in this case corresponds to the static spring constant of the polymer.

In previous studies[3,6,23] the force extension law was extended to include an additional linear component to describe bond length and bond angle deformation. While such an extension sounds physically reasonable, it cannot be justified by a better correspondence between the model and experimental data because the introduction of an extra free fit parameter will always give a better fit. For the fit shown in Figure 6, such an extension is not included and there is no indication that the

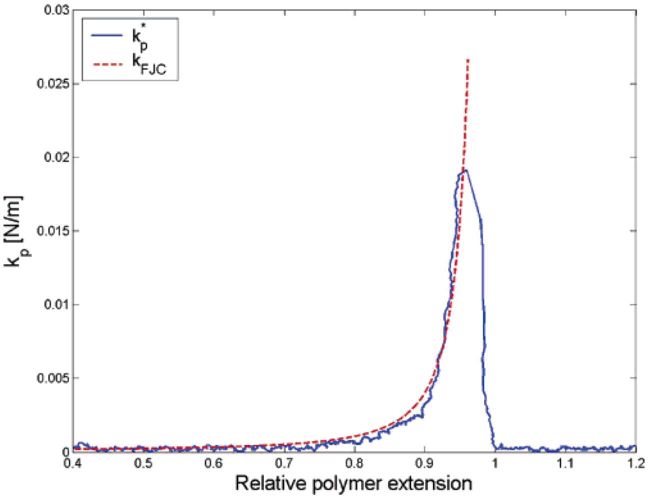

Figure 6. k_p^*compared to $k_p = dF/dx$, where F is found using a fit of the freely jointed chain (FJC) model to the force-extension curve. It is seen that k_p^* corresponds to the static spring constant of a freely jointed chain polymer with a Kuhn length of 5.1 Å.

model overestimates the stiffness of the polymer at high degrees of extensions. However, the method introduced in this paper will allow extensions to the theory beyond the entropic elastic regime to be evaluated, as it can also be tested against the polymer response obtained from dynamic measurements.

Conclusions

We have introduced a new technique to perform high-frequency and low-amplitude nanorheological measurements on a single polymer chain and demonstrated how the data obtained can be corrected for hydrodynamic contributions and for the properties of the cantilever.

Within the investigated range of frequencies and amplitudes, the dynamic mechanical response of poly(vinyl alcohol) is observed to be independent of the time and length scale of the experiments, which is reasonable for a simple and highly flexible polymer. The fact that the static response of poly(vinyl alcohol) has been recovered from these dynamic measurements is used to demonstrate that the method used in this paper is able to decouple the response of the polymer from the hydrodynamic response. If a polymer with a slow relaxation had been used in this study, one would not be able to determine if the amplitude and frequency dependence was due to a change in the polymer response or due to an inadequacy in the method employed to remove the influence of the hydrodynamic response of the cantilever. However, we foresee that this technique will be useful to probe time and length scales in systems that are stiffer and exhibit slower relaxation processes. Such systems will be investigated in future work.

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