

Hydrogen Bonding Governs the Elastic Properties of Poly(vinyl alcohol) in Water: Single-Molecule Force Spectroscopic Studies of PVA by AFM

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ABSTRACT: The mechanical properties of single poly(vinyl alcohol) (PVA) and poly(vinyl acetate) (PVAc) molecules under different solvent conditions were investigated using a new atomic force microscope (AFM) based technique—single-molecule force spectroscopy. The single-molecule force spectrum of PVA shows a kink at around 200 pN, deviating from the entropic elasticity of a random coil predicted from the WLC model. Since the single-molecule force spectra of PVAc and PVA in 8 M urea indicate that both behave like ideal entropic springs, the hydrogen bonding of hydroxyl groups in PVA must govern the elastic behaviors of PVA in water. Because of hydrogen bonding, PVA assumes a multiple stranded suprastructure in water. Upon stretching, the suprastructure undergoes a conformational transition to an overstretched state. This conformational change is fully reversible and is thus in thermodynamic equilibrium. This study demonstrates the unique utility of AFM in exploiting of suprastructures and conformational changes of synthetic polymers.

Introduction

The development of piconewton instrumentation has enabled study of the chemistry of single molecules; single-molecule chemistry is becoming one frontier of modern science.^{1–4} The atomic force microscope (AFM) is an instrument of particular interest. The high spatial resolution and extreme force sensitivity of the AFM has made it into a versatile experimental platform for experiments with single molecules toward low forces and small molecules.^{5–12} A new technique, single-molecule force spectroscopy by AFM, has opened the way to direct measurement of the mechanical properties of individual polymers with piconewton sensitivity and subnanometer accuracy.^{13–15} It has been shown that single-molecule force spectroscopy is powerful not only in understanding the elastic properties of single polymer strands but also in exploiting the conformational changes of single polymers, providing us with much valuable fingerprint information on single polymers.^{13–25} For example, the single-molecule force spectra of 1,4- α -linked polysaccharides, such as amylose, heparin, and dextran, revealed a force-induced chair–twist boat conformational transition of individual glucopyranose rings, which was seen as a force plateau in force–extension curves.^{15,17,18} Single-molecule force spectroscopic studies on xanthan^{16,21} and DNA^{26–28} revealed the complex conformational changes in their double-helix secondary structures. Single-molecule force spectroscopy on proteins, combined with polyprotein engineering techniques, is enabling study of the molecular mechanisms of the mechanical unfolding of proteins.^{14,22–24} This technique has therefore provided and will continue to provide new insights into the molecular mechanisms.

So far, most of the single-molecule force spectroscopic studies have focused on biopolymers that are important in biological processes. The application of single-

molecule force spectroscopy techniques to the understanding of the mechanical properties of synthetic polymers is just beginning.^{15,19,20,25,29–34}

Poly(vinyl alcohol) (PVA) is a commercially important water-soluble polymer. It has a variety of applications ranging from textile fibers, to stabilizing colloids, to medical applications.^{35,36} Due to the large number of hydroxyl groups in PVA, hydrogen bonding has a pronounced effect on the bulk properties of PVA materials. Because of the presence of hydrogen bonding, PVA has a high melting temperature. And, depending on the pattern of hydrogen bonding, PVA shows different crystalline structures: syndiotactic PVA shows a helix crystal structure stabilized by hydrogen bonds, while isotactic PVA shows an extended crystal structure.³⁷ Although there have been many studies of hydrogen bonding in the crystalline structure of PVA,³⁶ there have been few studies on the hydrogen bonding of PVA in aqueous solution.³⁶ In a previous communication,²⁰ we reported the preliminary results on the use of single-molecule force spectroscopy to study the mechanical properties of single PVA in water. In this full paper, we will present a detailed study on the mechanical properties of single PVA molecules. Our data reveal that the elastic properties of PVA in water are essentially governed by hydrogen bonding and further demonstrate the power of this technique in exploiting suprastructures and their conformation changes in synthetic polymer systems.

Experimental Part

All the experiments were carried out on a custom-built single axis atomic force microscope. The experimental details of single-molecule force spectroscopy by AFM have been described elsewhere.^{13–16} Briefly, when an AFM tip is brought into contact with a polymer sample that was immobilized on a substrate, the binding of single molecules can be achieved due to the molecular dimensions of the tip apex. Upon retracting, the polymer is stretched, and the restoring force is measured as a function of the separation distance, resulting in a force–extension curve.

