Multiscaling in a long semiflexible polymer chain in two dimensions

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Using atomic force microscopy, full visualization of a single giant T4 DNA molecules (166 kilo base pairs), the contour length of which is sufficient to examine the scaling property, was achieved. Fluorescence microscopic measurement was performed on exactly the same T4 DNA molecules. The results showed that there are three distinguishable regions in the scaling property $R \sim L^\nu$, where R, L, and ν are the end-to-end distance, contour length and scaling exponent, respectively: (i) $\nu \simeq 1$ when $L < 0.10$ μm, (ii) $\nu \simeq 0.5$ when 0.10 μm$< L < 4$ μm, and (iii) $\nu \simeq 0.75$ when $L > 4$ μm. This conformational behavior is discussed in relation to self-avoiding walk in 2D. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1475759]

I. INTRODUCTION

The scaling property of polymer chains has attracted much interest as a central issue in soft-matter physics. The conformation of polymer chains in solution has been theoretically interpreted as a three-dimensional self-avoiding walk (3D SAW).[1–3] The validity of the scaling concept has been examined by experimental observations of the physical properties of polymers in the ensemble, such as the neutron-scattering,[4] sedimentation,[5] and viscometry.[6] On the other hand, experimental tools such as electron microscopy (EM) and atomic force microscopy (AFM) can provide the information on conformation at a scale of 0.1 nm, which is at the level of individual polymer chains.[7,8] Although the scaling argument is expected to be applicable to long polymer chains, it is still difficult to perform observations for large differences in the scale, despite recent developments in experimental techniques. Among various kinds of giant macromolecules, double-stranded (ds) DNA molecules have long contour length and therefore are a good candidate for examining the scaling property of a polymer chain. Although many experimental studies have been reported in relation to the scaling property of dsDNA chains,[9,10] there still remain the some serious problems. (1) In measurements with EM and AFM, it is rather difficult to perform the observations on giant dsDNAs larger than tens of kilo bases. DNA is a rather stiff polymer with the persistence length of ca. 50 nm, corresponding to 150 base pairs (bp). However, to examine the scaling property, it is necessary to obtain information on the conformation of individual DNA chains over the size of 100 kbp. In addition, with AFM and EM, pretreatments such as drying and staining with metal are used, which raises the possibility that the conformation changes drastically before observation. (2) In studies with fluorescence microscopy (FM), a coarse-grained conformation in a solution with a resolution of 0.2–0.3 μm is observed for individual DNA molecules of the size above 10 μm.[11] Although one can obtain information regarding the entire conformation of a long chain, the low resolution limits the applicability of this approach to the scaling property.

In the present study, we report the results of the microscopic observation of the entire conformation of a giant dsDNA chain (166 kbp) with AFM. To the best of our knowledge, this is the first successful measurement of the whole chain conformation for DNA over the size of 100 kbp. We obtained information on the overall conformation of exactly the same giant DNA molecule using different tools, AFM and FM. The former gives microscopic information on a dried solid surface and the latter gives a coarse-grained image in solution without any pretreatment.

II. METHODS

To scrutinize the scaling property in the actual conformation of a polymer chain in 2D, it is needed to characterize the entire trail of individual polymer chains existing on smooth solid surfaces that have retained their scaling property. To obtain such data, we adapted giant dsDNA (T4 phage DNA, purchased from Nippon Gene, Japan) as a sample polymer in the present study: its conformational behavior can be directly observed by FM because of its extremely large molecular size (166 kbp; contour length, 57 μm).[12] The microscopic trail of a T4 DNA chain adsorbed onto a mica surface was observed by FM/AFM (NVB100, Olympus, Japan), according to the following procedure to prepare DNA chains in 2D (see Fig. 1): (1) Newly cleaved thin mica (thickness; ca. 30–50 μm) was closely stuck to a cover glass plate (Matsunami Glass, No. 1, Japan) for FM observation of DNAs with an oil-immersed ×100 objective lens (short working distance). (2) The mica surface was treated with 1 mM spermine (Wako Pure Chemicals, Japan) and washed with Millipore water. This treatment was performed so that T4 DNA chains would adsorb onto the mica surface. (3) Individual T4 DNA chains were observed in 10 mM Tris-HCl buffer solution (pH 8.2) by FM with the aid of

