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Two-dimensional polymers investigated by scanning near-field optical microscopy: Conformation of single polymer chain in monolayer

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Abstract

The conformation of single polymer chain restricted in a two-dimensional plane was studied by scanning near-field optical microscopy (SNOM). The single dye-labeled chain in a monolayer of poly(isobutyl methacrylate) (PiBMA) with a high molecular weight of ca. 5×10^6 was directly observed by SNOM as a circular shape with a diameter of 200–300 nm. The observed chain size was considerably smaller than that for a self-avoiding random walk chain generated by computer simulation, indicating that the polymer chain takes a contracted conformation in two dimensions. SNOM imaging was carried out for the labeled PiBMA chain dispersed in the matrix monolayer with 1000-times lower molecular weight. The long chain isolated among shorter chains had an expanded form as large as a random conformation. These results were in good agreement with the scaling theory, providing an unambiguous evidence for the first time with the real image for a single chain conformation by SNOM.

Keywords: Single polymer chain; Scanning near-field optical microscopy; Monolayer

1. Introduction

Polymers in restricted dimensions have attracted much attention from the fundamental and applied points of view. Since the entropy of individual polymer chain plays an important role to determine the bulk properties of polymer materials, the polymer ultra-thin film shows different physical and thermodynamic properties from those for the three dimensional bulk. Many researchers have been interested in fundamental aspects of polymer chains in restricted dimensions and have studied the various properties of polymer ultra-thin films [1–7]. So far the chain conformation in thin films prepared by spin-coating and water casting methods was experimentally evaluated by neutron and X-ray scattering [2–4], and it was indicated that the decrease of the film thickness resulted in the low inter-chain entanglement in confined geometry. For an infinitely thin polymer film, which has a thickness of the size of the monomer unit in a realistic system, the intra- and intermoleculer crossover is ruled out. Therefore, the polymer chain has no degree of freedom in z-direction and the physical properties of two-dimensional polymers would be drastically different from those in a three dimensional bulk state. In the previous studies, however, the chain conformation have been discussed for the film samples with a finite thickness on the order of 10 nm, where the crossover of the chain contour was still allowed.

With regard to the two-dimensionally constrained polymers, Maier and Rädler reported the fluorescence microscopy observation of single λ-pharge DNA chains [8,9], where the overlap of the chain segments was prevented by the tight adsorption onto a cationic lipid layer. They indicated that the DNA chain contour was well described by two-dimensional self-avoiding walks in a dilute condition. Since in the case of DNA we have to consider complicated interactions such as the electrostatic interaction among the monomer units and the effect of counter ions, an experimental study on a non-electrolyte polymer in two dimensions would be helpful to understand the insight of the properties of confined polymers. Kumaki et al. observed the conformation of single poly(methyl methacylate) chains deposited on a substrate in an

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extremely dilute condition and indicated the conformational rearrangement on a mica surface assisted by adsorbed water [10,11].

