Cooperativity vs. Phase Transition in a Giant Single DNA Molecule

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Abstract: Single-chain observation of giant DNAs grafted with poly(*N*-isopropylacrylamide) (PNIPAAM) was performed using fluorescence microscopy while changing the solution temperature. The PNIPAAM-grafted DNA was prepared through the intercalation of psoralen-terminated PNIPAAM. Individual DNAs grafted with PNIPAAM exhibit a sharp but continuous transition at around 34 °C, from an elongated coil to a collapsed compact state. It is found that the width of the transition is almost the same between the level of individual DNAs and the level of ensemble DNAs. The unique nature of this transition is discussed in comparison with the discrete nature of the folding transition in native double-strand DNAs. With the addition of spermidine, a trivalent amine, the conformation of individual T4DNAs changes in a markedly discrete manner, on the level of individual giant DNAs, whereas in the ensemble, the size of T4 DNAs changes rather mildly; i.e., the transition appears to occur over a temperature range of 30 degrees. Thus, it is confirmed that the cooperative transition on the DNAs grafted with PNIPAAM exhibits a sharper transition than the change in the ensemble average for the discrete phase transition in native DNAs induced by the polycation.

Introduction

In the living cellular environment, long DNA molecules on the order of mega base pairs, or with lengths in the millimeter to centimeter range are folded into a compact state on the order of micrometers. It is well known that such long DNAs dissolve in usual aqueous solutions with an elongated coil conformation.[1,2] The conformational change of DNA molecules from an elongated coil state into a compact state has been studied extensively[3−13] and is called DNA condensation. It has been believed that DNA condensation is highly "cooperative"; i.e., the "condensation" transition is steep but continuous.[9−13] This viewpoint of the continuity of the transition has been deduced from numerous experimental studies on DNA condensation. However, the term DNA condensation seems to refer most often to a phenomenon observed in an ensemble of DNA chains. Quite recently, using the observation of single DNA chains with fluorescence microscopy, it has been found that the folding transition of DNA molecules is largely discrete at the level of individual molecular chains, whereas the transition appears continuous with regard to physicochemical parameters in the ensemble of DNA chains.[14] This discrete nature of the folding transition in individual giant DNAs is rather general and is independent of any differences in the chemical properties of the condensation agents, such as polyamine,[15] multivalent metal cation,[16,17] hydrophilic polymer,[18,19] alcohol,[20,21] cationic surfactant,[22] etc.

In standard textbooks of polymer science and polymer physics, a polymer chain changes its conformation from an elongated coil state into a collapsed globule state through a so-

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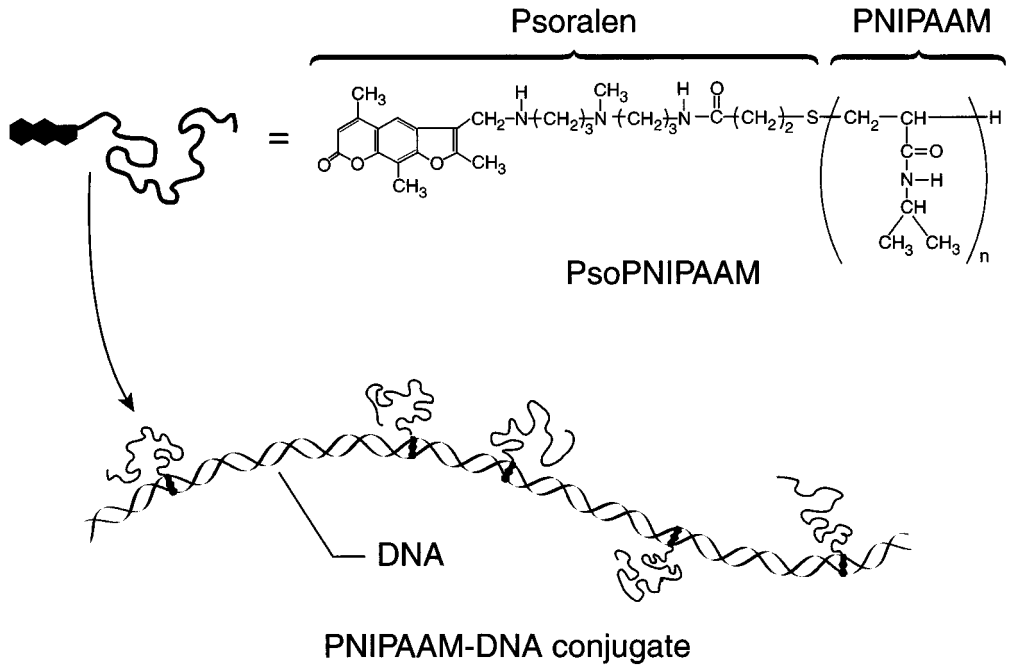