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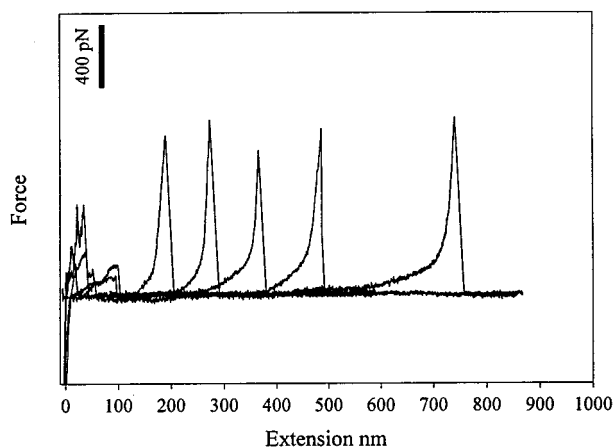


Figure 1. Force curves of individual PVA molecules of different contour lengths in 0.2 M NaCl aqueous solution.

Materials and Sample Preparation. Commercially available PVA (from Aldrich, average M_w is 124 000–186 000) and PVAc (from Aldrich, average M_w is 167 000) were used in this study. ^1H NMR indicated that the PVA and PVAc samples are atactic polymers.³⁸ PVA and PVAc were dissolved, to a concentration of 1 mg/mL,³⁹ in 0.2 M NaCl aqueous solution and 3-heptanone (Fluka), respectively. Appropriate PVA or PVAc solution was deposited onto a clean glass substrate and incubated overnight. The sample was then rinsed with the appropriate solvents and used for experiments immediately. The experiments on PVA were carried out in either 0.2 M NaCl aqueous solution or 8 M urea aqueous solution. The experiments on PVAc were carried out in 3-heptanone.

Force Measurement. Silicon nitride cantilevers from Digital Instruments (DI, Santa Barbara, CA) were used. The spring constant of each cantilever was calibrated after each experiment by measuring the amplitude of its thermal fluctuations.^{40,41} Measured values were between 50 and 70 mN/m.

The technique used here for the coupling of single molecules to the tip is based on adsorption. Various studies have shown that adsorption can produce very high connecting forces (> 700 pN), thus making adsorption a versatile approach.^{13–16}

Results and Discussion

Single-Molecule Force Spectrum of PVA in Water and Comparison with WLC Model. Figure 1 shows a series of force–extension traces of PVA chains measured in different samples and using different cantilevers. Since the PVA sample was polydispersed and the point at which the polymer chain adsorbs to the tip is distributed randomly along the polymer chain, the contour lengths of the PVA chains vary from 50 nm to more than 1 μm . Despite the difference in contour lengths, all of the force–extension traces exhibit similar deformation characteristics: the force rises monotonically with extension until a yield point is reached. All of the curves show a kink at around 200 pN.

To compare the force–extension relationship of polymers of different contour lengths, the force–extension traces were normalized by their lengths and superimposed,¹³ plotted in Figure 2. The superposition of these curves clearly shows that the elastic properties of PVA chains scale linearly with the contour length. This finding corroborates that the elasticity of single PVA molecules were measured. This provides a rigid criterion for differentiating single and multiple molecular bridges.^{13,15,25}

The force–extension relationship of a polymer chain can be described by the freely joint chain (FJC) model⁴² and the wormlike chain (WLC)^{43,44} model. The FJC

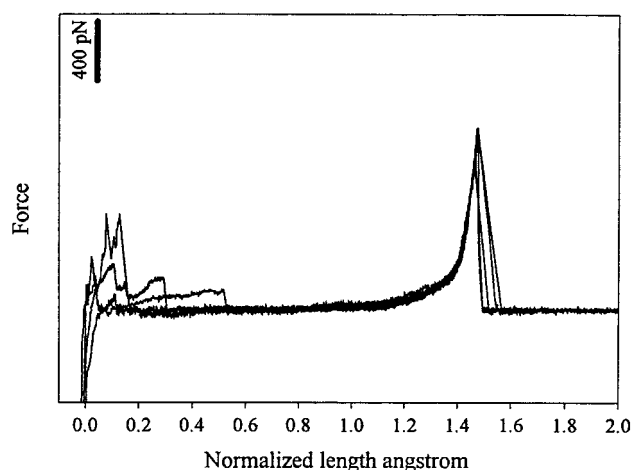


Figure 2. Force curves of PVA shown in Figure 1 are normalized by their contour lengths. The superposition of the force curves indicates that single PVA molecules were stretched.

