Dynamic Measurement of a Single Polymer Chain by Atomic Force Microscopy

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Abstract. We performed the measurement of the dynamic viscoelasticity of a single polymer chain by the self-produced piezo-control and data acquisition system externally built on the commercial atomic force microscope (AFM). The sinusoidal movement with the small amplitude was applied to a single polymer chain at the several points in the middle of elongation, and the response of the molecule was evaluated. As the result of the experiment, some dissipative behavior was observed in the low-elongation region of the polymer chain, which could have never been observed in the simple elongation experiment. This behavior may be due to the friction between the monomers constituting a polymer chain.

1. Introduction
The development of the technique based on the atomic force microscopy (AFM) such as “single molecule force spectroscopy (SMFS)” has enabled us to detect the forces acting on the single molecules directly. SMFS have widely performed by many researchers to elucidate the mechanical and the structural properties of the single polymers to date [1, 2, 3].

The viscoelasticity of the polymeric materials is not only scientifically fascinating but important for the practical use. At the single molecule level, though many SMFS studies on the polymers and the biomolecules have focused on the elongation characteristics, little attention has been paid to the intramolecular energy dissipation processes. To evaluate them, the measurement of the dynamic viscoelasticity as performed in the macroscopic rheological studies as well as the simple elongation experiment is essential.

In this study, elastic/dissipation properties of a single polymer chain were investigated by the AFM-based dynamic measurement. The sinusoidal response was recorded at the several points in the elongation process by the self-produced control/measurement system externally built on the commercial AFM.

2. Instrumental setup and sample preparation
To realize the dynamic measurement we constructed an external AFM piezo-scanner control and data acquisition system (Figure 1) with the LabVIEW (National Instruments, Austin, TX) on the NanoScope IIIa (Digital Instruments, Santa Barbara, CA) [4]. This system allows us to design any required movement of the sample stage in $z$-direction, especially the sinusoidal
movement. This technique was also employed in the study on the mechanical unfolding process of the proteins [5, 6, 7]. The command to control the piezo-scanner was sent from the computer to the function synthesizer. The control signal for the piezo-scanner was generated by the function synthesizer then amplified and input to the piezo-scanner. The deflection of a cantilever was detected by the photo diode then the signal was sent to the lock-in amplifier. The raw deflection signal, the amplitude and the phase shift of the deflection were recorded by the computer. Here the input signal from the function synthesizer was used as the reference signal for the lock-in amplifier. The frequency range of the sinusoidal movement applied to the piezo-scanner was limited to 1-100 Hz to avoid the viscous force of solvent.

In this experiment, polystyrene (PS, $M_n = 22,000$, $M_w = 39,000$) was selected as the model polymer for the measurement. Both ends of PS were terminated by -SH groups to form Au-S bonds with the gold-coated AFM tip (BL-RC150VB, Olympus, Tokyo, spring constant was 30 pN/nm) and the gold substrate. The chain length estimated from the molecular weight was 50-60 nm. The gold substrates were prepared by the vacuum deposition of gold on the freshly cleaved mica. The PS molecules were dissolved in $N,N$-dimethylformamide (DMF) at a concentration of 20 μg/ml, and a drop of the solution was put on a gold substrate. All the measurement were performed in DMF solvent with the fluid imaging cell.

In the dynamic measurement, first, the sample stage on the piezo-scanner was moved upward to make the substrate closer to the tip. Immediately after the surface of the substrate touched the tip, the signal of the sinusoidal movement was applied to the piezo-scanner. Then the stage was moved downward about 5 nm, and the sinusoidal movement was applied again. These procedures of the tip-sample separation and the sinusoidal movement were repeated until after the end of molecule was detached from the AFM tip or the substrate.

**Figure 2.** Series of the signals of (a) input signal (piezo-scanner movement) and (b) deflection of the cantilever. (c) Magnified signals of (1) the sinusoidal input and (2)-(5) the deflection of the cantilever at the several points in the elongation process of a chain.
Figure 3. (a) The amplitude and (b) The phase shift of the sinusoidal response of the molecule plotted against the sample position. Open symbols represent those for solvent (with no molecules) measured as the control experiment.

3. Results and discussions

Figure 2a and 2b show the series of the input signal (piezo-scanner movement) and the raw cantilever-deflection signal, respectively. At the each elongation point, 20 cycles of sinusoidal movement (50 Hz, 10 nm$_{p-p}$ amplitude) were applied. Figure 2c shows the magnified signals of (1) the sinusoidal input and (2)-(5) the characteristic behavior of the cantilever deflection at the several points in the elongation process of a PS chain. At the beginning of the measurement, the sample stage was approached and touched the AFM tip, then the sinusoidal movement was applied. The tip oscillated together with the sample stage, therefore the signal of deflection (2) is almost the same as the input signal (1). The amplitude of the deflection (3) is much smaller than that of (2), because the tip no more touched the substrate. The intensity of sinusoidal responses increased with further extension (4), then the detachment of the polymer chain from the AFM tip or the substrate was observed shortly after (4). After the detachment of the molecule, no remarkable response was observed (5).

