Highly Effective Compaction of Long Duplex DNA Induced by Polyethylene Glycol with Pendant Amino Groups

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Abstract: By observing isolated long DNA chains with fluorescence microscopy, the collapsing transition of individual single DNAs induced by polyethylene glycol with pendant amino groups (PEG-A) was compared to that induced by neutral polyethylene glycol (PEG). The main results are as follows: (1) DNA is collapsed by PEG-A at concentrations 10^5 times lower than those required for PEG. (2) In PEG-A, the concentration which induces collapse decreases with an increase in the salt concentration. This trend is similar to that in the collapse induced by PEG. (3) The transition induced by PEG is all-or-none; individual DNA chains exhibit either elongated coil or compacted globule states. On the other hand, segregation of a collapsed region along a single DNA chain occurs as an intermediate state in the collapsing transition induced by PEG-A.

Introduction

Living cells are generally on the order of 10 μm in size. However, they use long DNA molecules on the order of several centimeters long as a source of genetic information. Such long DNA chains are usually highly compacted in both prokaryotic and eukaryotic cells.[1−5] Despite recent advances in our understanding of the primary structure of various DNA chains, including human genes, progress in studies on the manner of packing, or on the higher order structure, of long DNA chains within the cellular environment has been rather slow, and knowledge on this subject remains in a primitive stage. The mechanism of the self-regulated changes in the higher order structure of long DNA chains in living cells may be one of the most important unsolved problems in the biological sciences. A better understanding of the manner of packing of long DNA chains may lead to useful medical applications, such as gene therapy and artificial regulation of gene expression.[4−7]

In relation to the *in vivo* compaction of DNA chains, DNA condensation with various chemical agents has been extensively studied during the past several decades.[1−21] As has been indicated by Bloomfield,[5] several DNA molecules are incorporated into the condensed structure. Generally, the term *condensation* has been used for situations where a finite number of DNA molecules are compacted. Thus, there has been essentially no study on the *compaction* of single DNA in its genuine meaning due to the limitations of the available experimental techniques, such as light scattering, sedimentation, and CD spectroscopy.

Recently, from the direct observation of individual DNA molecules using fluorescence microscopy,[22,23] we found that a long duplex DNA chain undergoes a marked discrete transition between an elongated coiled state and a collapsed globular state.[24−27] The essential aspects of this transition are summarized as follows: (1) Individual DNA chains, more than several tens of kilo base pairs long, exhibit a discrete transition between an elongated coil and a compact globule. The change

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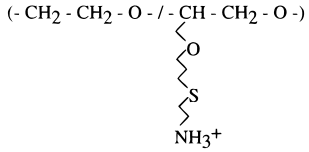
in the packing density of the segments in a DNA chain is more than an order of 10^4. This transition is reversible. (2) The discrete nature of the coil−globule transition is rather general, and the transition can be induced by various kinds of condensation agents such as neutral hydrophilic polymer (PEG), cationic surfactant (CTAB), alcohol, multivalent inorganic cation, and polyamine (spermidine and spermine), etc. (3). The phase transition diagram shows a region where the coil and globule states coexist: although the transition is discrete with regard to individual DNA chains, it appears to be continuous in the ensemble of monodispersed chains.

In the present paper, we describe the effect of polyethylene glycol with pendant amino groups (PEG-A) on the higher order structure of isolated long DNA chains and compare its efficiency in the compaction of DNA with that of PEG.



