Since the invention of atomic force microscopy (AFM), researchers have been able to study the statistical mechanical properties of individual polymer chains by using a technique known as single molecule force spectroscopy (SMFS), which stretches a single polymer chain by attaching one end of the chain to a substrate and the other end to the AFM tip. SMFS is used to obtain static information about single polymer chains, mainly through a quasistatic stretching process. With technological developments in recent years, dynamic SMFS measurements under nonequilibrium conditions have also been realized. The dynamic viscoelasticity of single molecule chains can also be measured, which will benefit our understanding of the nonequilibrium dynamic properties of polymeric materials.

Key Words: Single molecule force spectroscopy / Mechanical properties / Atomic force microscopy

1. INTRODUCTION

The mechanical properties of polymeric materials are closely related to the primary structure and conformation of the polymer chain. As the mechanical and functional requirements of polymers increase, there is a need to improve polymer performance by looking at the structure of smaller regions and providing feedback for structural control. Therefore, studying a single synthetic polymer chain, which is the most fundamental component of a polymer system, is critical. The physics of polymer chains was pioneered by great scientists such as Debye, Kuhn, Kramers, and Flory. In the 1970s, the theory was developed through methods such as functional integration, many-body theory, and scaling and was verified by information from single molecule chains using neutron diffraction and light scattering. However, because these studies obtain information about single molecule chains by measuring dilute polymer solutions, the results represent the average performance of many polymer chains. Information about a single polymer chain cannot be directly obtained. With the development of experimental techniques such as atomic force microscopy (AFM) and optical tweezers, it has become possible to perform precise measurements of minute forces and elongation experiments on single polymers. When compared to traditional batch experiments, this method provides information about the “personality” of single polymer chains. AFM has a force detection sensitivity of 10 pN and a high spatial resolution outperforming that of other similar instruments. AFM is commonly used for studying synthetic polymers (entropic elasticity, covalent bond strength, conformational transitions) and life science studies (protein folding transitions, polysaccharide conformational transitions, DNA/base pair separation, etc.). SMFS is a technique for measuring the mechanical properties of single polymer chains using AFM. Some of the force curves that have been studied. SMFS also enables direct measurements of intra- and intermolecular interactions at the molecular level, providing information about the molecular conformation and structure of synthetic polymers and biopolymers. In addition, SFMS can measure individual molecules under a variety of controlled conditions (solvent, temperature, pH, etc.).

The conformation and elasticity of a single polymer chain can be revealed by fitting force curves with polymer chain models. The most commonly used models for describing the entropic elasticity of synthetic polymer chains are the freely jointed chain (FJC) model and the worm-like chain (WLC) model. Various theoretical models such as the extended FJC model, Odijk WLC model, and quantum-chemical freely rotating chain (QM-FRC) model have been proposed in recent years to describe different types of synthetic polymers and biopolymers. However, in most...
SMFS experiments, the stretching rate is much lower than the fluctuation rate of the polymer chain segments (i.e., the stretching time is much longer than the time scale of the molecular chain motion), and thus, the polymer chain is in quasi-equilibrium during the stretching process. At this point, the static mechanical properties of the polymer chains are being measured. However, the dynamic mechanical properties of single polymer chains are important for polymers that are both viscous and elastic. Recent related studies have improved SMFS methods for obtaining dynamic mechanical properties of polymers in the nonequilibrium state, providing an important basis for understanding the nonequilibrium dynamic properties of polymeric materials\(^{29-31}\). This review will present the recent progress of static and dynamic mechanical properties of single polymer chains.

