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Dynamic Force Spectroscopy on a Single Polymer Chain

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ABSTRACT: A technique based on atomic force microscopy (AFM) for dynamic measurement of mechanical properties of a single polymer chain was proposed. AFM cantilever was mechanically oscillated at approximately 10 kHz during stretching process. By this technique, we could quantitatively estimate elongation-dependent changes of stiffness and viscosity of a single chain itself with using a phenomenological model. The solvent effect on the viscosity in low extension regions was investigated. It was ensured that the viscosity under 10 kHz perturbation was attributed to monomer−solvent friction. Viscosity quantization corresponding to consecutive detachments of polymer chains was also observed.

Introduction

The development of experimental instruments such as optical tweezers,[1] glass microneedles,[2] and atomic force microscope (AFM)[3] have enabled us to detect the forces acting on single molecules directly. As for AFM, if each end of a macromolecule is attached to a tip and a substrate by physisorption or chemisorption, we can deform, elongate, and break the molecule mechanically and measure the corresponding force. In the past decade or so, AFM-based mechanical stretching experiments on single biomolecules[4,5] and synthesized polymers,[6,7] so-called “single molecule force spectroscopy”, have been extensively performed by many researchers, and achieved great successes in clarification of the mechanical and structural properties. In our group, solvent effects on the statistical properties of single polymer chains were investigated by using this technique.[8] It was shown that the second virial coefficient around Θ states was determined at a single polymer basis, which was almost comparable with a simple Flory’s lattice model.[9]

From a practical standpoint, the viscoelasticity of polymers is one of the most important aspects. Therefore, vast array of studies have been carried out on viscoelasticity of bulk polymers, polymer melts, and polymer solutions to date. At the single molecular level, though the reports mentioned above have been focused on the elongation characteristics of a single polymer chain, little attention have been paid to the dissipative processes inside a molecule. To detect such dissipative processes as monomer−monomer friction due to the collision between several constituent monomers, monomer−solvent friction, etc.,[10] a dynamic force measurement as in macroscopic rheological studies beyond the simple stretching experiment is essential. Dynamic force measurements have another advantage that it is possible to detect the properties in low extension region, in which polymer chains form random coil and mechanical force caused by entropic elasticity is too small to detect by simple stretching experiment. The objective of our study is to elucidate not only elastic properties but dissipative process at the single molecular level by means of dynamic force measurement.

There have been several reports on AFM dynamic force measurement so far, for example, phase imaging in the tapping mode operation,[11] force modulation,[12] and magnetic cantilever modulation.[13,14] We have also reported a technique with piezo-drive at low frequency,[15] which was originally developed in the report on polymer blend films[16] then utilized in the study on a single protein molecule.[17] In the previous study,[15] we investigated the phenomena in low dynamic range of frequency region (0.1−100 Hz), and it was suggested that intramolecular interaction in a single polymer chain was observed in that frequency region. In this paper, we adopted another technique in which a cantilever is oscillated at its resonant frequency (around 10 kHz) and the oscillatory amplitude of the tip and phase shift between driving signal and tip response are measured, and thereby we intend to realize the application of modulation to a molecule at higher frequencies. The advantage of this method is that no special treatment for a cantilever is necessary and that we can easily apply modulation to a cantilever by the use of piezo-element cantilever drive on a cantilever mount. This technique was discussed by Anczykowski and co-workers[18] from a point of view of the interpretation of phase data in the tapping-mode operation. Its application to the dynamic force measurement of a single molecule has not ever been reported as far as authors know. By this technique, we could quantitatively estimate elongation-dependent changes of stiffness and viscosity of a single chain itself with a phenomenological model. The solvent effects on the viscosity in low extension regions were the main issue of this paper. It was ensured that the viscosity under 10 kHz perturbation was attributed to monomer−solvent friction. Viscosity quantization corresponding to consecutive detachment of polymer chains was also observed.

