Chemical Physics 419 (2013) 54–58

Contents lists available at SciVerse ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys

Quantitative analysis of end-to-end distance of single polymer chain in ultra-thin film by super-resolution fluorescence imaging

Hiroyuki Aoki [a,b,*], Kazuki Mori [a], Toshiaki Takahashi [a], Shinzaburo Ito [a,b]

[a] Department of Polymer Chemistry, Kyoto University, Nishikyo, Kyoto 615-8510, Japan
[b] Advanced Biomedical Engineering Research Unit, Kyoto University, Nishikyo, Kyoto 615-8510, Japan

ARTICLE INFO

Article history:
Available online 27 December 2012

Keywords:
Single polymer chain
End-to-end distance
Super-resolution
Thin film
Single molecule detection
Fluorescence microscopy

ABSTRACT

The end-to-end distance, *R*, of individual poly(methyl methacrylate) (PMMA) chains was evaluated for the individual chains by the super-resolution fluorescence microscopy, which determines the positions of the dye molecules introduced at both ends of a PMMA chain. The observed value of *R* was in good agreement with that reported by SANS experiments, and the distribution function was well fitted to the theoretical model of the random walk chain. The accuracy to determine the root of mean-square end-to-end distance is on the order of 1 nm. The distribution function of *R* obtained for the single PMMA chain in an ultra-thin film was almost the same as that in a bulk state. This indicates that the chain dimension in the ultra-thin was not significantly altered in the direction normal to the spatial confinement.

1. Introduction

The conformation of a single macromolecule plays important roles on various macroscopic properties and functions of materials. For example, the conformational dynamics of entangled polymer chains has great influence on the viscoelastic properties of polymer materials, and the conformational change of biomacromolecules such as protein and DNA is related to various functions in biological systems. Therefore, the conformation of polymer chains has been extensively studied in theoretical and experimental points of view. In recent years, the morphology and dynamics of the polymer chain confined in an ultra-thin film has attracted considerable attention because the properties of an ultra-thin film are different from those in a bulk state [1–8]. The restricted conformation and mobility alter the thermal and mechanical properties. The investigation of the chain conformation in an ultra-thin film is important from the viewpoints of not only the fundamental understanding of chemical physics of polymers but also the application of polymer thin films. Previously it was shown that the chain dimension in the normal direction to the confinement was not altered from the bulk state by neutron scattering [9,10]. Scattering methods measure the ensemble-averaged information for numerous chains in the system; therefore, the detailed information on the distribution function of the chain dimension for individual chains is not available. On the other hand, the direct imaging with microscopy techniques with nanometric spatial resolution would directly visualize the conformation of single chains [11–13].

The end-to-end distance of a polymer chain is a measure commonly used to discuss the chain dimension. A polymer chain in a realistic bulk system takes a random coil conformation in three dimensions and entangled with the surrounding chains. In order to observe the polymer chain embedded in a bulk medium, the fluorescence labeling technique is a most powerful method. For the studies on the end-to-end distance, the Förster resonant energy transfer (FRET) method has been employed for the polymer chain among the dye molecules labeled at both ends of a polymer chain [14–16]. The distance between the energy donor (D) and accepter (A) molecules is evaluated through the energy transfer efficiency observed by the fluorescence decay curve or spectrum. However, the energy transfer efficiency is affected not only by the distance but also by the angle between the transition moments of the D and A molecules. The recent progress in the development of super-resolution optical microscopy enables us to obtain optical information with the high resolution less than 10 nm [17,18]. For a single dye molecule observed as a circular spot with a diameter of ~200 nm in a microscopy image, the position of the molecule can be determined with nanometric localization accuracy. This is known as FIONA (fluorescence imaging with one-nanometer accuracy) and has been utilized to discuss the translational motion of a molecule. By the super-resolution detection of two dye molecules on a single chain, the direct observation of the end-to-end distance has been demonstrated [19–21]; however, the distribution of the chain dimension was not examined quantitatively.