Poly(ethylene glycol) derivative with pendant amino groups (PEG-A)

Experimental Section

Materials. Amino pendant polyethylene glycol (PEG-A) was prepared as previously reported.[28] The molecular weight distribution was confirmed to be very narrow ($M_w/M_n = 1.06$, where $M_n = 2390$). The average number of amino groups in PEG-A was 4.65 per polymer chain. T4 phage DNA and pBR322 plasmid DNA were purchased from Nippon Gene (Toyama, Japan). The fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) and the antioxidant 2-mercaptoethanol (2-ME) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Fluorescence Microscopic Measurements. T4 DNA, 166 kbp with a contour length of 57 μm,[24] was dissolved in pure water, and DAPI and 2-ME were added to the DNA solution. The final concentrations were as follows: 0.6 μM T4 DNA (in nucleotide units), 0.6 μM DAPI, and 4% (v/v) 2-ME. It has been confirmed that the persistence length and the contour length of DNA remain essentially constant at such a low concentration of DAPI.[24] Compaction was induced by the addition of PEG-A to the DNA solution. Fluorescence DNA images were obtained using an Axiovert 135 TV microscope (Carl Zeiss, Germany) equipped with a 100 × oil-immersion objective lens and a high-sensitivity Hamamatsu SIT TV camera, which allowed recording of images on video tapes. The video image was analyzed with an Argus 50 image processor (Hamamatsu Photonics, Hamamatsu, Japan). Observation was performed at 20 °C.

Electron Microscopic Measurements. Samples used for electron microscopy were prepared by the addition of PEG-A to 0.6 μM DNA solutions. They were mounted on carbon-coated copper grids (no. 200), negatively-stained with 1% uranyl acetate, and observed with a JEOL 1200EX transmission electron microscope (Tokyo, Japan) at 100 kV.

Results

Parts a−e of Figure 1 show fluorescent images of T4DNA molecules in 60 mM NaCl solution in the presence of different concentrations of PEG-A, and parts a′−e′ of Figure 1 are the corresponding light-intensity distributions in the photographs, indicating the spatial density distributions of the DNA segments. Individual DNA molecules were observed as independent elongated coils in the absence of PEG-A (Figure 1a). With an increase in the concentration of PEG-A, as shown in Figure 1c, at [PEG-A]/[DNA] = 0.5 (= charge ratio) partially globular DNA appears, in which a compacted part and an elongated part

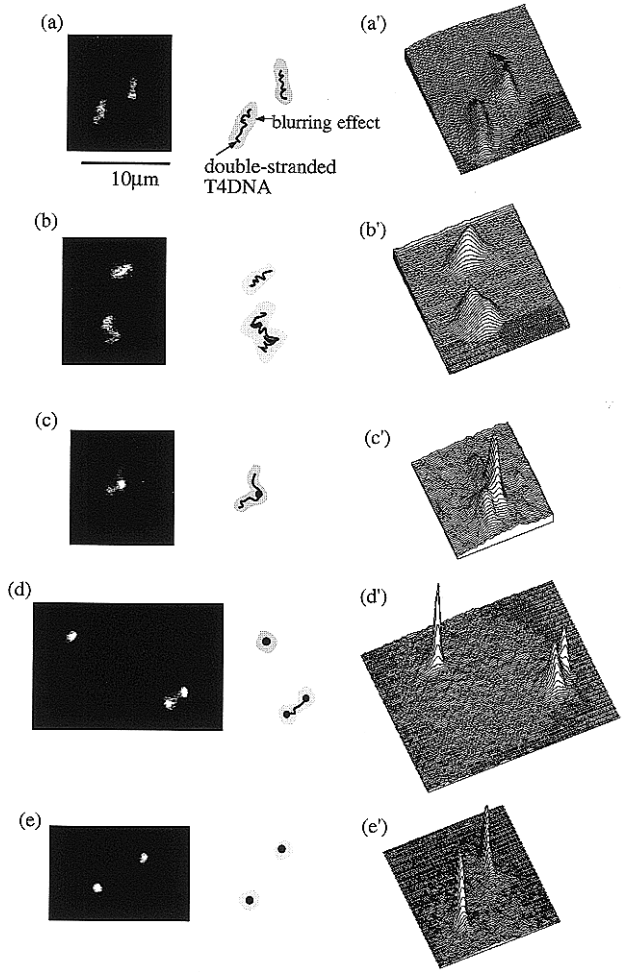


Figure 1. Change in the conformation of T4 DNA with the addition of PEG-A in a solution of 60 mM NaCl: (left) fluorescence micrograph, (middle) schematic representation, (right) quasi-three-dimensional representation, where the height corresponds to the fluorescent intensity. (a) Without PEG-A. The molar ratios, [PEG-A]/[DNA], are (b) 0.2, (c) 0.5, (d) 1.5, and (e) 3.0, where [PEG-A] is the concentration of the amino group.