The current study focuses on the conformation of a single polymer chain in a two-dimensional bulk state, that is, an individual chain surrounding the other chains in a monolayer, whereas in the previous studies the morphology and dynamics of the polymer chain at the single molecule level have been discussed for the individual polymer chain isolated from the other chains at an extremely low concentration condition. The static and dynamic properties of the polymer monolayers have been studied using various methods so far [12–15]. We previously reported the characteristic properties of the polymer monolayers from the surface viscometry and fluorescence techniques [14–17], implying that the polymer chain restricted in two dimensions takes a contracted pancake-like conformation as predicted by the scaling theory [18]. The direct observation of the individual chains in a polymer monolayer bulk is indispensable for the understanding of the previous results based on ensemble average measurements. In the discussion on the polymer chain conformation, the single chain observation directly provides much information on the molecular weight and conformational distribution of individual macromolecules. The development of scanned probe microscopy techniques such as atomic force microscopy (AFM) enables one to see the contour of a single polymer chain [10,11,19,20]. The isolated individual chains have been clearly detected as a ridge with height of the monomer unit (a few angstroms) on an atomically flat substrate, because AFM maps the height difference on the sample surface. However, considering such an imaging mechanism, AFM is not easily applicable to the single chains embedded in a matrix, i.e. the individual polymer chains consisting of a homogeneous monolayer. On the other hand, fluorescence labeling is a promising method to visualize individual macromolecules in a bulk by the selective introduction of a fluorescent moiety into the target molecule [8,9]. However, the spatial resolution of light microscopy is limited to a half of the wavelength by the diffraction limit. Therefore, except for huge biopolymers such as DNA the conventional fluorescence techniques could not be applied to the direct imaging of the individual macromolecule. In this study, we employed the scanning near-field optical microscopy (SNOM) and discussed the conformation of the polymer chain in the monolayer by the single chain detection. SNOM is an emerging scanning probe technique [21–23], enabling the optical measurement with a high spatial resolution by local illumination with the near-field 'light' emanating from a nanometric aperture. Thus spectroscopic information from a local area much smaller than the wavelength is available by SNOM, which allows one to obtain a micrograph in chemical contrast [24–27]. Therefore, SNOM is one of the most suitable methods for observing the structure of polymer chains in a monolayer at a single molecule level. In this study, poly(isobutyl methacrylate) (PiBMA) was used as a sample polymer, which is known to form a stable monolayer on water. Because of the amphiphilicity of the ester group in the methacrylate side chain, each monomer unit is strongly adsorbed at the air/water interface; consequently, the main chain is restricted on a water surface. Therefore, the monolayer of PiBMA is estimated to be a good model of a two-dimensional polymer having no degree of freedom in the height direction. A small amount of polymer chains forming a monolayer was modified to emit fluorescence, which was selectively observed by the fluorescence mapping by SNOM. The direct observation of single macromolecules was demonstrated by SNOM for a monolayer of PiBMA, and the conformation of the polymer chain restricted in two dimensions is discussed.

2. Experiments

Perylene-labeled PiBMA (Fig. 1) was dispersed in a monolayer of unlabeled PiBMA in a dilute condition to observe the individual labeled chain by the SNOM imaging in the illumination mode. The perylene-labeled polymer was prepared by the radical copolymerization of isobutyl methacrylate and 3-perylenylmethyl methacrylate [16,17]. The fraction of the introduced labels was about 1%. The synthesized polymer was fractionated by size-exclusion chromatography (SEC) to have a narrow molecular weight distribution. The molecular weight was determined by SEC calibrated by polystyrene standards (Tosoh) and converted to the molecular weight for PiBMA using the viscosity parameters for PiBMA and polystyrene in the eluent used for the SEC measurement (THF) [28]. The weight average molecular weight, M_w, and the molecular weight distribution, M_w/M_n, were 3.36×10^6 and 1.25, respectively, where M_n is the number average molecular weight. The PiBMA monolayer was prepared by the Langmuir–Blodgett technique. A mixed benzene solution of the unlabeled PiBMA and a small amount of the labeled polymer at a total polymer concentration of 0.1 g l^{-1} was spread on pure water at 20 °C. Surface pressure was measured by a Wilhelmy plate. The monolayer compression was done at a rate of 10 mm min^{-1}. Annealing to reach equilibrium was carried out by raising the water subphase temperature to 40 °C and keeping it constant for a given duration. The annealing was carried out in a constant area condition without surface pressure control. Pure water was supplied to the trough to compensate the water evaporation. After cooling the subphase temperature to 20 °C the monolayer was transferred onto a glass cover slip at a surface pressure of 5 mN m^{-1}.