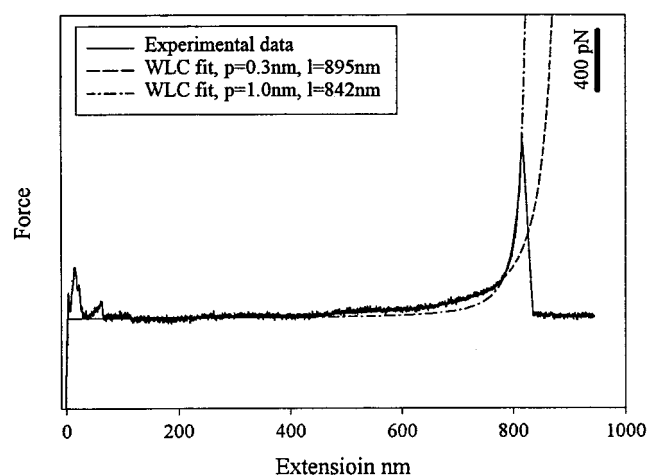


Figure 3. Comparison of experimental data of PVA in 0.2 M NaCl aqueous solution with the WLC model reveals that there exists great deviation of experimental data from the WLC model. The fit parameters are shown in the inset.

model treats a flexible polymer random coil as a chain of statistically independent segments (Kuhn segment). An extended FJC model has been introduced and used to describe the elastic behaviors of polymers, which assumes the Kuhn segments can be deformed under stress.^{13,18,19} Taking into account both local stiffness and long-range flexibility, we can also use the WLC model to describe a polymer chain. The force–extension relationship is given by the following formula:

$$F = \frac{kT}{p} \left(\frac{1}{4} \left(1 - \frac{x}{L} \right)^{-2} - \frac{1}{4} + \frac{x}{L} \right)$$

where F is the external force acting on the polymer chain, p is the persistence length which reflects the flexibility of polymer chain, x is the extension of the polymer chain (end-to-end distance), L is the contour length of the polymer chain, k is the Boltzmann constant, and T is the temperature. In this paper, we will focus on the comparison of experimental data with the WLC model.

Figure 3 shows a force–extension curve of PVA and fits of the low-force regime and high-force regime using the WLC model (dashed line). Because of the kink at ~ 200 pN in the force curves, the elasticity of PVA changes dramatically before and after the kink, and

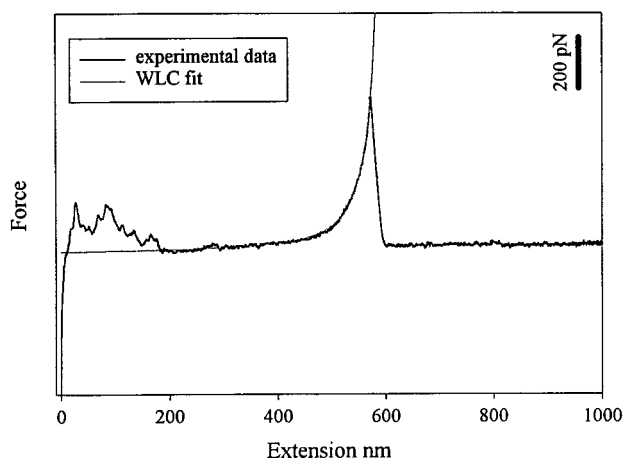


Figure 4. Force curve of a single PVAc molecule in 3-heptanone, superimposed with a WLC fit curve. Fit parameters are $p = 0.31$ nm and $L = 615.9$ nm.

thus the experimental data clearly deviate from the WLC model. The WLC model can only describe the elastic behaviors of PVA in water at either low-force regime or high-force regime, depending on the fit parameters. This indicates that PVA in water does not assume an ideal random coil conformation, but a suprastructure mediated by the interaction between polymer segments or the polymer-solvent interaction.

Neither the FJC nor the WLC model considers long-range interactions in the polymer coils.^{28,42,43} In the case of PVA, there is one hydroxyl group in every monomer. These hydroxyl groups can easily interact with each other and with water molecules via hydrogen bonds. Hydrogen-bonding interaction is so pronounced in PVA aqueous solution that the WLC model may not hold any more.

To prove the above assumption, we carried out single-molecule force spectroscopy on PVAc. In PVAc, the hydroxyl groups are esterified, and thus the long-range interaction due to the hydroxyl groups is prevented. Interactions between acetate groups in nonaqueous solvents are very weak,³⁶ and it is thus expected that PVAc behaves like an ideal random coil.