Figure 1. Structure of the DNA−PNIPAAM conjugate synthesized by a photoreaction between DNA and PsoPNIPAAM ($n \approx 50$).

called Θ state, which implies that the collapsing transition is continuous both on the level of the individual chain and on the level of the ensemble of chains.[23−25] Thus, it may be useful to look for conditions in which single long duplex DNA chains exhibit such a continuous transition by suitable modification of the DNA chains. If it becomes possible to realize the continuous transition on a modified DNA, it is of interest to examine whether the width of the transition is different between the level of single chain and the level of ensemble chains. Toward this aim, we have prepared a long DNA chain modified with poly(*N*-isopropylacrylamide) (PNIPAAM). It is well known that PNIPAAM chains undergo a collapsing transition at around 30−35 °C.[26−31] Thus, we performed single-chain observation of long DNAs grafted with PNIPAAM molecules while changing the temperature.

Experimental Section

Materials. T4 bacteriophage DNA (166K base pairs) was purchased from Takara Shuzo. Fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), was obtained from Wako Pure Chemical. The antioxidant, 2-mercaptoethanol (2-ME), was purchased from Nacalai Tesque. Psoralen-terminated PNIPAAM, PsoPNIPAAM, was prepared by the coupling reaction between the amino derivative of psoralen and carboxyl-terminated PNIPAAM. The details of the sample preparation have been reported elsewhere.[32] The polydispersity index and number-average molecular weight of PNIPAAM were 3.9 and 4300, respectively.

A polymer of DNA grafted with PNIPAAM chains was synthesized as follows. A solution of T4 DNA (60 μM base pairs) was mixed with a TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) solution of PsoPNIPAAM (0.5 μM in strands). The solution was then irradiated (ca. 30 mW/cm^2) for 5 min on an ice bath with a 500-W ultra-high-pressure Hg lamp equipped with a high-pass filter (Toshiba, UV-31). Photoreaction between DNA and the psolaren terminus of the PNIPAAM chains resulted in the grafting of PNIPAAM to DNA, giving a DNA−PNIPAAM conjugate (Figure 1).

Fluorescence Microscopic Measurements. The DNA−PNIPAAM conjugate was dissolved in TE buffer solution (10 mM Tris-HCl, 1 mM EDTA, pH 7.6), and DAPI and 2-ME were then added to the solution. The final concentrations in the sample for the fluorescence microscopic observation were as follows: 0.3 μM T4 DNA grafted with PNIPAAM (in base pair units), 0.3 μM DAPI, and 4% (v/v) 2-ME. Fluorescent images of individual DNA−PNIPAAM conjugates were observed with a Carl Zeiss Axiovert 135 TV microscope and recorded through a Hamamatsu SIT TV camera and an image processor (Argus 10, Hamamatsu Photonics). The temperature of the samples was controlled by using a water-circulating system. The accuracy of the temperature was ±0.5 °C. It has been confirmed that for free T4DNA molecules, both the contour length, 57 μm, and the persistence length, 65 nm, remain constant with such a small amount of the fluorescent dye, DAPI.[33]

AFM Observation. The collapsed structure of the DNA−PNIPAAM conjugate was observed with an atomic force microscope (NanoscopeIIIa, Dimension 3000, Digital Instrument, Santa Barbara, CA) on a self-assembled monolayer (SAM) surface with close-packed methyl groups. The methyl-functionalized SAM surface (CH_3-SAM) was employed to enhance the adsorption of globules to the surfaces for AFM scanning observation. The CH_3-SAM was prepared with the immersion of a gold-deposited glass substrate (Cr, 30; Au, 500 Å) into an ethanolic solution (1.0 mM) of 1-dodecanthiol overnight at room temperature. The PNIPAAM−DNA solution (0.3 μM in base pairs) was kept at 40 °C for 5 min, followed by dropping aliquots of 20 μL onto the CH_3-SAM surface, and this was left to stand for 1 min. After dehydration and air-drying of the surface, the DNA−PNIPAAM conjugates were observed in air with tapping mode AFM using a Si