The details on the SNOM system used are described elsewhere [17]. The optical fiber probe was made in-house and its spatial resolution was confirmed to be $\leq$100 nm from the fluorescence image obtained for fluorescent latex beads with a diameter of 26 nm. The probe-sample distance

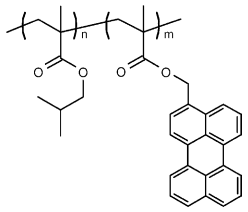

Fig. 1. Chemical structure of perylene-labeled poly(isobutyl methacrylate).

was regulated to be several nanometers by a shear force feed-back system. The piezo-actuators for the sample scanning and the probe height control were calibrated with a grating scale with a period of 3 μm and a step height of 20 nm (NT-MDT). The specimen was excited at a wavelength of 442 nm, and the perylene fluorescence at 460–550 nm was collected by a high NA objective (1.3 NA, 100×, Nikon) from the backside of the substrate and guided to a photomultiplier (R4220P, Hamamatsu Photonics). The SNOM measurement was carried out in an ambient condition.

3. Results and discussion

The fluorescence SNOM images are shown in Fig. 2. The labeled chains were dispersed in the unlabeled PiBMA with M_w of 1.76×10^6. The perylene-labeled PiBMA chains were observed as circular fluorescent spots in the fluorescence images. The comparison between Fig. 2(a) and (b) indicates that the number of spots increased with the increase of the fraction of the labeled polymer. The force feed-back images obtained simultaneously showed no topographical feature.

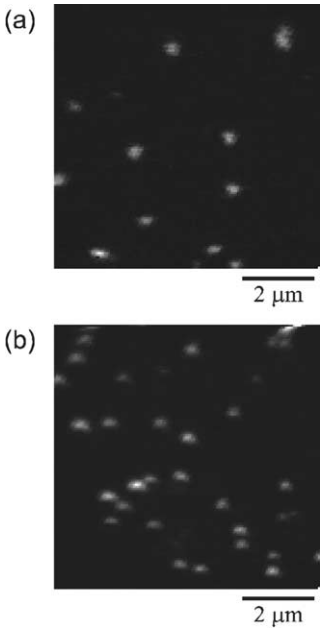

Fig. 2. Fluorescence SNOM images of single PiBMA chains. The perylene-labeled PiBMA was dispersed in the unlabeled polymer at concentrations of 0.10 (a) and 0.25% (b).

The thickness of the PiBMA monolayer was estimated to be 1.0 nm from the height difference between the monolayer and the substrate surfaces exposed by scratching a part of the film. The film thickness was in good agreement with the length of the hydrophobic isobutyl group [29], indicating that the PiBMA formed a uniform monolayer and the main chain was confined on a solid substrate. First we have to consider whether each fluorescence spot in Fig. 2 corresponds to a single labeled PiBMA chain or not. In experiments of the single dye molecule detection [30–32], the blinking of the fluorescence emission and the abrupt photobleaching are observed, which are characteristic to the emission from a single molecule. However, because a few hundred dye molecules were introduced to a single PiBMA chain in our experiment, the quantized emission behavior could not be observed. Therefore, in order to confirm the single polymer chain observation, statistical analysis was carried out for the fluorescence spots measured in a few tens of the SNOM images. The area density of the fluorescence spot was quantitatively discussed as follows. In preparation of the sample films, the PiBMA monolayer on a water surface was deposited at a surface pressure of 5 mN m^{-1}. At this point, the area occupied by a monomer unit, A, was estimated to be 0.27 nm^2 from the surface pressure-area isotherm. Therefore, the area density of the labeled PiBMA chain, σ_{calcd}, is given by the following equation.

$$\sigma_{calcd} = \frac{c}{(ADP)} \tag{1}$$

where DP and c are the degree of polymerization and the unit area fraction of labeled polymer in the monolayer, respectively. DP was calculated to be 18,900 from the molecular weight. Fig. 3 indicates the relationship between the fraction of the labeled polymer and the area density of the observed bright spot. The observed density was in quantitative agreement with the calculated value, indicating that each fluorescent spot in the SNOM image corresponded to a single PiBMA chain.

