Large Discrete Transition in a Single DNA Molecule Appears Continuous in the Ensemble

K. Yoshikawa,[1] M. Takahashi,[1] V. V. Vasilevskaya,[2] and A. R. Khokhlov[3]
[1]*Division of Informatics for Natural Sciences, Graduate School of Human Informatics, Nagoya University, Nagoya 464-01, Japan*
[2]*Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Vavilova St. 28, Moscow 117813, Russia*
[3]*Physics Department, Moscow State University, Moscow 117234, Russia*
(Received 1 November 1995)

We observed giant double-stranded DNA chains by fluorescence microscopy in an aqueous environment. We found that the coil-globule transition of T4DNA, 166kbp, induced by spermidine is markedly discrete for individual chains, and continuous for their ensemble average. Simple theoretical consideration is given taking account of the hierarchy of the system. It is suggested that the unique characteristics of the transition for long DNA has a general significance for the coil-globule transition in other biological and synthetic "stiff polymers."

PACS numbers: 87.15.By, 64.70.–p, 36.20.Ey

It is well known that polymer chains exhibit transition between the states of elongated coil and compacted globule depending on the characteristic of the solvent, i.e., temperature, salt concentration, dielectric constant, etc. According to theoretical considerations in previous reports, this transition can be discrete for stiff polymers and also for polyelectrolytes, i.e., first-order phase transition [1–3]. However, previous experimental studies have indicated that the transition is always continuous [4–8]. To shed light on this discrepancy, we have performed the observation on the structural transition of single duplex DNA chains by use of fluorescence microscopy. In the present study, we have chosen spermidine, a trivalent organic cation, as the condensation agent [9]. Figure 1(a) shows the fluorescence images to T4DNA molecules in the coil (left) and globule (right) states. Coiled DNA was observed in 10m*M tris* buffer solution, and globular DNA was seen in $180\mu M$ spermidine solution. Figure 1(b) shows the spatial distribution of the fluorescence light intensity for the coil and globule DNA's. Figure 1(c) shows a schematic representation of the relationship between the fluorescence image and the microscopic structure of the DNA chain. As depicted, a blurring effect occurs on the order of 0.3 μm, based on the resolution limit due to the wavelength of the observation light and to the technical characteristics of the SIT camera [3,10–12]. Based on measurements of the diffusion constant for the individual fluorescent obstacles, the gyration radii of coil and globule were estimated to be 1.6 and 0.2 μm, respectively, using the corrections proposed by Oono and Kohmoto [13]. This implies that the effective volume changes ca. 500-fold with the coil-globule transition.

To characterize the structural transition of DNA induced by spermidine in a quantitative manner, a series of measurements was carried out by changing the spermidine concentration. As a measure of the effective size, the long-axis length L of DNA chains [Fig. 1(c)] was evaluated directly from the video images. The results are summarized in Fig. 2(a), which shows two maxima with spermidine concentrations of $140\mu M$ and $160\mu M$. In the actual observation, we can clearly distinguish between coil and globule states [3], because the globule

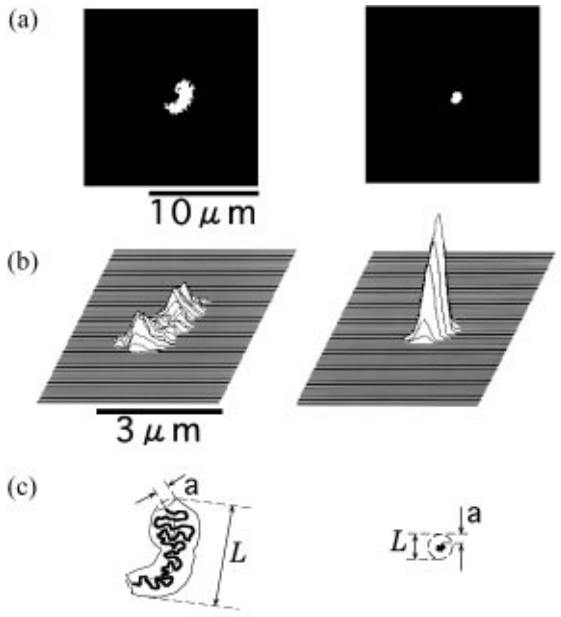

FIG. 1. (a) Fluorescence microscopic images of T4DNA with coil and globule states. The observation was performed at the conditions similar to the previous studies [3,10–12,14]. Briefly, we used *tris* buffer solution (10m*M tris*, lm*M* NaCl, at *p*H 5.2), with $0.1\mu M$, 4′,6-diamidino-2-phenylindole (DAPI), and 4% (v/v) 2-mercaptoethanol. Under this condition, the binding number of DAPI per one base pair is estimated to be 0.05, and the persistence length is expected to remain nearly the same as in the absence of DAPI [15]. We used a Nikon microscope, TMD-EF2, equipped with a 100× oil immersed objective lens. (b) Spatial distribution of the fluorescence light intensity (a). Because of the high density of DNA segments in the globule state, the fluorescence intensity is rather strong for the globular obstacle. (c) Schematic representation on the relationship between the conformation of the actual DNA chain and the corresponding fluorescence image. Because of the blurring effect ($a \approx 0.3$ μm) [4,10–12], the fluorescence image is larger than the actual size of the DNA chain.