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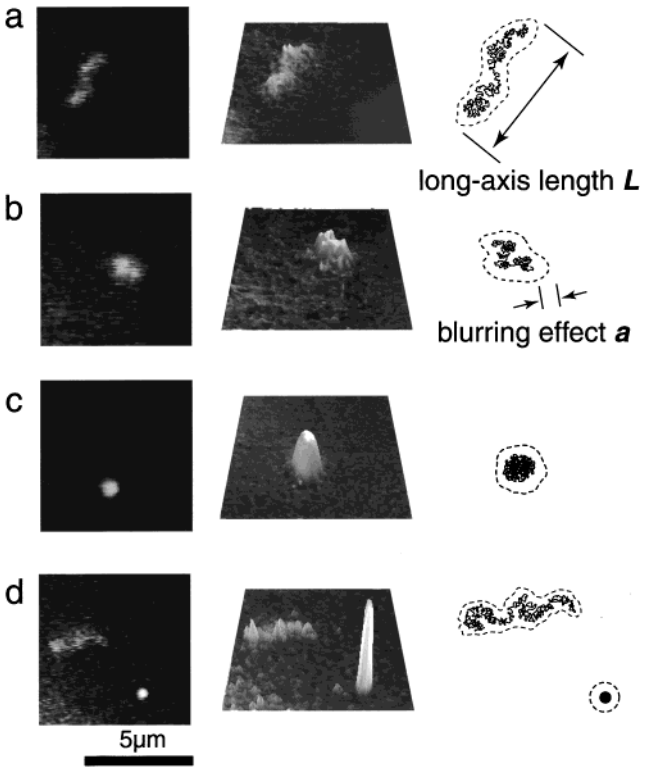

Figure 2. (Left) Fluorescence images of the single DNA−PNIPAAM conjugate at various temperatures (a, 10 °C; b, 32.5 °C; c, 35 °C) and native DNA in the presence of 1.2 mM spermidine (d). (Middle) Corresponding quasi-three-dimensional representations of the fluorescence images, where the vertical scale indicates the fluorescence intensity. (Right) Schematic representation of the relationship between the conformation of an actual DNA chain and the corresponding fluorescence image. Due to the blurring effect (ca. 0.3 μm), the fluorescence image is larger than the actual DNA chain.

single-crystal cantilever (FESP, Digital Instruments). The free vibrating amplitude was set at about 1.12 V, and the setpoint was taken as 1.10 V.

Results

The left side of Figure 2 shows fluorescence images of single grafted T4 DNA molecules at three different temperatures (a−c), indicating that the DNA takes an elongated coil conformation at 10 °C, and a collapsed compact conformation at 35 °C. At 32.5 °C, an intrachain segregated structure is observed where elongated coiled and shrunken parts coexist in a single DNA−PNIPAAM conjugate. On the bottom of Figure 2, for comparison, the fluorescence image of T4DNAs in the presence of spermidine is shown, where an elongated chain and a compact chain coexist.[34] It has been clarified that the effective volume of the individual native DNA chains decreases on the order of 1/1000−1/10000 with the folding transition induced by spermidine.[35] The middle of Figure 2 shows quasi-three-dimensional representations of the fluorescence images, where the height in the pictures indicates the intensity of the fluorescence; i.e., the height is proportional to the density of the segments in the chain. As shown schematically on the right of Figure 2, a blurring effect of 0.3 μm is present,[22] due to the resolution limit originating from the observation light and also partly to the high sensitivity of the TV camera.