The SNOM image shown in Fig. 2 indicates a large

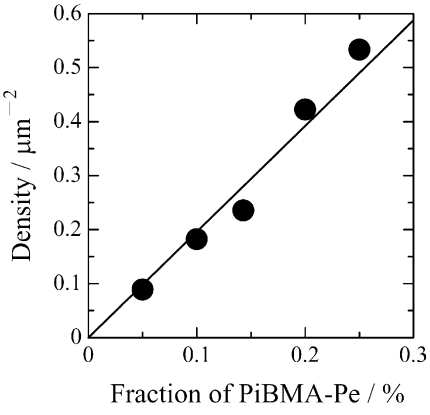

Fig. 3. Relationship of the observed and calculated area densities of the labeled PiBMA chains in the monolayer. The closed circles and solid line indicate the observed and calculated value.

variation in the fluorescence intensity of each spot. This is due to the molecular weight distribution of the sample polymer used. Because the perylene group was introduced into the PiBMA chain by random copolymerization, it can be said that the number of dye moieties in a labeled chain is proportional to the chain length, i.e. molecular weight. Moreover, the interaction among the perylene moieties introduced to the labeled chain was negligible due to the low concentration. Therefore, the fluorescence intensity, I_i, from the ith polymer chain with a molecular weight of M_i is given by the following equation.

$$I_i = aM_i \tag{2}$$

where a is a constant depending on the experimental condition: the throughput of the probe, the excitation light power, and the signal collection efficiency. The molecular weight distribution of the labeled PiBMA was observed by SEC. Now, because the SEC trace is given by $c(\log M)$, the fluorescence intensity histogram can be fitted to the molecular weight distribution curve by $c(\log I - \log a)$, where I is the fluorescence intensity and $\log a$ is a shift factor. Fig. 4 depicts the histogram of the fluorescence intensity for the single chains and the molecular weight distribution measured by SEC. Both were well fitted by shifting the intensity histogram with a factor of $\log a = 1.72$. This result indicates that the bright circular spot observed in the SNOM image corresponds to the individual PiBMA chains and the molecular weight of the single chains can be estimated from the emission intensity.

A single PiBMA chain was observed as a tiny fluorescence spot in the SNOM image, the size of which slightly increased with the molecular weight. Most of the chains appeared as an almost circular shape, whereas a small number of chains showed slightly distorted forms due to the conformational dispersion. The sample monolayers were transferred onto glass substrates from the water surface by dipping substrates vertically and horizontally to the Langmuir trough. In both cases, the difference in the shape of the observed fluorescence spot could not be seen. This indicates that the film transfer process from the water surface onto the substrate had negligible influence on the conformation of the PiBMA chain in the monolayer. Fig. 5 indicates an example of the fluorescence intensity line profile for the polymer chain with a molecular weight of 4.8×10^6 (DP = 33,800), which was determined by the comparison of the fluorescence intensity histogram with the SEC chromatogram as described in the above section. The observed size of the chain was 220 nm. This value was quite small considering the contour length of this chain, which was estimated to be >8.4 μm. In order to discuss the chain expansion in the monolayer, we considered a completely shrunken pancake-like form and a randomly distributed chain conformation in two dimensions generated by a self-avoiding walk algorithm. For the numerical calculation of the self-avoiding walk, a polymer chain was regarded as sequentially linked hard circular disks with the same area as the occupation area for the monomer unit evaluated experimentally from the Π-A isotherm (0.27 nm^2), and each disk was inhibited to overlap with another. However, it was quite difficult to generate a self-avoiding random walk chain for large DP in two dimensions because the crossing of the chain contours was forbidden due to no degree of freedom in the height direction. Therefore, we first examined the scaling relationship between DP and the root-mean-squared radius of gyration, R_g, given by the following equation.

$$\langle R_g^2 \rangle^{1/2} = KM^{-\alpha} \tag{3}$$

where K and α are a constant and a scaling factor, respectively. From the calculation in a wide DP range from 20 to 10,000, we obtained the scaling factor of $\alpha = 0.68 \pm 0.01$. In order to illustrate the chain with a molecular weight of 4.8×10^6, a 10,000mer was generated and extrapolated to the chain size corresponding to a 33,800mer by linear scaling using the factor obtained