The single-molecule force spectrum of PVAc in 3-heptanone, and superimposed with a fit curve by the WLC model, is shown in Figure 4. As expected, the kink in the force curve no longer appears, and the force increases monotonically versus extension. The WLC model can therefore fit the data very well, indicating that the PVAc behaves like an ideal random coil. This observation supports the hypothesis that the kink in the force spectra of PVA results from hydrogen-bonding interaction between hydroxyl groups of PVA and suggests that the kink is a deformation characteristic of the PVA suprastructure in water stabilized by hydrogen bonding (although other types of interactions, such as hydrophobic interactions, may also contribute to the stabilization of the suprastructure).

Hydrogen bonding has a great influence on the properties of bulk PVA materials. For example, the syndiotactic PVA shows a helical crystal structure, while the isotactic PVA exhibits an extended crystal structure. However, there are few studies on the influence of hydrogen bonding on the structure of PVA in aqueous solution. Previous studies have suggested that PVA exists as a helical structure in aqueous solution due to the hydrogen bonding of hydroxyl groups and that PVA

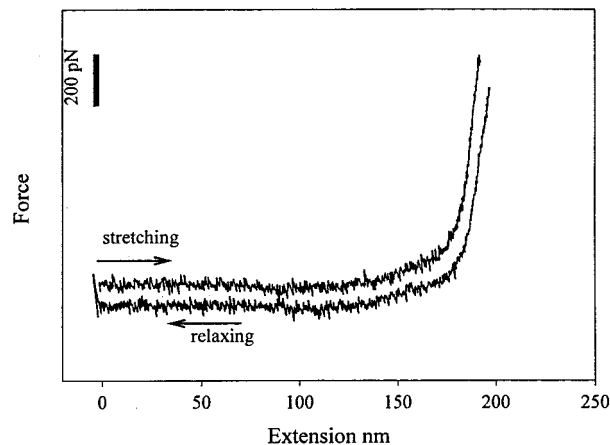


Figure 5. Stretching and relaxing traces of the same PVA in 0.2 M NaCl aqueous solution do not show any hysteresis, indicating the conformational change in the PVA suprastructure is reversible. For clarity, the two traces are offset.

undergoes a helix-coil transition upon increasing temperature.³⁶ However, the details of this helical structure are still unknown. The present single-molecule force spectroscopic study reveals that the elastic properties of PVA in water are essentially governed by hydrogen bonding and confirms that there exists a hydrogen-bonding-mediated suprastructure of PVA in aqueous solution, which might correspond to the previously reported helical structure.

Conformational Changes in the Suprastructure of PVA. It is worth mentioning that the kink in the single-molecule force spectra always occurs at ~ 200 pN. The elasticity of PVA helix changes dramatically before and after the kink. This indicates that the suprastructure of the PVA undergoes a conformational change from a relaxed to a more stretched helical structure at ~ 200 pN. When a single PVA helix was repetitively stretched and relaxed (Figure 5), there was no hysteresis, indicating that the conformational change is fully reversible and takes place under equilibrium conditions. We also carried out experiments on the rate dependency of the force induced transition of PVA (data not shown). When stretching speeds were varied from 0.02 to 10 $\mu\text{m/s}$, no effect was observed on the force required for the conformational change (~ 200 pN) or its reversibility. This indicates that this conformational change occurs much faster than the experimental time scale.

In contrast, similar conformational changes have been reported with DNA double helices^{26–28} and with xanthan,^{16,21} but their conformational changes show hysteresis. These conformational changes may serve as the fingerprints of these polymers' mechanical properties.

"Denatured" PVA Behaves Like an Ideal Random Coil. Hydrogen bonds can occur intermolecularly as well as intramolecularly. This is particularly important in macromolecular and biological assemblies, such as proteins and nucleic acids. In the native state of protein and DNA, hydrogen bonds are responsible for stabilizing ordered secondary structures, such as α helices and β sheets in proteins and double helices in DNA. Upon exposure to denaturants, the hydrogen bonds (along with hydrophobic interactions) are broken, and thus the ordered secondary structure of proteins is lost.^{45–47} Urea is a typical denaturant.

As we discussed above, PVA suprastructure in water is stabilized by hydrogen bonding. To further investigate the nature of the PVA helix in water, we carried out

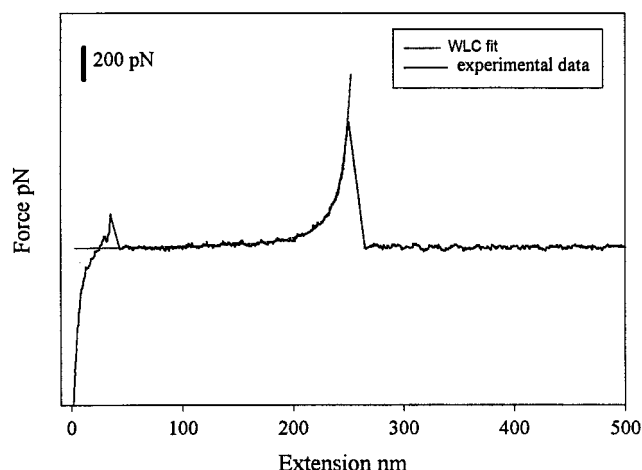


Figure 6. Force curve of a single PVA molecule in 8 M urea solution, superimposed with a fit curve by WLC model. Fit parameters are $p = 0.16$ nm and $L = 266$ nm. The superposition indicates that “denatured” PVA behaves like an ideal random coil.