does not exhibit any visible conformational fluctuation, whereas the coil exhibits intramolecular Brownian motion. In Fig. 2(b), the solid circles show the maximum values of the long-axis length L. The shaded region in the figure corresponds to the bimodality in the distribution. The open squares indicate the mean value of L in the distribution, and the broken line corresponds to the transition profile of the ensemble average among the DNA chains. These results clearly show that *the transition between the coil and globule states is discrete for individual DNA chains, in the sense that each macromolecule assumes either the coil or globule state. On the other hand, the transition behavior of the ensemble average of the DNA chains is sigmoidal, which is typical for a cooperative and continuous transition.* To the best of our knowledge, all of the previous experimental studies have indicated that the coil-globule transition is steep but continuous for both natural and synthetic polymers [4–8]. On the contrary, theoretical studies [1–3] have predicted a discrete transition for chains that are sufficiently stiff. The result in the present study indicates that single polymer chains, such as DNA and other stiff polymers, may exhibit a first-order phase transition, even when the transition appears continuous in the ensemble. This experimental result can be explained on the basis of the modern theory of coil-globule transitions [1–3]. Let us consider a polymer chain which contains N monomer links with persistence length l and width d. The free energy F can be written as

$$\frac{F}{T} = \frac{3}{2}\left(\frac{1}{\alpha^2} + \alpha^2\right) + \frac{BN^{1/2}}{\alpha^3 l^3} + \frac{C}{\alpha^6 l^6}, \qquad (1)$$

where the first two terms describe the elastic free energy, and the third and fourth terms are the free energy of interaction. B and C are the second and third virial coefficients for the interaction of the monomer links. The dependence of the equilibrium swelling coefficient α of the polymer chain on the solvent can be found by minimizing free energy F over α. As a result, we obtain the following equation:

$$\alpha^5 - \alpha - \frac{2y}{\alpha^3} = x\frac{d}{l}. \qquad (2)$$

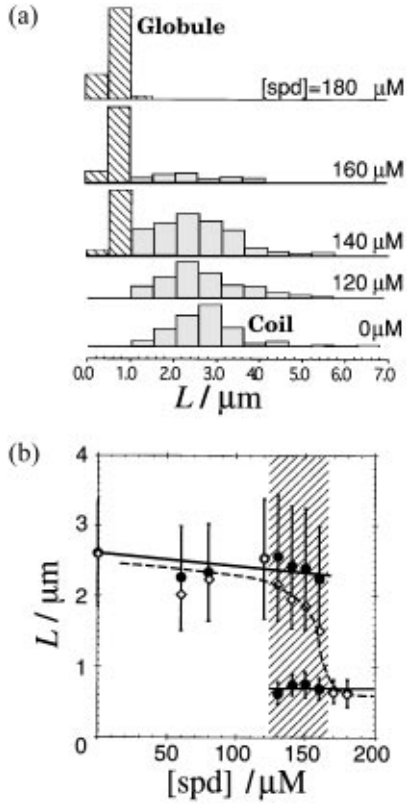

FIG. 2. (a) Histogram on the distribution of the long-axis lengths of T4DNA molecules at various concentrations of spermidine, [spd]. The number of the analyzed T4DNA's was ca. 100 $[\mathrm{DNA}] = 0.10\mu M$ in the unit of base pair concentration. Each area of the histogram is normalized to be equal. (b) Long-axis lengths of T4DNA molecules vs the concentration of spermidine. The solid circles indicate the maxima for the coil and globule, respectively, in the DNA lengths' distribution. The statistical error in the distribution is given as the standard deviation. The broken line represents the transition curve for the ensemble average of the long-axis lengths. The actual length in the globule is evaluated to be ca. $0.4\mu M$ from the measurement of the diffusion constant.

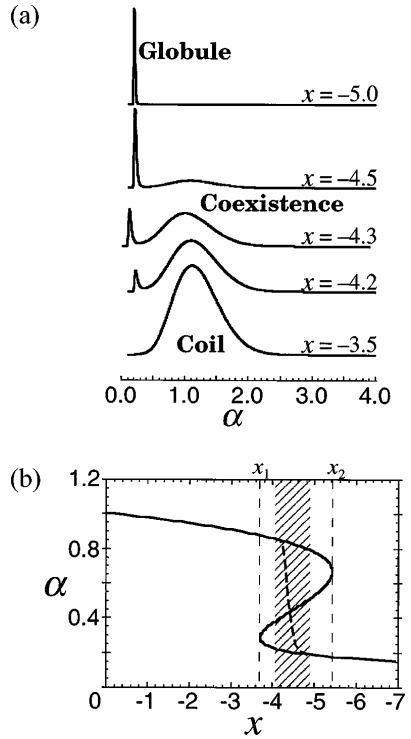

FIG. 3. (a) Size distribution for the ensemble of a stiff polymer, $y = 10^{-3}$, depending on the solvent quality. (b) Unique transition characteristics for the stiff polymer. For a single chain, bistable property is represented by the Z-shaped solid line. For the ensemble of the chains, the transition looks like a continuous transition as is given by the broken line.