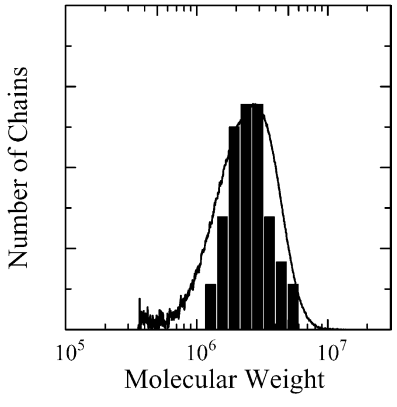

Fig. 4. Molecular weight distribution for the perylene-labeled PiBMA and histogram of the number of spots as a function of fluorescence intensity. The molecular weight distribution curve was measured by SEC and the ordinate is scaled in molar concentration. The fluorescence intensity histogram was fitted to the SEC curve by a shift factor of $\log a = 1.72$, where a is a proportionality factor between the molecular weight and the fluorescence intensity.

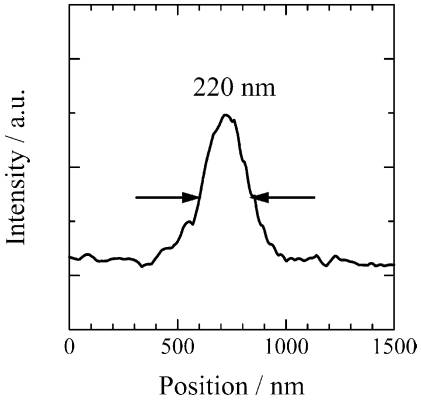

Fig. 5. Fluorescence intensity profile for a single PiBMA chain with a molecular weight of 4.8×10^6.

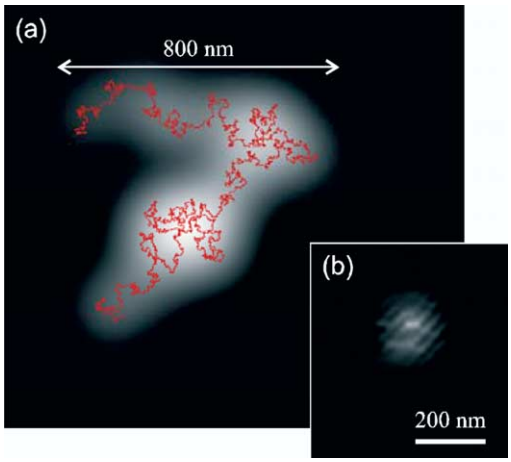

Fig. 6. Snapshot of a self-avoiding random walk chain with a degree of polymerization of 33,800 generated by computer simulation in two dimensions (a) and the real SNOM image of the single 33800mer of PiBMA (b). The red contour and the gray-scale background image indicate the chain contour and its calculated SNOM image.

above. Fig. 6 shows a snapshot of the chain conformation generated by the self-avoiding random walk with the most probable radius of gyration and the observed real SNOM image of a single PiBMA chain with a molecular weight of 4.8×10^6. The red trace and the gray-scaled background image in Fig. 6(a) depict the generated chain contour and the calculated SNOM image as the convolution of the chain segments and the near-field distribution. A polymer chain with a random conformation in a two-dimensional plane would take an expanded form as large as 1 μm. On the other hand, assuming the completely close-packed conformation in a circular shape, the diameter would be 120 nm. This indicates that the observed chain size of 220 nm was clearly smaller than the self-avoiding chain in two dimensions and that the polymer chain in a monolayer has an almost contracted conformation like a pancake. No change in the individual chain conformation could be seen for the monolayer samples annealed for more than 10 h, indicating that the contracted form is an equilibrium conformation for the two-dimensional polymer chain. Thus the conformation of the single polymer chain restricted in two dimensions could be directly shown through the observation by SNOM.