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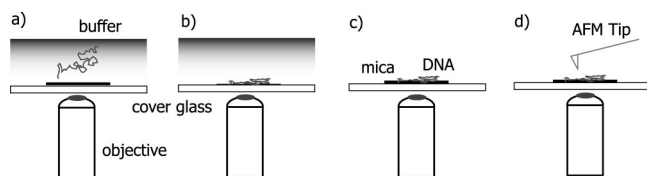
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FIG. 1. Scheme of the protocol for observing giant dsDNA chains by FM/AFM method. First, individual T4 DNA molecules are observed in buffer solution with FM (a), and adsorption process onto a mica surface is checked (b). Then, the sample droplet on the surface is removed (c). Finally, a single DNA chain selected by FM observation is observed by the tapping mode in AFM (d).

an EB-CCD camera (Hamamatsu Photonics, Japan) [Fig. 1(a)]. To visualize DNA molecules, a fluorescent dye, YOYO-1 (Molecular Probe, USA), was used (dye/bp: 1/10). The observation was performed in a droplet (10 μl) instilled on mica. (4) The adsorption process of DNA chains onto the mica surface was monitored [Fig. 1(b)]. In this step, measurement by FM confirmed that the extent of the DNA chain did not significantly change with adsorption. (5) The sample droplet on mica was removed by blowing with nitrogen, washed with Millipore water, and blown dry again with nitrogen [Fig. 1(c)]. Through this step, we checked whether the adsorbed DNA chain retains the same morphology as in solution. (6) Finally, a single T4 DNA chain selected by FM observation was observed using the tapping mode in AFM. It is to be noted that both FM and AFM images were obtained for the very same DNA chain.

III. RESULTS

Figure 2 shows FM (a) and AFM (b) images of exactly the same single T4 DNA chain adsorbed onto a mica surface.

Figure 2(c) shows the shaded pattern of coordinates of the AFM image in Fig. 2(b), using a Gaussian filter of ca. 300 nm. This shaded pattern corresponds well to the FM image, which demonstrates the reliability of the local segment density at a scale of several hundreds of nm based on fluorescent intensity. Prior to the AFM observation, the conformation of individual T4 DNA chains in bulk solution was checked by FM, and these were observed with strong conformational fluctuation due to the intramolecular Brownian motion of the segments (data not shown, see our previous studies).[13] These fluctuating chains were adsorbed onto the positively charged surface of mica treated with spermine, and frozen on the surface [Fig. 2(a)]. We confirmed that the coarse-grained conformation and average extent of individual DNA chains (around 4 μm under the present conditions) were maintained throughout both the adsorption process onto mica and the preparation process for AFM observation in the air. Under these conditions, a weakly adsorbed chain is considered to readjust its conformation on a 2D surface by thermal fluctuation before evaporation of the solute. Thus, it is expected that the AFM image in Fig. 2(b) reflects the conformational characteristics of 2D SAW (two-dimensional self-avoiding walk).