Experimental Section

To realize the stretching of a single polymer chain by AFM, each end of the chain was attached to a gold substrate and a gold-coated AFM tip via a gold−thiol bond. In this experiments, we used SH-terminated polystyrene (PS) as a sample. It was based on a living-polymerized COOH-terminated PS with $M_n = 93\ 800$, $M_w = 100\ 400$. The polydispersity was $M_n/M_w = 1.07$. The degree of

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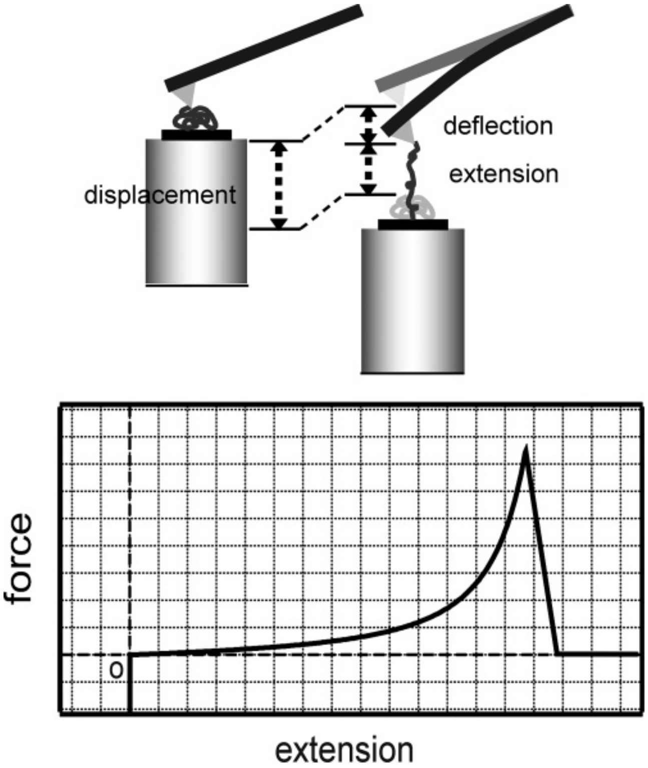

Figure 1. Principle of stretching a polymer chain, a force−extension curve.

polymerization was about 900 and thus its contour length was about 220 nm. The thiol groups were substituted for the COOH ends using 1,10-decane dithiol by means of thiolester bonding. Therefore, the end group became $C(=O)S(CH_2)_{10}SH$. The polyphosphate ester (PPE) was used as a condensation agent.[19,20] No dimer formation was indicated from the gel permeation chromatography (GPC) result. Au(111) surfaces on mica substrates were prepared as described.[15] The polymer was dissolved in a Θ solvent, cyclohexane, and a good solvent, toluene, at 20 μM/mL, and 10 μL of the solution was cast on a Au(111) substrate. After 5 min of incubation, the surface was rinsed with pure solvents.

The AFM (NanoScope IV, Veeco Instruments) used in the experiments was equipped with a fluid cell for measurements in liquid environment, a lock-in amplifier (LI 5640, NF Co., Japan) for the analysis of the detector signal of the AFM, and a heater accessory for temperature control (BioHeater, Veeco Instruments). To pick up the SH-modified terminal, a gold-coated cantilever, BL-RC150VB-C1 (Olympus Co. Ltd., Japan), was used. The spring constant of the cantilever was 0.0296 N/m. The spring constant was calibrated by thermal noise method[21] after the measurement.

The dynamic force measurement was based on force−distance curve measurement. The schematics of force−distance curve measurement were depicted in Figure 1. The force−extension curves were obtained directly from force−distance curves using the relationship that the *z*-piezo displacement was able to be divided into two quantities, cantilever deflection and polymer extension length. During the force−distance curve measurement, the cantilever was oscillated at its resonant frequency (8−10 kHz, in liquid). Through measurement, amplitude, frequency, and phase of the excitation signal were fixed at constant values. The speed of *z*-piezo scanner movement was kept constant at 200 nm/s. The signals, oscillatory amplitude and phase shift, were measured by a lock-in amplifier; the signal of the cantilever excitation was used as the reference signal. Low-pass filtered deflection was also measured simultaneously to determine the mean of stretching force. To examine the solvent effects, the measurement was performed in cyclohexane at temperatures of 30, 35, 40, and 45 °C and in toluene at a temperature of 35 °C.