The molecular weight dependence of the chain expansion size in two dimensions is discussed. In obtaining the SNOM images of the single chains to discuss their conformations, the quality of the probe was carefully checked and the measurement was carried out using the same probe. It should be noted that the obtained SNOM image of an individual polymer chain was broadened by the finite spatial resolution of the SNOM probe (∼90 nm). As indicated above, since the polymer chain takes a contracted form, we can model the chain conformation into a disk form. The observed chain size was evaluated as the convolution of the disk-shaped conformation and the near-field distribution on the assumption of a Gaussian distribution for the optical near-field intensity from the SNOM probe with a full width at a half maximum of 90 nm. The actual polymer chain dimension was estimated by the comparison between the diameters of the observed and convoluted images. Fig. 7 shows the logarithmic plot of the chain size estimated by the above procedure against the molecular weight. The chain diameter was roughly proportional to the power of molecular weight with the scaling factor of 0.5, which is expected for a completely aggregated chain in two dimensions. Also this indicates that the chain has a contracted conformation. This experimental result obtained in this study directly supports the validity of de Gennes' picture, which predicted that the polymer chain in a two-dimensional bulk was strongly segregated [18]. The molecular conformation of the polymer chain in two dimensions results in the macroscopic physical properties characteristic to the mononolayer. We previously showed that the surface viscosity of polymer monlayers was linearly dependent on the molecular weight up to 10^6 [14]. Such the weak dependence of the surface viscosity is because there exists few entanglements among the polymer chains in the contracted conformation, which was revealed by the SNOM imaging in this study.

The above discussion was made on the homogeneous system, namely, where the degree of polymerization for the probed chain was the same as that of the matrix chain. The SNOM measurement was carried out for the labeled PiBMA chains dispersed in a polymer monolayer with a molecular weight of 2900, which was approximately 1000 times smaller than that of the labeled polymer. At first we observed the monolayer prepared without annealing, but no difference was seen between the shapes of the chains embedded in high and low molecular weight matrices (data is not shown here). This indicates that the long probed chain was shrunken in the monolayer at the moment immediately after the spreading on the water subphase. Fig. 8(a) shows the SNOM image of the single long PiBMA chains in the low molecular weight PiBMA monolayer matrix annealed

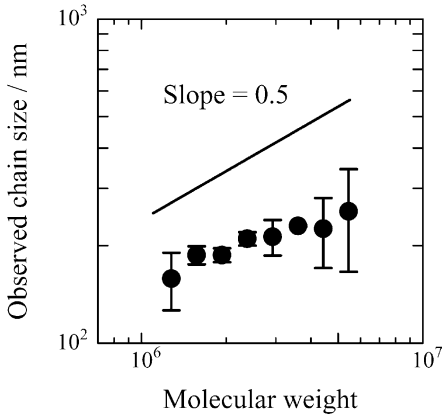

Fig. 7. Molecular weight dependence of the chain size. The size of the chain dimension was estimated by the deconvolution with the near-field intensity profile assuming the Gaussain distribution.

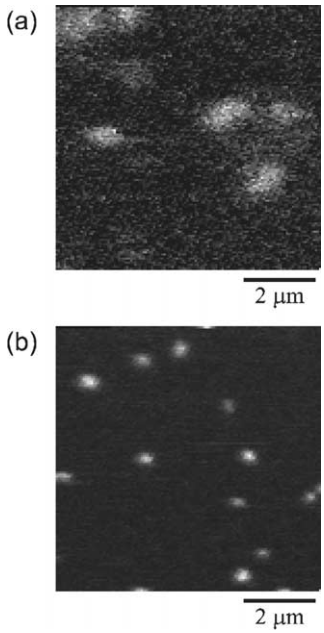

Fig. 8. Fluorescence SNOM images of single PiBMA chains dispersed in the unlabeled PiBMA matrices with low molecular weight (a) and high molecular weight (b). The molecular weight of the unlabeled polymers for the short and long chain matrices was 2.90×10^3 and 1.76×10^6, respectively. The molayers were annealed for 6 h on the water surface prior to deposition.