coexist in a single chain; i.e., intrachain segregation is generated. Finally, when [PEG-A]/[DNA] = 3, all of the DNAs exhibit a completely compacted structure.

The effects of PEG-A on the apparent long-axis length of DNAs in pure water and in 60 mM NaCl solution, together with the morphological changes, are summarized in Figure 2. As for morphology, individual DNAs were classified into three different categories: (1) elongated coil, (2) partial globule, and (3) compacted globule. In the actual observation, it is easy to classify the morphology of the DNAs into these three classes on the basis of the apparent size and light-intensity distribution in time-successive video images. Figure 2 shows the distribution of the long-axis lengths at different concentrations of PEG-A, and a histogram is given together with the classification of the morphology (coil, partial globule, or globule). Note that a blurring effect on the order of 0.3 μm[7,24−26] is present, since the observation is carried out using visible light with a wavelength of ca. 0.4 μm, and partly because of the high sensitivity of the SIT camera. Thus, the actual size of the compact globule is expected to be less than 0.3 μm. This means that the elongated coil is more than 10-fold larger than the compacted globule. Thus, the effective volume of the coil is more than 10^3-fold greater than that of the globule.

On the basis of the distribution given in Figure 2, the changes in the average long-axis lengths for the individual morphologies

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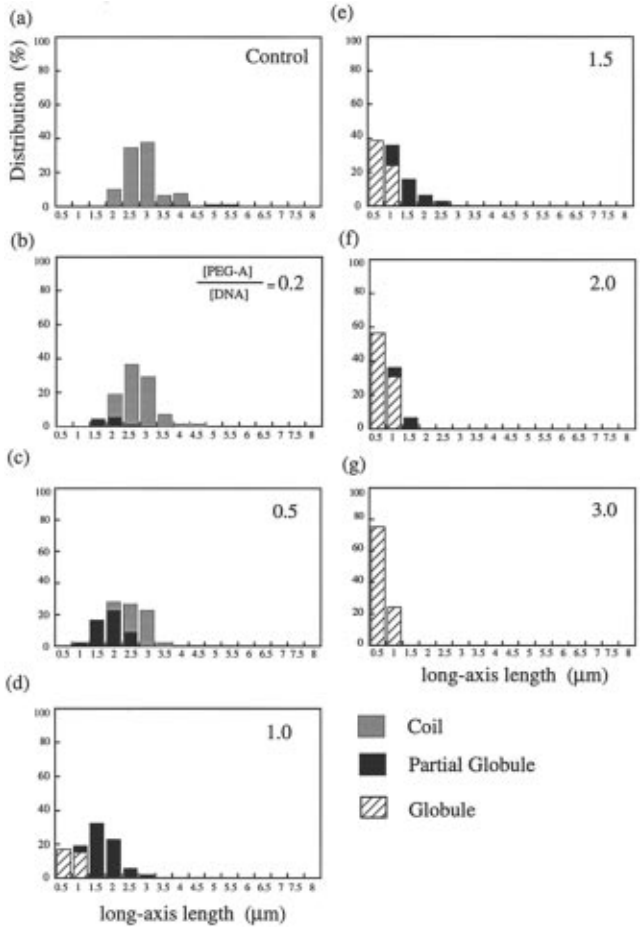

Figure 2. Distribution of the long-axis length depending on the PEG-A concentration in 60 mM NaCl. The DNAs are classified into three groups (coil, partial globule, and globule) based on time-successive images of thermally fluctuating objects.