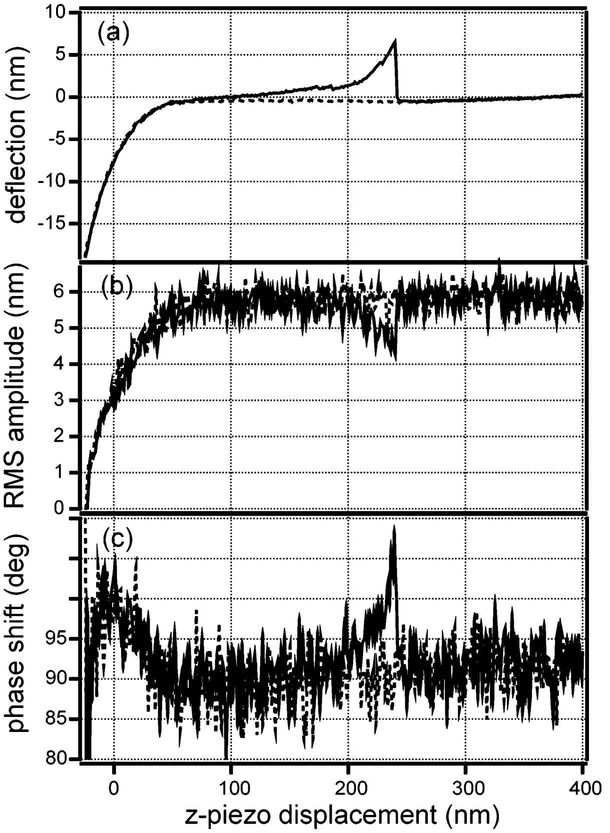

Figure 2. Behaviors of (a) deflection, (b) amplitude, and (c) phase shift of stretching experiment. The solid lines describe the behavior of the separating process, and the dashed lines describe that of the approaching process.

Results and Discussion

At the beginning of the experiment, we obtained the resonant frequency $\omega_1/2\pi = 9.03$ kHz and the quality factor $Q = 25$, from measurement of resonance curve in liquid environment. Figure 2 shows the typical behavior of deflection, RMS amplitude A_{rms} and phase shift θ signals against z-piezo displacement in Θ solvent (cyclohexane, 35 °C), where the drive frequency was fixed at the free oscillation resonance,[22] 9.03 kHz. In the free oscillation, $A_{\mathrm{rms}} = 6.0$ nm, and $\theta = 90°$. Although the tendency of deflection was almost similar to that without oscillation, i.e., a force−distance curve obtained in a conventional static experiment, there was a critical difference that a position of contact between the cantilever and the substrate was not clear. The deflection decreased gradually with the decrease in tip−sample distance and did not have any inflection point. It seemed impossible to detect the contact point from the deflection. There were presumably some repulsive forces affecting on the cantilever. However, it was difficult to specify the forces because this was a complicated situation, in which the expanded polymer chain fixed its one end on the substrate was deformed, and the deflection of the cantilever and the decrease in the oscillation amplitude were occurring simultaneously. This phenomenon was not observed from experiments in which the solvent was exchanged to ethanol, poor solvent (data not shown). Thus, this might be an inevitable phenomenon and be concerned in expanded polymer chain. In Figure 2b, though the amplitude also decreased gradually close to the contact point, it was able to detect the point where noise level decreased abruptly. The decrease in noise was also observed at the contact point by the experiment regardless of the situation with or without polymer chain adsorption in all the solvents including poor solvents. Therefore, we determined this point as the contact point and set the displacement as zero.

As the substrate was separated from the cantilever tip, the value of amplitude increased close to that in free oscillation then dropped again due to the tension of a polymer chain, whereas the value of phase shift decreased close to 90° then increased again (Figure 2c). The decrease in amplitude and the increase in phase shift continued until a bond rupture point, where the jump to the free oscillation was observed. If no molecule was attached to the tip, such behavior was not observed and the values of amplitude and phase shift were constant at those in free oscillation. Therefore, it was ensured that the changes of these values with separation were characteristic of stretching of a single polymer chain. Incidentally, the behavior did not show remarkable dependence on the speed of *z*-piezo scanner movement in this experimental condition.

To obtain the information about mechanical properties of a single polymer chain, we analyzed the data in Figure 2 on the basis of a phenomenological model depicted in Figure 3. Assuming sinusoidal drive of a cantilever base $z_d(t) = A_d e^{i\omega t}$ with drive amplitude A_d and drive frequency ω, the deflection of a cantilever as a sinusoidal response can be written as $z(t) = Ae^{i(\omega t+\theta)}$ with amplitude A and phase shift θ.[18] A cantilever is represented by an effective tip mass together with parallel-connected spring and dashpot, which are characterized by mass m, spring constant k_1, and damping coefficient η_1, respectively. The damping here is due to viscous drag by the surrounding medium and intrinsic damping caused by the deflection of the cantilever beam. A polymer chain is also described as a Voigt element, which has parallel-connected spring k_2 and dashpot η_2; the mass of a molecule is so small compared with the tip mass that it is ignored. The two parameters k_2 and η_2 are what we are interested in.