The parameter x characterizes the solvent quality, $x = BN^{1/2}/l^2 d$. The parameter $y = C/l^6$ depends on the chain flexibility: the stiffer the macromolecule, the lower the value of parameter y. In Fig. 3(b) we plot typical theoretical dependencies of the swelling coefficient α of macromolecules undergoing the coil-globule transition as a function of parameter x, which represents the quality of the solvent. For chains that are sufficiently stiff ($l/d \gg 1$), the coil and globule states coexist in a region with finite parameter width ($x_1 > x > x_2$). For an infinitely long chain, the transition width should be very narrow. However, actual polymer chains, both natural and synthetic, always have a finite length. As a result, the size distribution function $p(\alpha)$ of macromolecules becomes bimodal [Fig. 3(a)]:

$$p(\alpha) = \alpha^2 \exp[-\Delta F(\alpha)/T] \Big/ \int \alpha^2 \exp[-\Delta F(\alpha)/T]\, d\alpha. \quad (3)$$

On the other hand, if DNA collapse is observed by the usual experimental methods, such as light scattering and sedimentation, only the characteristics regarding the ensemble average are observed (see, e.g., Refs. [4–6] and [3,10–12]). The mean swelling coefficient $\langle\alpha\rangle$ is determined as follows:

$$\langle\alpha\rangle = \int_0^\infty \alpha\, p(\alpha)\, d\alpha. \quad (4)$$

In such cases, the broken curve in Fig. 3(b) is observed, which is consistent with the experimental results with DNA [Fig. 2(b)]. Thus, although stiff chains should show a discrete first-order conformation transition (as manifested by the bimodal size distribution), the results which are actually observed by standard experimental methods appear as continuous sigmoidal curves with a characteristic width $\sim 1/N$ (cf. Refs. [4–8]). In the present Letter, we have provided definite evidence of the first-order transition for a long DNA molecule. Since the above theoretical consideration is rather general, this unique characteristic of polymer chains should also apply for other natural and synthetic "stiff polymers."

V. V. V. is grateful to Ciba-Geigy Foundation for the Promotion of Science (Japan) who provided the opportunity for her stay in Nagoya. This work is partly supported by Russian Foundation of Fundamental Research and Japanese Society for Promotion of Science.

[1] I. M. Lifshits, A. Yu. Grosberg, and A. R. Khokhlov, Rev. Mod. Phys. **50**, 683 (1978).
[2] A. Yu. Grosberg and A. R. Khokhlov, *Statistical Physics of Macromolecules* (American Institute of Physics, New York, 1994).
[3] V. V. Vasilevskaya *et al.*, J. Chem. Phys. **102**, 6595 (1995).
[4] C. B. Post and B. H. Zimm, Biopolymers **21**, 2123 (1982).
[5] H. Yamakawa, Macromolecules **26**, 5061 (1993).
[6] T. Norisuye and Y. Nakamura, Polymer **34**, 1440 (1993).
[7] J. Widom and R. L. Baldwin, J. Mol. Biol. **144**, 431 (1980).
[8] S. T. Sun *et al.*, J. Chem. Phys. **73**, 5971 (1980).
[9] It is well known that polycationic aliphatic amines, such as spermidine, putrescine, and spermine, are present in all eukaryote and play an essential role in the growth of both normal and neoplastic tissues [C. W. Porter and R. J. Bergeron, Science **219**, 1083 (1983); D. T. Hung *et al.*, Science **221**, 368 (1983)]. Polyamines are also believed to be essential for the self-condensation of long DNA chains into phage heads [C. W. Tabor and H. Tabor, Annu. Rev. Biochem. **53**, 749 (1984)].
[10] S. M. Mel'nikov, V. G. Sergeyev, and K. Yoshikawa, J. Amer. Chem. Soc. **117**, 2401 (1995); J. Am. Chem. Soc. **117**, 9951 (1995).
[11] K. Yoshikawa and Y. Matsuzawa, Physica (Amsterdam) **84D**, 220 (1995).
[12] Y. Yoshikawa and K. Yoshikawa, FEBS Lett. **361**, 277 (1995).
[13] Y. Oono and M. Kohmoto, J. Chem. Phys. **90**, 520 (1983).
[14] K. Minagawa *et al.*, Biopolymers **34**, 555 (1994).
[15] Y. Matsuzawa and K. Yoshikawa, Nucleosides Nucleotides **13**, 1415 (1994).