The scaling property of the 2D SAW of an adsorbed DNA chain was scrutinized using position vectors on the whole trail of the real polymer chain. First, the position vectors were assigned every ca. 2 nm along the trail of the DNA chain in Fig. 2(b). Next, the end-to-end distance and contour length from one selected starting position to any ending position on the trail were calculated from the coordinates. By choosing starting positions with a proper interval (about 2 μm, which is much more than the persistent length), more than 30 series of the data set of end-to-end distances and
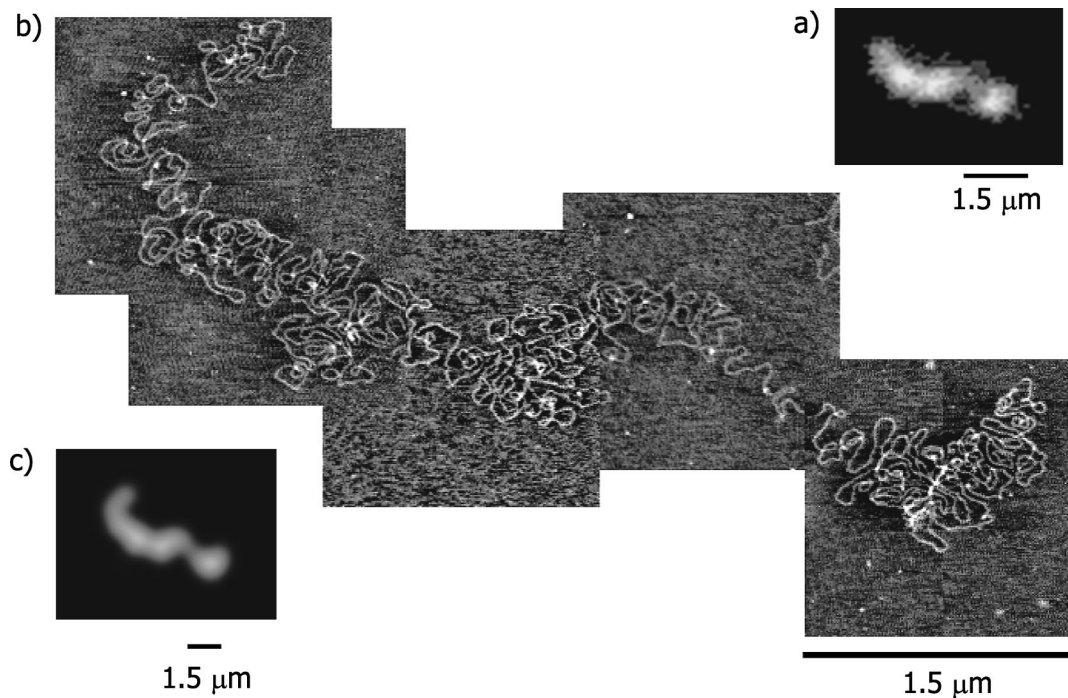

FIG. 2. Microscopic observations of a single T4 DNA chain in the random coiled state adsorbed onto a mica surface. (a) FM image. (b) AFM image on the very same DNA as in (a). (c) Coarse-grained image of (b) using a Gaussian filter of ca. 300 nm.
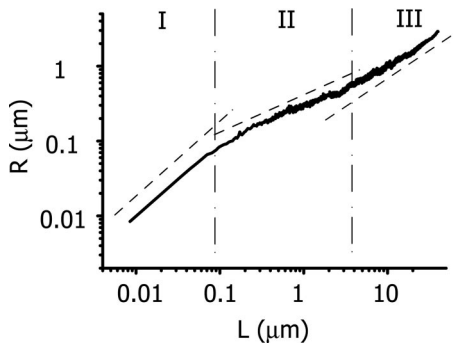
FIG. 3. Log–log plot of the end-to-end distance vs contour length along the trail of the DNA chain in Fig. 1. The dotted lines have slopes of 1.0, 0.5, and 0.75 (left to right), respectively.

contour lengths were obtained. By averaging these series, the relationship between end-to-end distance and contour length was determined, as depicted in Fig. 3. Figure 3 shows that there are three different regions: (I) $L<0.10$ μm, (II) 0.10 μm$<L<4$ μm, and (III) $L>4$ μm. The scaling exponents are 0.98 for region (I), 0.5 for region (II), and 0.75 for region (III), respectively.

IV. DISCUSSION

We would like to discuss whether the conformation observed by AFM [Fig. 2(b)] is the 2D projection of 3D object or actual 2D structure. The existence of crossovers in the AFM image indicates that the adsorbed DNA does not take 2D structure in its exact meaning. On the other hand, it is important to note that the slope of the third region in Fig. 3, 0.75, corresponds to the scaling exponent of a 2D self-avoiding walk, not to that of 3D, 0.60.[1–3] This suggests that the conformation would be better interpreted as 2D structure in the present condition. Therefore, we start the discussion on the conformation in 2D (length of Kuhn segment, l; number of segments, N). By taking $\beta=1/k_BT=1$, the free energy of the single polymer chain can be written as follows:[2]