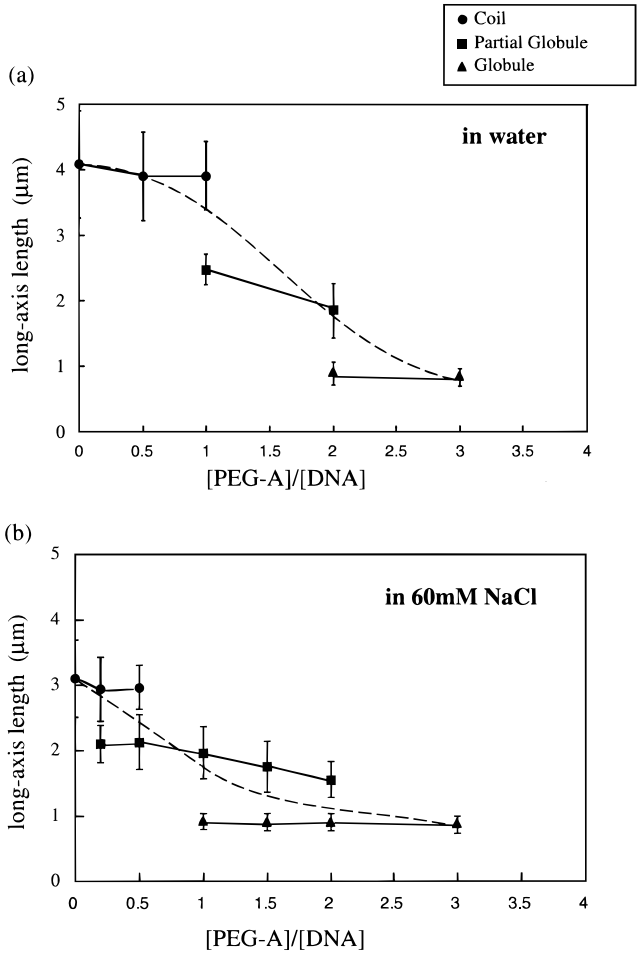

Figure 3. Change in the long-axis length depending on the PEG-A concentration. The average values are given for the three morphological categories. The broken line is the ensemble average for all of the DNAs.

are shown in Figure 3. It is clear that the ensemble average of the size for each morphology remains nearly constant with an increase in the PEG-A concentration. Therefore, the main cause of the change for the ensemble of DNAs is believed to be the change in the relative population of the coil, partial globule, and compacted globule morphologies. In contrast, the average size for all of the DNAs exhibits a continuous change that depends on the concentration of PEG-A.

To obtain insight into the actual size and shape of the globule, we performed electron microscopic observation of globular DNA collapsed with PEG-A. Figure 4a depicts the morphology of collapsed DNA observed at [PEG-A]/[DNA] = 2, where a flexible coiled part is connected with a compacted globular part. Figure 4b shows a completely collapsed globule at [PEG-A]/[DNA] = 3. We noticed that partially unfolded globules were often observed at [PEG-A]/[DNA] = 2, and that completely collapsed globules comprised the major morphology at higher PEG-A concentrations. This experimental trend in the morphology of DNA observed by electron microscopy corresponds well to that observed by fluorescence microscopy.

Discussion

Potential for DNA Collapse. PEG is one of the most frequently used chemicals for so-called ψ condensation.[9,19] In the present study, we have shown that PEG-A may be a much stronger condensing agent than PEG: (i) the ability of PEG-A to induce the collapse of single DNA is 10^5 times stronger than that of PEG, which implies that PEG-A can be used to purify DNA without increasing the solution viscosity; (ii) the collapsing transition induced by PEG-A is reversible; (iii) collapsed DNA exhibits a rather compact morphology, which should be suitable for purification.