Analysis starts from the following equation of motion:

$$m\ddot{z}(t) + (\eta_1 + \eta_2)\dot{z}(t) + (k_1 + k_2)z(t) = k_1 z_d(t) \qquad (1)$$

The parameters η_1 and m are obtained from spring constant of the cantilever k_1, resonant frequency ω_1, and quality factor Q as follows:

$$m = \frac{k_1\sqrt{Q^2 - 1}}{Q\omega_1^{\,2}}, \quad \eta_1 = \frac{k_1}{Q\omega_1} \qquad (2)$$

Note that these values are determined in the cantilever tuning process; therefore, they are independent of the polymer chain stretching event. Though detailed calculation is omitted, k_2 and η_2 can be calculated from the solution of eq 1:

$$k_2 = \frac{A_1}{A}\{(-m\omega_1^{\,2} + k_1)\cos\Delta + \eta_1\omega_1\sin\Delta\} - (-m\omega_1^{\,2} + k_1) \qquad (3)$$

$$\eta_2 = \frac{A_1}{A\omega_1}\{(\eta_1\omega_1\cos\Delta - (-m\omega_1^{\,2} + k_1)\sin\Delta\} - \eta_1 \qquad (4)$$

where A_1 is the oscillation amplitude without polymer chain and Δ is the difference in phase shift with (θ) and without (θ_1) polymer chain ($\Delta = \theta_1 - \theta$). Amplitude and phase shift in approaching process are regarded as A_1 and θ_1.

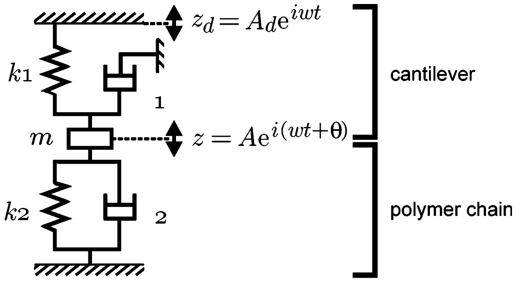

Figure 3. Rheological model applied to this experimental system, which is comprised of two Voigt elements. One is the cantilever and the other is the polymer molecule. Here a linear response to the input drive signal is assumed for the tip motion.

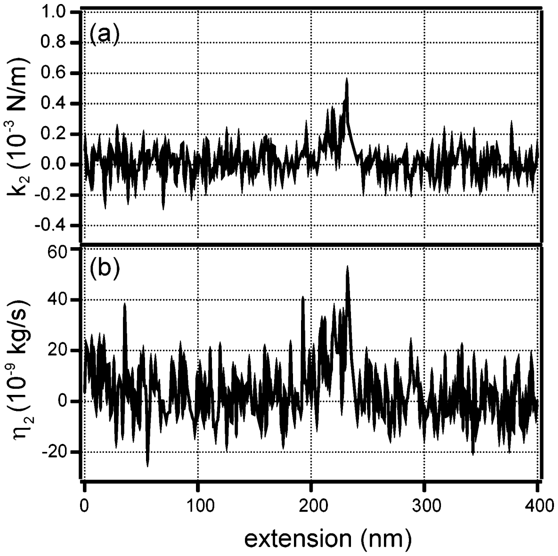

Figure 4. Behavior of (a) chain stiffness, k_2, and (b) the viscosity of the chain, η_2 against the extension. The values, k_2 and η_2 were calculated by eqs 3, 4 using the values in Figure 2b,c.

The data of amplitude and phase shift in Figure 2b,c were substituted for A and Δ in eqs 3 and 4, respectively, then replotted against the extension of polymer chain in Figure 4a,b. The stiffness of a single chain k_2 increased abruptly with stretching, as commonly observed in conventional simple stretching experiments on polymers. The average value of k_2 in low extension region was 2.86×10^{-5} N/m. To examine the relevance of the elongation dependence and the value itself, the corresponding force−extension curve obtained from Figure 2a was used to calculate the elasticity as shown in Figure 5. The force−extension curve in Figure 5a gave a nice fit (gray solid line) against wormlike chain (WLC) model as previously reported,[8] and we obtained the contour length of 271.7 nm and the persistence length of 0.262 nm.[9,23,24] The entropic elasticity obtained from the initial slope of fitting curve was 8.93×10^{-5} N/m, whose order was slightly larger, however, comparable to the value of k_2. The elasticity in Figure 5b was calculated as the gradient of each point averaged over neighboring 11 points of Figure 5a. The elasticity represents almost same elongation dependence with k_2 in Figure 4a, i.e., the elasticity possess a constant value in low extension region and increase abruptly in high extension region starting from the extension length of about 200 nm. The strange behavior in low extension region (0−50 nm) was possibly due to the fact that the detection of small force was difficult. The similar situation is frequently observed in simple static stretching experiments. Here comes the advantage of dynamic force measurements where the information on elasticity is not directly obtained from the force but from the perturbed motion of cantilever. One might put a question about the difference in the value of elasticity in Figure 4a and Figure 5b. We do not have any clear answer at this moment, while it would be related to the difficulty in determining quality factor