$$F\sim\alpha^2+\alpha^{-2}+BS\phi^2, \tag{1}$$

where α is an expansion factor, i.e., $\alpha=R/R_0$, the ratio of the extent of a real chain to that of an ideal chain. B, S, and ϕ are the second virial coefficient, the area occupied by elongated coil of polymer chain and the number of segments per unit area, respectively. The first two terms describe the free energy of elastic deformation of the polymer chain and the last term shows the free energy of interaction between two segments. In the interaction term, many-body interactions among chain segments are neglected, due to the low segment density of the random-coiled polymer chain. S and ϕ are given as follows:

$$S\simeq R^2\sim N(l\alpha)^2, \tag{2}$$

$$\phi=\frac{N}{S}\simeq(l\alpha)^{-2}. \tag{3}$$

Thus, F can be written as

$$F\sim\alpha^2+\alpha^{-2}+\frac{BN}{l^2}\alpha^{-2}. \tag{4}$$

By minimizing the total free energy with respect to α,

$$\frac{\partial F}{\partial\alpha}\sim\alpha-\left(1+\frac{BN}{l^2}\right)\alpha^{-3}=0. \tag{5}$$

This equilibrium extent R of the chain can be evaluated as

$$R\sim N^{1/2}l\left(1+\frac{BN}{l^2}\right)^{1/4}. \tag{6}$$

This relationship implies multiscaling, when N is large enough $(N\gg l^2/B)$, $R\sim N^{3/4}$ and when N is small $(N\ll l^2/B)$, $R\sim N^{1/2}$. Note that for a chain shorter than the length of a Kuhn segment, i.e., $N<1$, $R\sim N$. As a result, the following three different scaling behaviors are expected,

  I.   stiff rodlike region; $R\sim N$ for $N<1$,
  II. ideal chain region; $R\sim N^{1/2}$ for $1<N_c\ll l^2/B$
  III. SAW region; $R\sim N^{3/4}$ for $N_c\gg l^2/B$

where N_c is the critical number between regions II and III. The scaling exponents obtained in the above-mentioned nearly coincide with the experimental trend given in Fig. 3. Based on this close agreement, the values of l, N_c, and B can be evaluated as follows: l can be directly estimated from the critical contour length between (I) and (II) in Fig. 3, $l\sim10^{-1}$ μm. From the critical contour length between (II) and (III), $N_cl\sim4$ μm, i.e., $N_c\sim40$. From these values, the second virial B can be evaluated as $B\sim2.5\times10^{-4}$ μm^2.

Since we used good solvent in the present experiment, it is natural to take B as the excluded volume of a segment (in the present case, the excluded area on a solid surface). Thus, if we assume $B\simeq ld$, where d is the width of the segments, $d=B/l\simeq2.5$ nm, which is on the same order as the diameter of real DNA (2 nm).

In 3D, we can adapt the similar argument as in 2D under the consideration that the projection of a real chain (fractal dimension: 5/3) into 2D does not affect the scaling property of a chain.[14] α in equilibrium state is given by the following equation:

$$2\alpha-2\alpha^{-3}-3Bl^{-3}N^{1/2}\alpha^{-4}=0, \tag{7}$$

where the first term is always non-negligible due to $\alpha>1$. Under the condition that the second term in Eq. (7) is dominant $(\alpha\gg3/2Bl^{-3}N^{1/2})$, $\alpha\sim1$, i.e., $R\sim N^{1/2}$. For a longer chain, when the third term in Eq. (7) is dominant, $\alpha\sim3Bl^{-3}N^{1/10}$, i.e., $R\sim N^{3/5}$. In order to satisfy the former condition, $N^{2/5}<2$ should hold because of the relation, $\alpha<3Bl^{-3}N^{1/10}$. This means that ideal chain region cannot exist in 3D under good solvent condition. It is, thus, concluded that the above-mentioned multiscaling property cannot be interpreted as a 2D projection of 3D conformation.

Although other effects, such as adsorption and drying are expected, it has become clear that the mean-field theoretic discussion of a 2D self-avoiding walk adapted in the present study, describes the essential features of the conformational

behavior of the DNA. In summary, we have shown the experimental evidence of a multiscaling property for a DNA chain.

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