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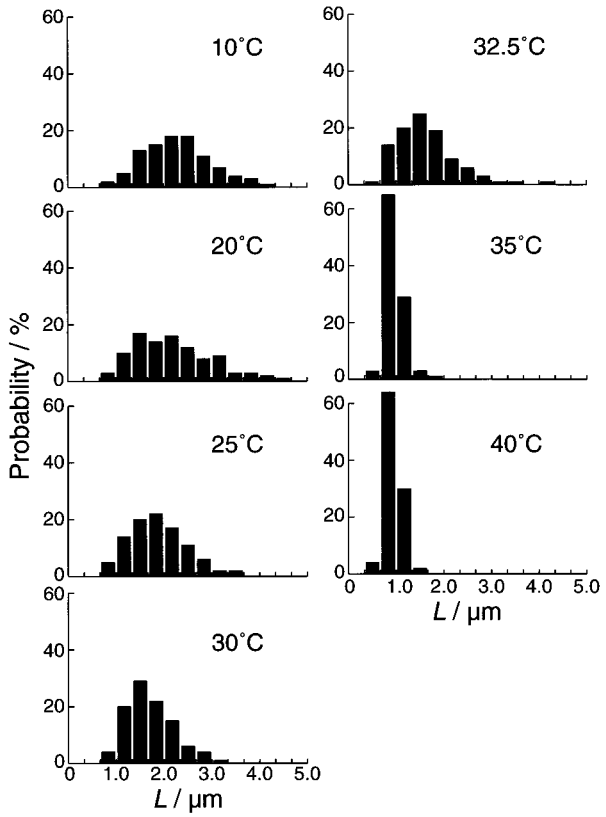

Figure 3. Histograms of the apparent long-axis length, *L*, at various temperatures. At least 200 points of DNA images were analyzed for each temperature.

To quantitatively analyze the change in conformation, we made histograms of the long-axis length in the DNA−PNIPAAM conjugate at different temperatures (Figure 3). In these experiments, we changed the temperature at a rate of 5 °C per hour. At each temperature, we measured the long-axis length for at least 200 molecules. For histograms below 32.5 °C, it is clear that DNA−PNIPAAM conjugates are present in an elongated state with a relatively large thermal fluctuation in conformation, while above 35 °C, DNA−PNIPAAM conjugates are in a compact state with much less fluctuation in the apparent size.

Figure 4 shows the temperature dependence of the mean long-axis length $\langle L \rangle$ of the DNA−PNIPAAM conjugates, as indicated with open circles. It is clear that the transition is continuous but very steep at around 34 °C. To obtain information on the actual change in the spatial extension of the DNA−PNIPAAM conjugates, avoiding the effect of blurring in the fluorescent images, we measured the hydrodynamic radius[22,36] by analyzing the translational diffusion constant *D* of individual molecules. The value *D* can be obtained from the mean-square displacement of the center of mass for individual DNA molecules. Although we attempted to minimize the convective flow in the aqueous sample, a small but non-negligible convective flow was present during the measurement, possibly caused by the thermal effect of illumination. Since the rate and direction of the convective flow were almost constant during the period of observation, we could eliminate the effect of convective flow using the following relationship:[36]

$$\langle (\mathbf{R}(t) - \mathbf{R}(0))^2 \rangle = 4Dt + At^2 \qquad (1)$$

where $\mathbf{R} = (R_x, R_y)$ is the position of the center of mass for DNA, $\langle (\mathbf{R}(t) - \mathbf{R}(0))^2 \rangle$ is the mean-square displacement, and *A*

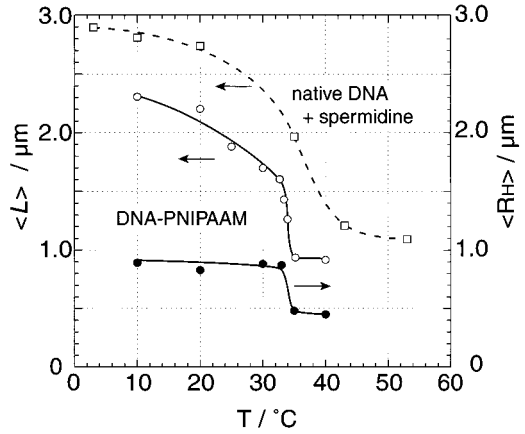

Figure 4. Dependence of the mean long-axis length $\langle L \rangle$ and the mean hydrodynamic radius $\langle R_H \rangle$ on temperature. Due to the blurring effect,[22,36] the actual long-axis length is smaller than the apparent long-axis length by ca. 0.6 μm. The open and filled circles indicate mean long-axis length and mean hydrodynamic radius of the DNA−PNIPAAM conjugate, respectively. The open squares indicate the mean long-axis length of native DNA in solution with 1.2 mM spermidine (ref 34).