* Corresponding author at: Department of Polymer Chemistry, Kyoto University, Nishikyo, Kyoto 615-8510, Japan.
E-mail address: aoki@photo.polym.kyoto-u.ac.jp (H. Aoki).

0301-0104/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chemphys.2012.12.026

In the current paper, the chain conformation of poly(methyl methacrylate) (PMMA) chain in an ultra-thin film is examined by the super-resolution fluorescence imaging technique. We show that the super-resolution fluorescence imaging allows the quantitative analysis of the end-to-end distance with an accuracy less than 1 nm. The conformation of PMMA is discussed by comparing the distribution of the end-to-end distance of the individual polymer chain in bulk and confined states.

2. Experiments

The dye-labeled PMMA at the chain ends was synthesized by atom transfer radical polymerization. Methyl methacrylate (MMA) was polymerized from an initiator having a perylene diimide (PDI) moiety with CuCl (I)/dinonyl bipyridine, yielding the PMMA chain labeled by a single PDI molecule at the initiating end [22]. The number- and weight-average molecular weight, M_n and M_w, were evaluated to be 94,000 and 111,000, respectively. In order to label the terminal end, we performed the block copolymerization of a short chain of the random copolymer of MMA and PDI-labeled methacrylate (PDIMA). After the polymerization of the poly(MMA-*ran*-PDIMA), the molecular weight increased to M_n = 97,000 and M_w = 117,000, indicating that the molecular weight of the short labeled fragment was evaluated to be 3000. The average number of the PDI molecule therein was 0.4 from the UV–vis absorption. The number of each monomer component introduced in a random copolymer is given by the binomial distribution. Therefore, 27% of the obtained PMMA chain was labeled at each end with a single PDI molecule. The details on the characterization of the labeled polymer are described in Supplementary Information. A toluene solution of a mixture of the labeled and unlabeled PMMA at a ratio of 10^{-6}—10^{-5} was spin-cast onto a clean glass cover slip, yielding homogeneous films with thicknesses of 5 and 80 nm. The sample films were annealed at 140 °C for 24 h before the fluorescence microscopy measurement.

Fluorescence images were acquired by an inverted optical microscope (TE-2000, Nikon) equipped with an electron magnifying CCD camera (Cascade II, Roper Scientific) under a total internal reflection illumination at a wavelength of 532 nm. The fluorescence image was acquired using a high NA objective (100×, NA 1.4) through a dichroic mirror and a band-pass filter (z532r and HQ580/60, Chroma Technology, respectively) at a frame rate of 10 Hz. The average number of the detected photon for a single PDI molecule in each frame was ca. 4000. The individual labeled chains were observed at a number density of 0.01–0.1 chains/μm^2 in a fluorescence image. The image of each chain was analyzed using a numerical computation software package, Scilab (http://www.scilab.org/).

3. Results and discussion

Fig. 1a shows the fluorescence image of the PDI-labeled PMMA chains. A single dye molecule is observed as a circular spot with a diffraction-limited size. The fluorescence image of a single molecule i located at the position $\mathbf{r}_i$ can be expressed by a two-dimensional Gaussian function,

$$I_i(\mathbf{r}) = I_i \exp\left(-\frac{(\mathbf{r}-\mathbf{r}_i)^2}{2w^2}\right), \quad (1)$$