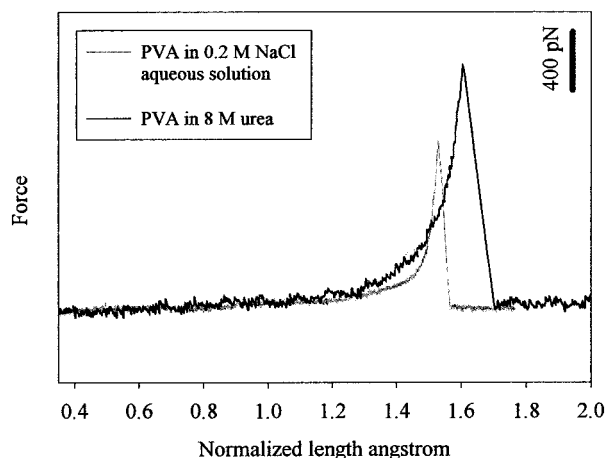


Figure 7. Comparison of the normalized force spectra of PVA in 0.2 M NaCl aqueous solution with that of PVA in 8 M urea. Both of the force curves are normalized at 500 pN.

the single-molecule force spectroscopy on PVA in 8 M urea. Similar to protein denaturation studies, we also used urea as a denaturant. Figure 6 shows the single-molecule force spectrum of PVA in 8 M urea superimposed with a WLC fit curve. After denaturation, the kink disappeared, and the force curve of “denatured” PVA can be well described by the WLC model with a persistence length of 0.16 nm. This is close to the monomer size of 0.15 nm (C–C bond length), indicating that “denatured” PVA behaves like a flexible random coil similar to PVAc in 3-heptanone. This observation further supports the hypothesis that the helical structure of PVA in aqueous solution is stabilized by hydrogen bonds (along with some hydrophobic interactions), as are proteins.

The persistence length of PVAc in 3-heptanone (0.31 nm) is larger than that of “denatured” PVA in urea (0.16 nm). This may be due to the fact that the acetate side group in PVAc is much bigger than the hydroxyl group in PVA, and the steric effect of acetate groups may become pronounced and make PVAc stiffer.

The PVA’s Helix Is a Multiple-Stranded Structure. A comparison of the normalized force spectra (Figure 7) shows that, at high-force regime, the slope of the force curve (the stiffness of the molecule) of PVA

in water (35 000 pN/nm) is much larger than that of denatured PVA in urea (13 000 pN/nm). This suggests that the suprastructure of PVA in water is a double- or multiple-stranded helix. If the helix of PVA in water were a single-stranded structure, the mechanical stretching of PVA would unravel the suprastructure and stretch the polymer chain to an almost fully stretched state where deformation of the polymer chain is dominated by the stiffness of bond angle potentials and independent of solvent.²⁵ Hence, the force curve of PVA at high-force regime would then superimpose that of denatured PVA, since denatured PVA is a random coil of a single polymer chain. This, however, was not observed, indicating that the PVA helix in water is a multiple-stranded structure.

In contrast to the multiple-stranded helix of PVA in water, PEG exists in water as a single-stranded helix. It has been shown that in water PEG assumes a helical structure stabilized by water-bridged hydrogen bonds.^{25,47} Its single-molecule force spectrum is also characterized by a kink. In a nonpolar solvent, the hydrogen bonds disappeared and PEG assumed a random coil conformation. Because of the single-stranded nature of the helical structure of PEG in water,²⁵ the force curves of PEG in water and in nonpolar solvent superimpose well with each other at high force regime.

Conclusion

The present nanomechanical study reveals that PVA do not exist as individual random coils, but as a supramolecular assembly stabilized by hydrogen bonding. Thus, the elastic properties of PVA in water are essentially governed by this hydrogen bonding. This study demonstrates the unique utility of AFM in identification of suprastructures and exploring conformational changes of synthetic polymers. This technique may be applied to a range of polymers where hydrogen bonding or other interactions are predominant.

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