is a numerical constant related to the convective flow. The hydrodynamic radius R_H was calculated from D on the basis of the Stokes−Einstein relation according to the Zimm model as follows:[37,38]

$$R_H = \frac{k_B T}{6\pi\eta_S D} \tag{2}$$

where k_B is the Boltzmann constant and η_S is the viscosity of the solvent. The results are also given in Figure 4, with closed circles. Similar to the change in the long-axis length, the hydrodynamic radius R_H exhibits an abrupt transition at around 34 °C. From the change in R_H between the elongated and collapsed states, the effective volume decreases as $(1/2)^3 \sim 1/8$, indicating that the effective volume becomes 1 order of magnitude smaller with the collapsing transition.

For comparison, Figure 4 also shows the change in the long-axis length of native T4 DNA in the presence of a trivalent amine, spermidine, as shown by open squares (experimental details have been described elsewhere).[34] Note that the change in $\langle L \rangle$ for the ensemble average of native T4 DNAs is quite gradual. Thus, as for the characteristics of the collapsing, or folding, transition of DNAs, the transition for DNA−PNIPAAM conjugates is abrupt and looks something like a first-order phase transition, except for evidence that the transition is definitely continuous. On the other hand, the folding transition induced by the trivalent cation is very mild in the ensemble average (the broken line in Figure 4) and does not appear to be related to any kind of phase transition. However, recent studies on the folding transition have made it clear that this transition is markedly discrete at the level of individual DNA molecules, while in general there is no discreteness of the characteristics for the ensemble average of DNA molecules.[9−13]

Figure 5 shows the atomic force microscopic (AFM) image on the collapsed product for the DNA−PNIPAAM conjugate. The collapsed DNA−PNIPAAM conjugates take a nearly spherical structure with a radius of ca. 0.3 μm. As shown in

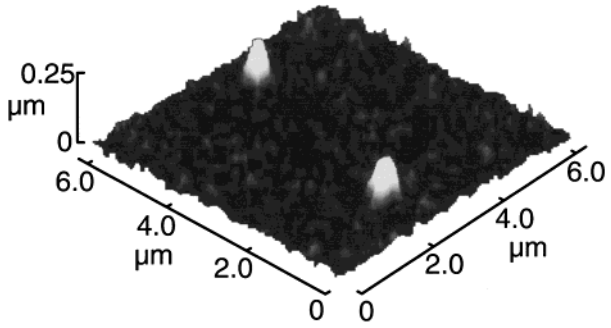

Figure 5. AFM image of compacted DNA−PNIPAAM conjugates observed on a self-assembled monolayer surface with close-packed methyl groups. Imaging was performed using a Digital Instrument Nanoscope IIIa in air under tapping mode, on a 256 × 256 pixel format. The average radius of collapsed DNA−PNIPAAM conjugates is found to be 0.3 μm.

Figure 4, the hydrodynamic radius of the collapsed DNA−PNIPAAM conjugates is found to be ca. 0.4 μm. The correspondence may be satisfactory, considering the effect of pretreatment of the specimen in the AFM measurement.

Discussion

The present results show that the DNA−PNIPAAM conjugates undergo a steep but continuous transition from an elongated state into a compact state at around 34 °C. Such a steep and continuous transition has been rather frequently reported for various phenomena as a cooperative and/or allosteric effect.[39−42] The notion of cooperation in the coil−globule transition in single macromolecules is the standard description found in textbooks of polymer science, whereas individual giant DNA molecules exhibit a large discrete transition between the elongated and compact states. In this case, the temperature range for the coexistence of elongated and compacted DNAs is rather wide. To clarify the nature of folding transitions, we plotted the size distribution against a free energy profile of individual DNA molecules. Figure 6 shows the free energy profiles of the DNAs (a) for DNA−PNIPAAM conjugates and (b) for native DNAs in the presence of 1.2 mM spermidine. The free energy difference is evaluated from the size distribution of the T4 DNAs as observed by fluorescence microscopy:

$$-\frac{\Delta G(L)}{kT} = \ln\frac{P(L)}{P_0} \tag{3}$$

where $P(L)$ and P_0 are populations with a long-axis length of L and the control, respectively. It should be mentioned that the population $P(L)$ inevitably contains experimental error due to the effect of image blurring.[22,36] The difference in the nature of the transitions thus becomes clear in terms of the change of the free energy. For the transition for DNA−PNIPAAM conjugate, the free energies always exhibit a single minimum. In contrast, for native DNA in the presence of spermidine, the free energy has double minima over a wide range of temperature. Thus, the folding transition of native DNAs by spermidine is classified as a first-order phase transition, according to the criterion of Landau[43] (see Appendix 1).

Summarizing the above discussion, in Figure 7 is shown a

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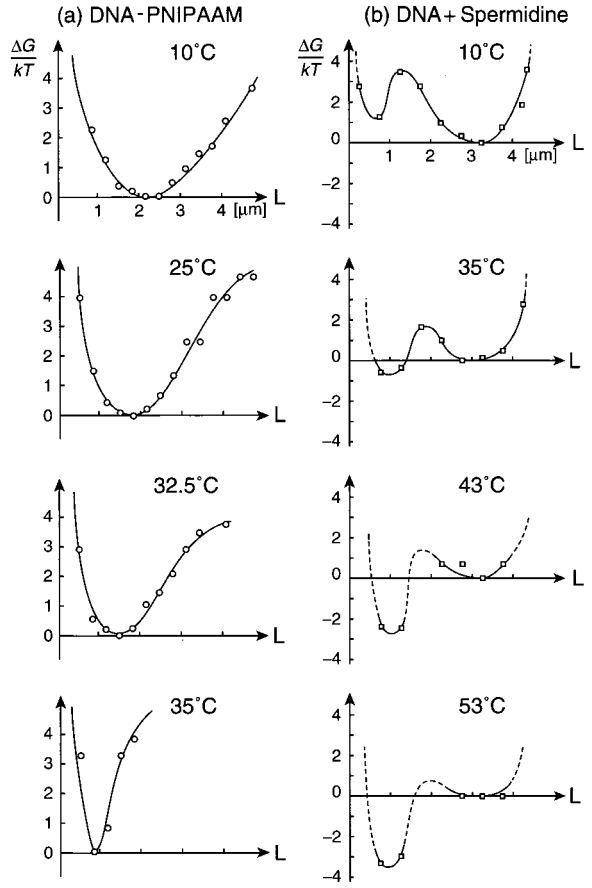

Figure 6. Change in the free energy profile of single giant DNA at various temperatures, as evaluated from the Boltzmann distribution in the histogram (Figure 3). (a) DNA−PNIPAAM and (b) native DNA in the presence of 1.2 mM spermidine (ref 34).

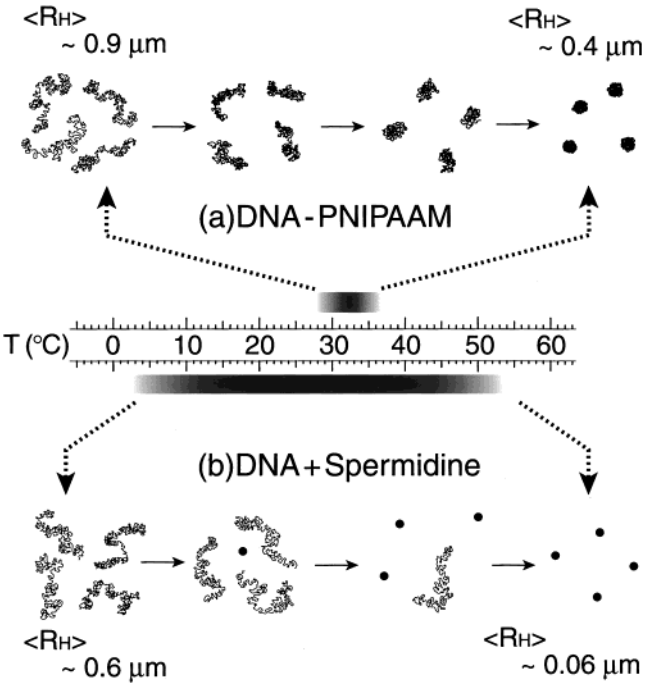

Figure 7. Schematic representation of the change in the conformation of individual DNA molecules at different temperatures. (a) The collapsing transition in the DNA−PNIPAAM conjugate is very sharp but continuous, while (b) the folding transition in native DNA in the presence of 1.2 mM spermidine is markedly discontinuous, although the ensemble of the DNA molecules shows a rather mild transition. The average hydrodynamic radii for native DNA are from ref 35.