In addition to these characteristics, the critical concentration for inducing the coil−globule transition decreases with an increase in the salt concentration in the solution. This property is similar to that with PEG.[25]

It is interesting to note that the effect of salt on DNA collapse is opposite that for collapse with polyamines[4,12,14] or with multivalent cations:[29] in this latter case, the critical concentration of polycations increases with an increase in the salt concentration. This retarding effect of salt on DNA collapse has been suggested to involve a change in the translational entropy of the counterions accompanied by ion exchange between monovalent and multivalent cations during the coil−globule transition.[29] On the other hand, the effect of salt on PEG-induced collapse has been attributed to the smaller loss of free energy in the process of increased cation binding for the negatively charged DNA chain.[25] The present results suggest that PEG-A-induced DNA collapse has properties similar to that induced by PEG, at least with respect to the salt effect.

Stepwise Compaction of DNA

The most significant finding in the present paper is the evidence of stepwise compaction for individual DNA chains. As has been reported recently, the coil−globule transition for

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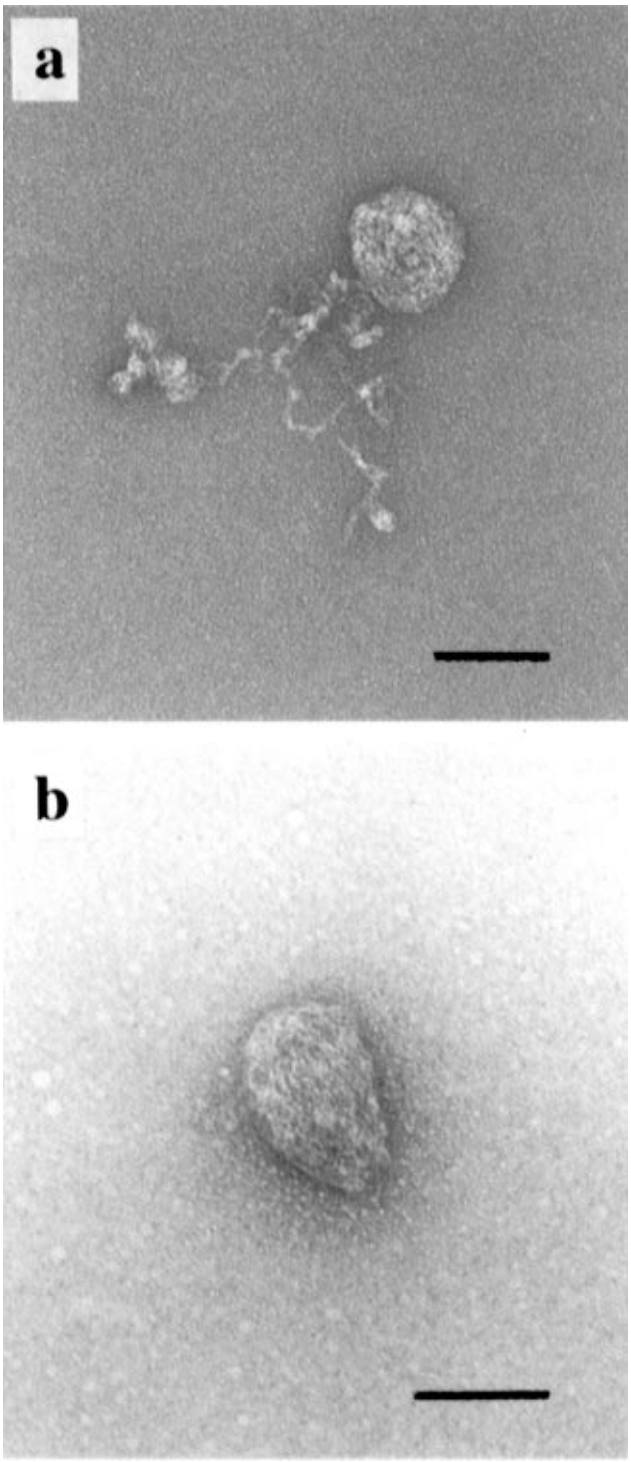

Figure 4. Transmission electron micrograph of collapsed DNA: (a) [PEG-A]/[DNA] = 2, (b) [PEG-A]/[DNA] = 3. The scale bar is 100 nm.

long DNA induced by PEG is largely discrete, i.e., all-or-none (see the schematic representation of the coil−globule transition in Figure 5).[25] The existence of a rather wide region of coexistence (with regard to the concentration of PEG) suggests that the difference in free energy between the coil and globule states remains within the order of thermal energy for a relatively wide range of PEG concentrations.