2. SINGLE MOLECULE FORCE SPECTROSCOPY

In an SMFS experiment, a single polymer chain is chemically or physically bonded to fix one end of the molecular chain to a substrate and the other end to the tip of the AFM. In the scanning mode of AFM (perpendicular to the substrate), a single polymer chain is stretched, and the force curve is measured. Figure 1a shows a schematic diagram of SMFS by AFM\(^{32}\). To obtain the physical properties of a single polymer chain, the relationship between the force and the extension of the polymer chain must be analyzed. However, in the original force-extension curve, the stretching of the molecular chain and the displacement of the sample are not equal (stretching process). The actual magnitude of the stretch \(\delta\) is equal to the displacement of the sample \(z\) minus the deformation of the cantilever \(\Delta\), i.e., \(\delta = |z| - |\Delta|\). The stretching force is then \(f = k\delta\), where \(k\) is the spring constant of the cantilever. Thus, the force-extension curve of a single poly(N-isopropylacrylamide) (PNIPAM) chain can be obtained, as shown in Fig. 1b. To describe the entropic elasticity of the polymer chain, the FJC model and the WLC model were used to fit the force curves. The FJC model is described as follows:

\[
\frac{F}{k_B T} = L^4 \left(\frac{\delta}{L}\right), \quad L(x) = \coth x - \frac{1}{x}
\]  

(1)

where \(\delta\) is the extension length at an external load of \(F\), \(l_K\) is the persistence length, and \(L\) is the contour length. The curve fitting from the WLC model is superimposed in Fig. 1b (red curve). The WLC model fits the force-extension curve well, with an \(l_p\) of 0.34 ± 0.02 nm and an \(L\) of 85.1 ± 0.05 nm.

Differences in model fits often depend on the type of molecule, also reflecting differences in the physical properties at the single molecule chain level.

3. STATIC MECHANICAL PROPERTIES OF SINGLE MOLECULE CHAINS

Slow stretching of single molecule chains at equilibrium, i.e., quasistatic SMFS, can provide us with static mechanical properties of single molecule chains, such as the entropic elasticity, enthalpic elasticity at high elongation, and intra- and intermolecular interactions\(^{33,34}\). These results provide a strong experimental foundation for understanding the mechanisms underlying the physical and functional properties of polymers.

We investigated the mechanical properties of a single chain of the temperature-sensitive polymer PNIPAM using SMFS\(^{32}\). Figure 2a shows typical force-extension curves of PNIPAM single chains in pure water. At 28 °C, a temperature discrepancy between the FJC model and our experimental result. In comparison to the FJC model, the WLC model can fit experimental data significantly better in the high-extension regions. The WLC model is described as follows:

\[
\frac{F}{k_B T} = L^4 \left(\frac{\delta}{L}\right), \quad L(x) = \coth x - \frac{1}{x}
\]  

(2)

where \(\delta\) is the extension length at an external load of \(F\), \(l_p\) is the persistence length, and \(L\) is the contour length. The curve fitting from the WLC model is superimposed in Fig. 1b (red curve). The WLC model fits the force-extension curve well, with an \(l_p\) of 0.34 ± 0.02 nm and an \(L\) of 85.1 ± 0.05 nm. Differences in model fits often depend on the type of molecule, also reflecting differences in the physical properties at the single molecule chain level.

Fig. 1   (a) Schematic diagram of single molecule force spectroscopy (SMFS) based on atomic force microscopy (AFM). (b) Typical force-extension curve of a typical single polymer chain in a solvent\(^{32}\).
well below the lower critical solution temperature (LCST) of PNIPAM in water (31.5 °C) the curve initially exhibits a linearly increasing tensile force. This type of curve is usually observed with a chain dissolved in a good solvent and can be used to quantitatively characterize the entropic elasticity of the PNIPAM chain. Figure 2b presents a curve recorded for PNIPAM in pure water heated to 38 °C, which is well above the LCST of PNIPAM in this solvent. In contrast to that in Fig. 2a, the force curve in Fig. 2b has a plateau of constant force, namely, the Rayleigh plateau corresponding to the “reeling-out” of chain segments one by one from a collapsed globule. In addition, we found a temperature dependence (Fig. 2c) of the persistence length of the PNIPAM molecular chains and calculated the interfacial free energy between the PNIPAM molecules and water.