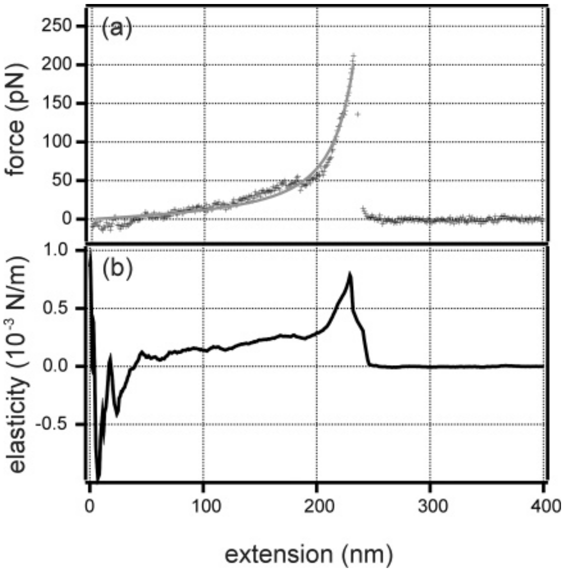

Figure 5. Behavior of (a) external force and (b) elasticity against the extension. Curve a is directly obtained from Figure 2a. The gray solid line in part a represents the fitting curve with WLC model. The elasticity was estimated by calculating the gradient of curve a.

Q. The *Q* value was experimentally obtained at the free oscillation condition in the absence of polymer chains. The change in Q has great influences on the determination of *m* and η_1 and therefore k_2 and η_2. Further studies are necessary to elucidate the point.

The viscosity of a chain η_2 also increased in same manner as k_2. This behavior exhibited a striking contrast to the previous report on low-frequency measurement (0.1−100 Hz).[15] In the previous report, the existence of viscosity in the low extension region due to the monomer−monomer friction was indicated. It looks somewhat strange that viscosity increased though the chain is stretched and thereby it becomes rigid and rodlike shaped. Naturally, in the stretched state, the probability of collision between monomers is much smaller than that in low extension state. Therefore, monomer−monomer friction is excluded from the reasons of the viscosity increase observed in Figure 4b. One speculation is that such viscosity is caused by monomer−solvent friction or some hydrodynamic interaction. At a glance, there observed almost zero viscosity in the low extension region, however, viscosity had a certain value in this region. From Figure 4b, the viscosity η_2 in the region (30−150 nm) averaged out at 2.62×10^{-9} kg/s. It is noted that the value is the first estimation of viscosity at a single polymer chain basis in low extension region. Since the value was obtained for low extension region, it must be compared with results of general measurements such as light scattering, osmotic pressure methods in which the results reflect the averaged properties from a large amount of polymer chains. To verify the above-mentioned speculation, the solvent dependence of the viscosity was compared with that of general measurements.

A coefficient of the viscosity-molecular weight relationship was chosen as the property for comparison

$$[\eta] = KM^{a} \qquad (5)$$

where $[\eta]$ and *M* are intrinsic viscosity and molecular weight, respectively. *K* and α are the constants depending on polymer species, solvent qualities or temperatures.[9] Although the unit of $[\eta]$ and of η_2 are different, we consider the origin of both parameters are same. It is because that both parameters represent the viscosity in infinite dilute polymer solution. Thus, we assumed the existence of a constant *K′* satisfying the relation,