In contrast to the all-or-none nature of the PEG-induced transition for individual DNA chains, the PEG-A-induced transition exhibits two different coexistence regions; i.e., coexistence of the coil and the partial globule, and coexistence of the partial globule and the globule (Figure 5).

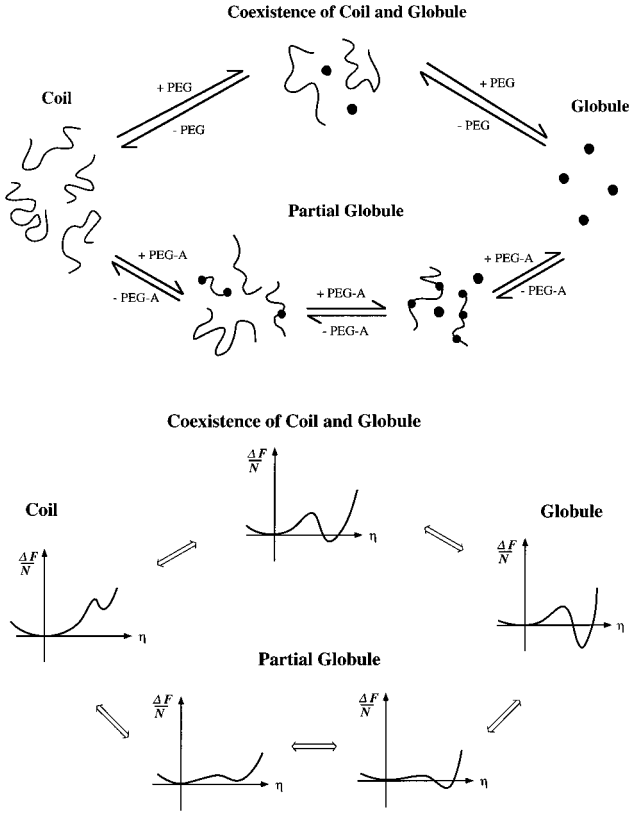

Figure 5. (a, top) Schematic representation of the morphological changes in DNA induced by PEG and PEG-A. (b, bottom) Change in the free energy profile corresponding to the morphological change given in (a). $\Delta F/N$ is the free energy per Kuhn segment with respect to the free energy minimum of the coil state.

Let us briefly discuss the marked difference in the transitions induced by PEG and PEG-A, on the basis of a consideration of the change in the free energy. As a reference state, we consider the elongated coil state with a characteristic one-dimensional size R_o[30] as the ensemble average of the radii of coiled DNAs. Taking the gyration radius for individual DNAs as R, a dimensionless parameter,[31] $\bar{\eta}$, for the mean density of the segments in a single DNA chain is written as

$$\bar{\eta} = (R_o/R)^3 - 1 \qquad (1)$$

Thus, $\bar{\eta} = 0$ corresponds to a coiled DNA chain of size R_o. As a next step in the discussion, we consider the spatial fluctuation parameter η along a single DNA chain to take into account intrachain segregation, as in the partial globule state. Thus, η reflects the segment density with coarse-graining of the DNA chain to the size of a "blob".[19] Using such an order parameter η that depends on the position along the DNA chain, the change in the free energy profile ($\Delta F/N$) may be depicted as in Figure 5b, where ΔF is the difference in free energy of a single DNA chain with respect to the coil state with $\eta = 0$ and N is the number of Kuhn segments.[30,32] In the case of T4 DNA, it is known that the persistence length λ is ca. 50 nm and the contour length is 57 μm.[13] Since the Kuhn length l is double the persistence length ($l = 2\lambda$), the total number of segments, N, is ca. $57/0.1 \cong 600$.