The amplitude $A$ and the phase shift $\delta$ acquired with the data in Figure 2 are plotted against $z$-position in Figure 3. As the control experiment, the amplitude and the phase shift measured for the solvent only system were plotted together. In Figure 3, the position $z = 0$ means the surface of the substrate. When the AFM tip was in contact with the substrate, $A$ was equal to the input amplitude (10 nm$_{p-p}$) as mentioned above, and $\delta$ was zero since the tip oscillated with the substrate. In the low-elongation region ($0 < z < 40$ nm), $A$ was almost equal to the amplitude for solvent. The phase shift $\delta$ increased and then had the maximum around $z = 20$ nm. As the polymer was elongated further ($40 < z < 60$ nm), $A$ increased rapidly, and $\delta$ decreased then came back to zero due to the strong tension of a molecule. After a molecule was detached form the AFM tip or the substrate ($60$ nm $< z$), $A$ and $\delta$ values became equal to those of solvent because the polymer chain connecting the AFM tip and the substrate did not exist any more. It should be noted that $\delta$ for a single polymer in the low-elongation region showed completely different behavior from that for solvent, which may reflect the viscoelastic characteristics of a single polymer. Such behavior in the low-elongation region could have never been observed in the simple elongation experiment.

The storage and the loss stiffness ($K'$, $K''$) of a single PS chain were calculated from the result of Figure 3 with equations:

$$K' = (Ak/A_0) \cos \delta$$

$$K'' = (Ak/A_0) \sin \delta,$$
where $k$ is the spring constant of a cantilever and $A_0$ is the input amplitude. Figure 4 shows $K'$ and $K''$ plotted against the elongation ratio $r$; $r$ is the normalized elongation by the full length of a polymer chain. In Figure 4, the tip was in contact with the substrate in $0.0 < r < 0.2$ and in the region of $r > 1$ a PS molecule had already been detached from the tip or the substrate. The storage stiffness $K'$ increased with the elongation of polymer chain, as usually observed in the simple elongation experiments. The simple theoretical estimation of the spring constant of a single polymer chain $k_{\text{chain}}$ in the random coil state can be performed with the following equation:

$$k_{\text{chain}} = \frac{3k_BT}{n^5a^2}$$

where $k_B$, $T$, $n$ and $a$ are Boltzmann constant, temperature, degree of polymerization and length of segment, respectively. We assumed the length of segment $a$ to be 0.56 nm and the chain length $L$ to be $L = 51$ nm [4], hence $n = L/a \sim 90$ and $k_{\text{chain}}$ resulted in $1.8 \times 10^{-4}$ N/m. The storage stiffness $K'$ of a single PS chain was $9.4 \times 10^{-4}$ N/m in the low-elongation region ($r \sim 0.5$), which is of the same order of theoretical estimation, and thereby implies our measurement was consistently performed.

The loss stiffness $K''$, which reflects the dissipative behavior was almost constant in the low- and the middle-elongation region ($0.2 < r < 0.6$) and dropped in the high-elongation region ($0.7 < r$). Such behavior in the low- and the middle-elongation region implies that there is some intra-molecular dissipation processes inside a single polymer chain. According to the literature [8], three forms of the internal friction are suggested: i) the friction between the monomer and the solvent molecule, ii) the friction arising from the energy barrier between the different conformations and iii) friction between the monomers constituting a polymer chain resulting from the loop or the entanglement of a single chain. Previously we reported that the monomer-solvent friction was observed in the frequency region of 10 kHz [9]. Therefore this type of friction seem not to be appropriate for the reason of the dissipative behavior observed in this experiment of 1-100 Hz region. Moreover, because trans-gauche conformational changes are well known as the phenomena of picosecond time scale, above ii) is eliminated from the reason of the internal friction. One possible candidate would be iii) monomer-monomer friction. This phenomenon is more likely observed in the low-elongation state where the spatial density of monomers are higher than in the high-elongation. Indeed, the values of $K''$ in the low-elongation are much larger than in the high-elongation.

4. Conclusion
To detect the dissipative phenomena of a single polymer chain we constructed the self-produced measurement system by modifying the commercial AFM system. The response of a single polymer chain to the small sinusoidal oscillation was successfully measured at the several points in the middle of elongation process. By the dynamic measurement the dissipative behavior was

\[ K', K'' \text{(N/m)} \]

**Figure 4.** The storage stiffness ($K'$) and the loss stiffness ($K''$) obtained from the dynamic measurement. The elongation ratio $r$ is the normalized elongation by the full length of a polymer chain. The points in the region of $r > 1$ represent the results of the measurement for solvent only (after the detachment of a molecule).
observed in the low-elongation region of a polymer chain. This phenomena implies the existence of monomer-monomer friction inside a single polymer chain.

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