schematic representation of the change in the conformation of T4 DNAs with temperature. In the ensemble of DNA chains, (a) for the DNA−PNIPAAM conjugates the transition is very sharp, and (b) for the native DNAs the transition is rather diffuse. In the characteristics of individual DNA chains, (a) for the DNA−PNIPAAM conjugate the transition is continuous, but (b) for the native DNA the transition is discrete. In the case of the DNA−PNIPAAM conjugate, the collapsing transition would resemble the transition into a liquidlike state. Thus, the morphology of the collapsed DNA−PNIPAAM conjugates is spherical, or globule, as shown in the AFM picture in Figure 5. The spherical structure in the "liquid droplet" is deduced from the condition to minimize the surface free energy. On the other hand, the folding transition of native DNA with spermidine resembles the transition into a crystal state. Actually, it is known that DNA segments make an ordered array in the toroidal DNA.[8,16,44−47]

The lack of discreteness for the steep transition of DNA−PNIPAAM conjugates is attributable to local inhomogeneity in the degree of modification with PNIPAAM chains (see Appendix 2). The microscopic inhomogeneity on the degree of binding of the conjugate along a single DNA chain may result in a decrease in the correlation length in the collapsing transition. Thus, an intermediate intrachain state with marked fluctuation of the segment density in a DNA is observed in the collapsing transition, as in Figure 2b. On the other hand, for the folding transition of native DNAs with a trivalent amine, the full molecular length is considered to remain within the correlation length in the first-order phase transition, under the condition of a small concentration of coexisting 1:1 ion.[15] Thus, the transition is markedly discrete at the level of individual DNA molecules; i.e., the transition is all or none. However, since the number of elements in the ensemble, or the number of segments in a single DNA, is finite, or much less than Avogadro's number, the "transition temperature" exhibits a finite, or rather wide, range (see Figure 7).[14]

Now, it may be of value to mention the future importance of the finding and the concept reported in the present study. According to the textbook interpretation of the collapsing transition, or the coil−globule transition, the product of the transition from a coiled single polymer molecule has been called a "globule".[23−25] This implies the expectation that the collapsed polymer should take spherical globular conformation. From our current study, long DNA chains take various kinds of non-spherical structures, such as toroid and rod, through the collapsing transition. The result of the present study indicates that the grafted DNA takes a spherical structure, exhibiting a size almost 1 order of magnitude larger than that in the typical compact folded state of the corresponding native DNA. Thus, a novel strategy to design the structure of nanoparticles made of single polymer chain is becoming clearer. Future efforts along this line would be promising. For example, if one can prepare

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a long DNA grafted on a specific region (for example, a GC-rich part), local selective folding/unfolding would become possible.

Conclusion

The essence of the results is summarized in Figure 7. At the level of individual DNA molecules, the collapsing transition of DNA−PNIPAAM conjugates is steep but continuous, in contrast to the discrete nature of the transition of native DNAs induced by a multivalent cation. For the ensemble of chains, the cooperative nature in the transition of DNA−PNIPAAM conjugates looks much stronger than that in the folding transition of native DNAs.

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Appendix 1

According to the modern theory in statistical physics, the first-order phase transition is interpreted with the change of the free energy F retaining the profile of double minima with respect to an "order parameter η" (see Figure 8).[43] In the context of the present study, the order parameter η is the density of the segments in individual DNA chains; $\eta \propto L^{-3}$, when we take long-axis length L of a DNA chain.

The essential feature of the first-order phase transition is represented with the following simple equation.[43]

$$F \sim (\eta + 1)^2(\eta - 1)^2 + \tau\eta \tag{4}$$

$$\tau = \alpha(1 - T/T_0) \tag{5}$$

where τ is a control parameter representing the temperature change, being proportional to the deviation from the critical temperature, and the constant α exhibits the physical meaning to show the degree of the temperature dependence. F can be either Gibbs or Helmholtz free energy, depending on the system to consider. For simplicity, we consider the two states, $\eta = -1$ and $\eta = 1$, corresponding to the double minima in the free energy, without sacrificing the generality. In the case of long DNA chains, both states coexist for a rather wide temperature range, as is schematically shown in Figure 7.