where I_i, $\mathbf{r}$, and w are the maximum fluorescence intensity, the position vector, and the observed size of the molecule in the fluorescence image, respectively. By fitting this equation to an image of a single molecule, the position of the molecule $\mathbf{r}_i$ can be obtained. The accuracy to determine the position of the molecule $\mathbf{r}_i$ is approximately given by $w/\sqrt{N}$, where N is the number of the detected photons. In our apparatus, the localization accuracy for a single molecule was evaluated to be 3.1 nm by the repeated measurements for a PDI molecule embedded in a PMMA film (see Supplementary Information). The end-to-end vector $\mathbf{R}_{obsd}$ shown in Fig. 1b can be observed by determining the positions of the PDI molecules at the both ends of a polymer chain. However, in this mechanism to examine the position of the single molecule, only one molecule has to be observed in the field of view of the fluorescence microscopy image; therefore, the two PDI molecules at the chain ends must be observed separately to evaluate the end-to-end distance. Since the molecular weight of the current sample was 10^5, the end-to-end distance is estimated to be on the order of 10 nm. Consequently the two PDI moieties are overlapped in the fluorescence image (Fig. 1a) and cannot be analyzed separately; therefore, the two PDI molecules were separated in time [19,20]. Fig. 1c shows the time trajectory of the fluorescence intensity from this chain, indicating the stepwise photo-bleaching at the time t = 3.1 and 9.6 s, which are indicated by the left and right arrows in Fig. 1c. This indicates that the two PDI molecules bleached independently. At beginning, the two PDI molecules at both ends, A and B, of the PMMA chain emitted the fluorescence, and then one of them showed the photo-bleaching at the times of t = 3.1 s and the emission from the other dye molecule was observed. Finally the emission intensity decreased to the background level by the irreversible bleaching of the other PDI molecule at t = 9.6 s. Thus, at the time range from 3.1 to 9.6 s, only one PDI molecule was observed in the fluorescence image. Therefore, the position of one chain end A, $\mathbf{r}_A$, can be evaluated by fitting an image in this time range to Eq. (1). The fluorescence image at $t < 3.1$ s can be given as a sum of two Gaussian functions, $I_A(\mathbf{r}) + I_B(\mathbf{r})$, for the positions of the chain ends A and B. Since $\mathbf{r}_A$ and I_A were obtained by the previous fitting, the position of the other end B, $\mathbf{r}_B$, is available by fitting the fluorescence image at$t < 3.1$ to the intensity distribution $I_A(\mathbf{r}) + I_B(\mathbf{r})$ using the fixed values of $\mathbf{r}_A$ and I_A. Thus, the

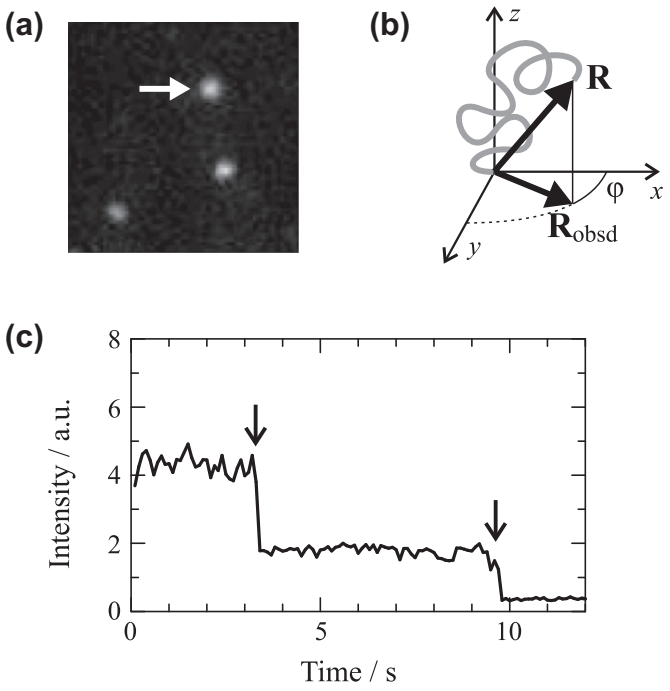

Fig. 1. Fluorescence microscopy image of single end-labeled PMMA chains (a), schematic illustration of the observed chain (b), and time trajectory of the fluorescence intensity for the chain indicated by an arrow in the panel a (c). The x–y plane and the z axis correspond to the plane of the sample film and the optical axis, respectively.