The enthalpic elasticity of single molecule chains at high extension can also provide important information. Marszalek et al. found that the change in enthalpic elasticity of polysaccharide chains was caused by a transition from a chair-like conformation to a boat-like conformation13). In addition, Cui et al. studied several polymers with side chains of different lengths and shapes35). The authors found that only side chains that are both long and bulky affect single-chain entropic elasticity and that most polymers are identical to PE, implying that the single-chain entropic elasticity is determined by the nature of the C–C backbone.

### 4. DYNAMIC MECHANICAL PROPERTIES OF SINGLE MOLECULE CHAINS

Most SMFS studies to date have focused on slow extension at equilibrium; however, if extension becomes so rapid that external stimuli are unable to maintain internal equilibrium, nonequilibrium effects must be considered. Recently, nonequilibrium “dynamic SMFS” has become possible, allowing experimental measurement of not only the elasticity of individual polymer chains but also the viscosity. Since the viscoelasticity of polymers is widely recognized as the most important property of nonequilibrium dynamics, there are high expectations for the study of viscoelasticity of single polymer chains22).

With the advancement of experimental techniques, studies on the measurement of viscoelasticity using noise analysis of thermal fluctuations in cantilever beams31) and periodic external forced oscillation methods36) have been successively reported. In 2000, Nakajima et al. conducted a pioneering study, measuring the dynamic mechanical properties of a single protein molecule for the first time29). The authors applied a sinusoidal vibration of 0.25 Hz to a single protein molecule, measured the protein’s response, and observed the interesting phenomenon of repetitive unfolding and refolding. Recently, we measured the dynamic viscoelasticity of single polystyrene (PS) chains over a frequency range of 7–9 kHz by applying oscillation to the cantilever37). Figure 3a, 3b show the friction and elasticity coefficients of the polymer chain as a function of the applied force. Both the elastic coefficient \(k_2\) and friction coefficient (or viscosity coefficient) \(\eta_2\) exhibit a plateau in the low-extension region (the values of \(k_2\) and \(\eta_2\) in the low-extension region were 4.02 ± 1.36 × 10^{-6} \text{ N m}^{-1} \) and 5.15 ± 1.46 × 10^{-10} \text{ kg s}^{-1} \), respectively). The experimental results of the chain length dependence of viscoelasticity agree with theoretical predictions from the hydrodynamic theory of Kirkwood. The Kirkwood model was used to calculate the effective viscosity of a single polymer chain, which revealed that the viscosity of a single PS chain in a solvent is dominated by the interaction between the polymer and solvent (shown in Fig. 3c).

In addition, Kawakami et al.38) and Humphrisy et al.39, 40) studied the dynamics of conformational transitions such as folding-unfolding of proteins and DNA by applying periodic forces driven by magnetism, and Mcleish et al.41, 42)
investigated the mechanism of internal friction in the high elongation expansion of polypeptide chains. Patil et al. measured the dynamic elastic response of single molecules by using a nonresonant oscillation method and discussed the differences in these responses in good and poor solvents. The study of viscoelasticity for single polymer chains will aid in our understanding of the origins of nonequilibrium properties in polymer solutions and polymeric materials.

CONCLUSION

In the last century, although the theories of polymer physics were based on single molecule chain theory, there were few experimental methods to verify the accuracy of this theory. Researchers were in dire need of a tool that could accurately describe the mechanical properties of single synthetic polymer chains, which represent the primary stage of polymer systems. Fortunately, the invention of AFM solved this problem, and AFM has unlimited potential in fields ranging from traditional synthetic polymers to biomedicine. This review discusses recent advances in the study of the mechanical properties of single molecule chains, with a focus on the measurement of static and dynamic mechanical properties. In particular, the extension of dynamic SMFS promises to deepen the understanding of the macroscopic nonequilibrium properties of polymers and is expected to be a future research direction for SMFS.

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![Graphs showing friction coefficient-extension and elasticity coefficient-extension curves of a single PS chain.](image-url)
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