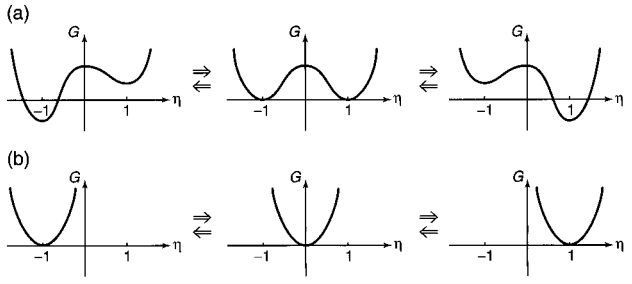

Figure 8. Schematic representation of the change of the free energy (a) for a first-order phase transition and (b) for a cooperative transition. For simplicity, the transition between the two states, with the order parameter of $\eta = \pm 1$, is shown.

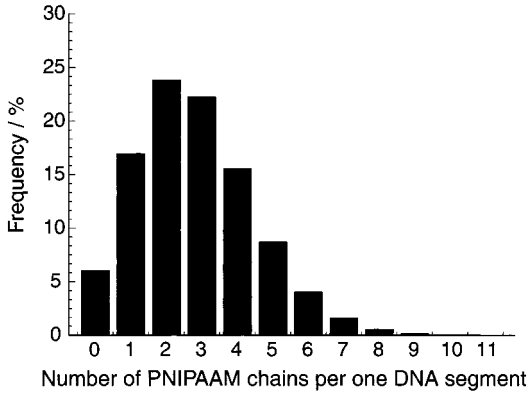

Figure 9. Theoretical expectation on the distribution of the number of PNIPAAM chains per Kuhn segment, ca. 300 bp, in DNA. Here, it is assumed that the PNIPAAM chains bind to DNA in a stochastic manner.

On the other hand, a cooperative phenomenon is represented as[48]

$$F \sim (\eta - \xi)^2 \tag{6}$$

$$\xi = \beta\left(\frac{-1 + \tau^n}{1 + \tau^n}\right) \tag{7}$$

Here again, we consider the transition between two state, $\eta = \pm 1$. τ also has a physical meaning similar to that in (5), and β is a constant. The parameter n is a positive rational number ($n > 1$), indicating the degree of cooperativity. Thus, there exists always a single free energy minimum during the cooperative transition, as shown in Figure 8b. With this framework, the sigmoidal curve as in Figure 4 observed for the DNA−PNIPAAM conjugate is interpreted.

Appendix 2

In the present experimental procedure on the DNA−PNIPAAM conjugate, the graft reaction was performed under the condition of [DNA] = 60 μM in base pairs and [PNIPAAM] = 0.5 μM in chains, indicating that ca. 1400 chains or less of PNIPAAM are grafted to one individual chain of T4 DNA, 166 kilo base pairs. It has been already established that the essentials of the nature of the folding transition of giant DNAs are interpreted with coarse-graining to the level of Kuhn length.[25] Since the T4 DNA chain is composed of ca. 500 Kuhn segments (Kuhn length, ca. 130 nm,[33] or on the order of 300 base pairs), the grafting condition of PNIPAAM can be regarded as a statistical exercise that 1400 samples (PNIPAAM chains) are distributed randomly to 500 "boxes" (Kuhn segments), where the occupation number is zero or a positive integer. The result of numerical calculation is shown in Figure 9. It is clear that the fluctuation in the number of PNIPAAM chains is rather large; for example, among the Kuhn segments of DNA (as long as 1000 Å!), ca. 20% segments have zero or only a single PNIPAAM chain. Even when the distribution of the PNIPAAM chains would exhibit a weak cooperative binding along a long DNA, the large fluctuation will survive on the scale of Kuhn length. Therefore, we may conclude that local inhomogeneity of PNIPAAM distribution is the definite cause for the diffuse nature in the transition of the grafted DNA.

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