It is clear that all of the segments in a single DNA chain will be in either an elongated state ($\eta \approx 0$) or a collapsed state (η

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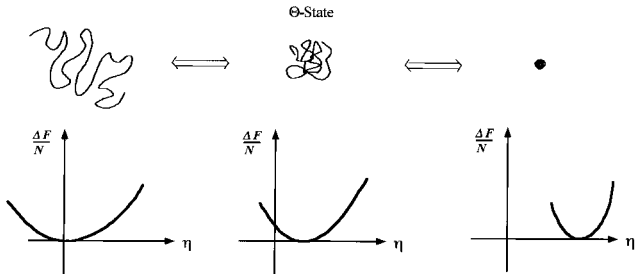

Figure 6. Change in the free energy profile for a continuous transition from a coil to a globule state.

is large) when the free energy minimum is deep enough for either a coil or a globule. The leftmost and rightmost profiles in Figure 5b correspond to the states in which all of the DNA chains exist in the coil state and the globule state, respectively. When the free energy barrier is high enough, as shown in the upper middle profile in Figure 5b, individual DNAs will exhibit either the coil or globule state; i.e., coils and globules will coexist. This is the case for the transition with PEG. On the other hand, if the free energy barrier in the bimodal profile is not high enough compared to the thermal energy (kT), elongated and condensed parts may coexist in a single DNA chain. Thus, when the free energy for the dispersed state ($\eta \approx 0$) is slightly deeper, one would expect coexistence between the coil and partial globule states. When the free energy for the condensed state is slightly lower, on the order of kT, one would expect coexistence between the partial globule and globule states. This would explain the observed experimental trend given in Figure 3b.

Difference from the Traditional View of the Collapse Transition of DNA. Although there have been numerous studies on DNA collapse, to the best of our knowledge, almost all of the previous experimental studies have suggested that the coil−globule transition is continuous not only for DNAs but also for other neutral and synthetic polymers.[4,14,33−35] This apparently continuous character seems to have been based on the traditional manner of thinking in polymer science,[32,36] which is shown schematically in Figure 6. The polymer chains shrink gradually, and pass through the so-called Θ state. This gradual collapse corresponds to the change in the free energy profile in the lower part of Figure 6. The steep and continuous transition may have been explained by the idea of "cooperative binding" of ligand molecules.[37,38] If we consider that ligand binding is "cooperative", we can estimate the degree of binding as

$$P = C^n/(a + C^n) \qquad (2)$$

where P is the degree of binding, C is the concentration of the ligand, n is an index to show the degree of cooperativity, and a is a constant. In our case, C corresponds to [PEG-A]/[DNA]. With the "reasonable" assumption that the degree of shrinking of the polymer chain is roughly proportional to the degree of binding, this resembles the traditional style of the analysis of DNA condensation induced by various condensing agents. However, the present study indicates that, at least in the case of the PEG-A interaction, DNA does not behave in accord with the above, traditional manner of thinking.

It has been supposed that changes in the secondary structure of the DNA chain play a role in the self-regulation of gene expression in living cells. It is expected that the interaction between DNA segments in the collapse state modifies the secondary structure of the double helix in appropriate sequences, and that such interaction may play an active role in DNA processing.[39] Actually, it has been shown that DNA condensation greatly promotes DNA strand exchange and renaturation in the absence of any proteins.[40] It was also found in some cases that supermolecular structures trigger the melting of specific sequences, which in turn induces transcription and replication.[39] It is natural to suppose that there is a close relationship between the secondary and tertiary structures in a DNA chain. As an extension of the new insight into the stepwise change in the higher order structure of DNA chains, studies of the changes in the secondary structure in relation to changes in the tertiary structure are eagerly awaited.

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