for 6 h. The probed chains were observed as broaden fluorescence spots on the order of 1 μm, whereas the recognizable conformational change was not observed after annealing for the PiBMA chains in the high molecular weight matrix as shown in Fig. 8(b). This result indicates that a long polymer chain among short chains expands from a contracted form to a random conformation as large as the self-avoiding walk model. This is explained as follows. The polymer was spread dropwise on water from a dilute solution. Consequently, each polymer chain was isolated and the chain segments were intramolecularly in a condensed state on the water surface [11]. Such isolated chains were gathered to form a homogeneous Langmuir film. Hence in the initial state the polymer chains in a monolayer appeared contracted. However, this chain conformation is probably in a non-equilibrium state when surrounded by low molecular weight polymers. The temperature elevation activates the conformational relaxation to reach the equilibrium, resulting in an expanded chain conformation. This behavior is in qualitative agreement with the scaling theory, which predicts that a long polymer chain swells in a matrix of shorter chains. In this experiment, the molecular weight of the labeled PiBMA chain was 1000 times larger than that of the matrix polymer. The long probe chain was expanded due to the two-dimensional dilution by the matrix polymers.

Fig. 9 illustrates the conformation of the long macromolecules in two dimensions embedded in high and low molecular weight matrices. Considering the conformational

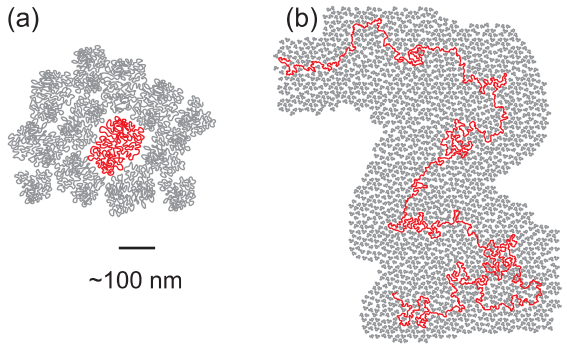

Fig. 9. Schematic illustration of the conformations of the long macromolecules in two dimensions embedded in high (a) and low (b) molecular weight matrices. The probed chains are shown in red.

entropy for a single polymer chain, the contracted form is unfavorable compared to a randomly expanded conformation. However, the expanded form results in a large 'free area' inside of the chain trail. This two-dimensional void is energetically unstable and must be occupied by intra-chain segments or by penetration of the other chain segments. In the latter case, this would require the entanglement between the probe and matrix chains in the case that the molecular weight of both polymers is the same. As mentioned above, in the two-dimensional system, the crossover of the chain contours is not allowed due to the absence of the degree of freedom in the height direction. It is difficult to form the entanglements among the chains in a monolayer. Therefore, all polymer chains take contracted conformations. On the other hand, in the case that the molecular weight of the matrix chain is far smaller than the probed chain, the matrix chains can easily occupy the void area of the expanded probe chain without entanglement and crossover of the chain contours. In other words, the short chains behave as small particles (solvent) compatible with a long probe chain. This system can be said to be a 'two-dimensional' polymer solution in a good solvent condition, resulting in the expanded conformation of the long probe chain as large as the self-avoiding random walk conformation.

4. Conclusion

Conformation of the polymer chains restricted in two dimensions was studied by scanning near-field optical microscopy (SNOM). Monolayers of poly(isobutylmethacrylate) (PiBMA) was prepared by the Langmuir–Blodgett technique, in which a small amount of the dye-labeled chains were dispersed. The conformation of an individual macromolecule in a two-dimensional bulk was discussed through the single macromolecule observation by SNOM. In a homogeneous monolayer, the PiBMA chain with a molecular weight on the order of millions appeared as a circular spot with a diameter of a few hundred nanometers, indicating that the polymer chain has a contracted pancake-like conformation. On the other hand, a conformation as

large as a self-avoiding random walk chain was observed when the labeled PiBMA chain was embedded in the lower molecular weight polymer matrix. The direct observation of the individual polymer chain indicates the validity of the scaling theory for the estimation of the single chain morphology in two dimensions. The method of SNOM is being improved for the spatial resolution and the detection sensitivity, and the high resolution optical detection of single macromolecules will provide further insight into the polymer science in restricted and/or nanometric dimensions.

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