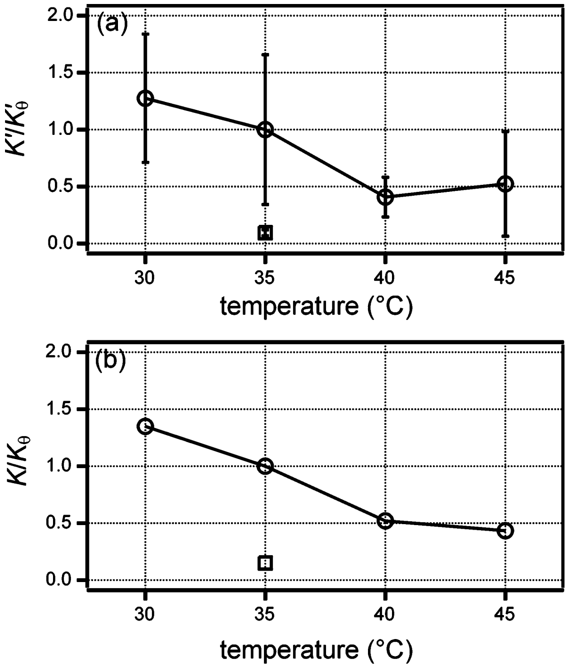

Figure 6. Solvent dependence of the viscosity constant (a) *K′* and (b) *K*. Both constants are divided by those in Θ solvent for scaling. Open circle and open square represent results in cyclohexane and toluene, respectively. The α values used for the calculation are 0.48 (30 °C), 0.50 (35 °C), 0.55 (40 °C), and 0.58 (45 °C) for cyclohexane and 0.71 (35 °C) for toluene.

$$\eta_2 = K'M^{a} \qquad (6)$$

To compute the constant *K′* from the experimental data, molecular weight *M* was calculated from the contour length which was obtained by WLC fitting against force−extension curve, and a constant α was determined by reference data.[25] The solvent dependence of the viscosity constant *K′* and corresponding reference data *K* are shown in Figure 6. The viscosity constants *K′* and *K* are divided by those in Θ solvent K'_θ and K_θ for scaling, respectively. Experimental and reference data show good agreement to each other in both solvent temperature and solvent type by comparing parts a and b of Figure 6. This agreement supports our idea that the viscosity measured here depends on solvent conditions clearly as well as intrinsic viscosity. Taking also into account the viscosity increase at high extension region, we can conclude that the viscosity under 10 kHz perturbation is attributed to monomer−solvent friction. We believe that our experimental results, at least in the low extension region, does not contradict with the assumption of free-draining molecule,[26] which is the first approximation of polymer chains interacting with solvent molecules. However, the increase in viscosity at higher extension region cannot be explained by this simple assumption. Further studies with the improved signal-to-noise ratio are necessary to elucidate the point including the comparison with more sophisticated theories.[10,27,28] We also have to consider the complex nature of hydrodynamic flow between tip and substrate.[29] Such complicated flow might finally cause the change in η_2 value. Thus, our analysis in this time must be treated as the first approximation. The investigation on molecular weight dependence will also help to check the validity of our analysis. Such experiments are now under way.

It is important to elucidate the meanings of experimental data by the comparison with general measurements. However, the

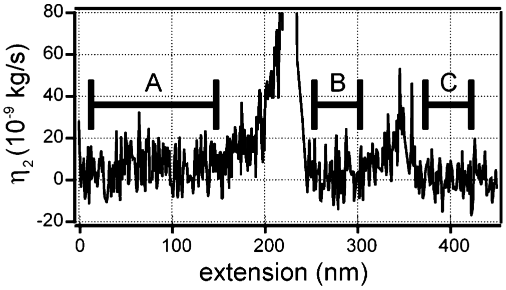

Figure 7. Behavior of viscosity concerned with consecutive detachments of two polymer chains.

true advantage of the performed measurement lies on the results those are obtained only by dealing with single polymer chains. Figure 7 shows a deeply interesting phenomenon of "viscosity quantization" which was revealed in simultaneous stretching of two polymer chains. There were two consecutive detachments of polymer chains at extensions of 230 and 350 nm. The mean value of viscosity in regions A, B and C were 7.07×10^{-9}, 3.45×10^{-9}, and 0.01×10^{-9} kg/s, respectively. The value in region A, stretching two polymers, was two times larger than that in region B, stretching one polymer after the first detachment; and that in region C, after the second detachment, was so small that it could be regarded as noise. Thus, the value of viscosity quantally increased with the increase of the number of polymer chains. This phenomenon clarified that the measurement captured viscosity at single polymer chain basis. Then, it had possibility of being close on the origin of polymer solution viscosity.

Conclusion

We performed dynamic force measurement on a single polymer chain at the frequency region around 10 kHz by applying oscillation to the cantilever at its resonant frequency in liquid environment. The quantitative estimation of elongation-dependent stiffness and viscosity of a single polymer chain was enabled by the data analysis based on a phenomenological model. Since the viscosity increased with the extension of a polymer chain and showed the solvent dependence clearly, it was ensured that the viscosity was caused by monomer−solvent friction.

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