Transition of Double-Stranded DNA Chains between Random Coil and Compact Globule States Induced by Cooperative Binding of Cationic Surfactant

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Abstract: Interaction between cetyltrimethylammonium bromide (CTAB) and large T4DNA was studied by potentiometric titration using an ion-selective membrane electrode. The data obtained were assessed in terms of recently reported results regarding the higher order structure of T4DNA with the addition of CTAB: individual DNA chains exhibit discrete phase-transition between random coil and compact globule states. The apparent cooperativity represented as a sigmoidal function is attributed to bimodality in the distribution between the elongated coil and compact globule states of T4DNA chains. In addition, the globule-coil transition of single T4DNA is reversible, demonstrated by single-molecule observation with fluorescence microscopy. At a fixed concentration of CTAB, transition from the globule to the coil state is brought about by the addition of polyacrylic acid, suggesting that this reversible transition may be used for the purification of giant DNA molecules.

Introduction

According to previous experimental studies on the interaction between DNA and cationic surfactant, the binding of surfactant molecules to DNA is highly cooperative.[1-3] To explain this high cooperativity, theoretical studies have been performed, taking into account the interaction between neighboring binding sites. Various thermodynamic parameters, such as binding constants and cooperativity parameters, have been deduced from these studies. However, all of these theoretical analyses have been carried out based on the tentative assumption that the higher order structure of DNA does not change significantly. Contrary to this assumption, we recently found[4] that, with the addition of cationic surfactant, the higher order structure of large DNA chains changes in a discrete manner between elongated coil and compact globular states.

The significant finding in our previous study[4] was that individual DNA chains show discrete changes, or first-order phase transition. The discrete nature of the transition between coil and globule has been confirmed by single-molecule observation[5-8] with fluorescence microscopy. The main purpose of the present paper was to explain a discrepancy between previous studies and our recent finding, i.e., past studies have indicated that the transition is "cooperative" but continuous, while we noted that the transition is discrete for each DNA chain.

Recently, we also reported[4] the process of globule formation from coiled T4DNA under a concentration gradient of CTAB in aqueous solution. However, the dynamic aspects of the transition from globule to coil have not been studied. The recovery from the globule to the coil state seems to be important from a biochemical perspective. Precipitation of various DNA by cationic surfactants has been applied to the DNA extraction, concentration, and counting.[9-12] It has been shown that DNA is quantitatively precipitated by cationic surfactant. The DNA is then separated and recovered in a purified state by washing with mixed organic solvents. However, it is still unclear whether there is any change in the higher order structure of the purified DNA. It has been suggested that DNA can be recovered in a native state with 12-, 14-, and 16-carbon alkyltrimethylammonium bromides.[11] Therefore, a study on the higher order structure of single DNA, recovered after precipitation with cationic surfactant, is necessary.

In the present paper, we compared the results of direct observation by fluorescence microscopy to those of a potentiometric study: the former method provides information on the higher order structure of DNA and the latter affords information on the binding equilibrium of surfactant to DNA. We focused our efforts to clarify the relationship between "cooperative binding" and the structural transition between the elongated coil and compact globule states in DNA.

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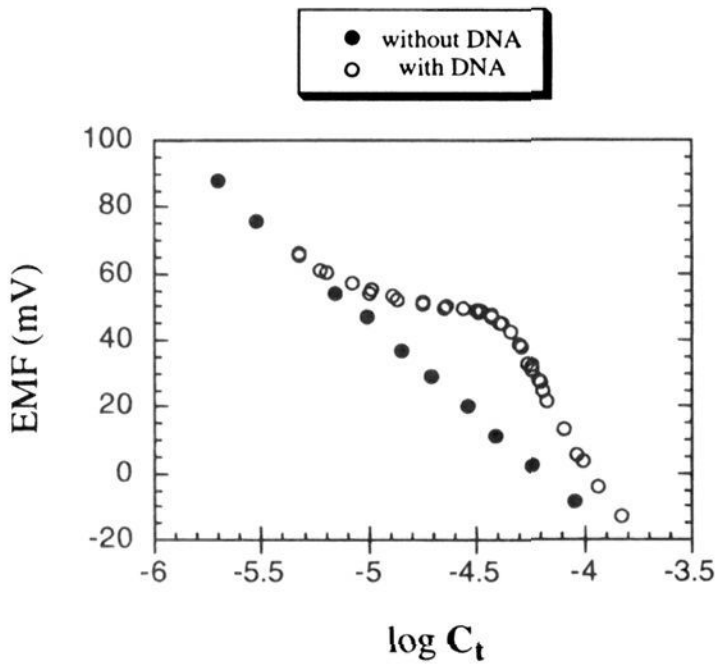

Figure 1. Electromotive force *vs* total CTAB concentration at 20 °C. Filled circles show the calibration curve, and open circles show the titration curve with T4DNA, [T4DNA] = 6.0×10^{-5} M in nucleotide.

Experimental Section

Materials. Bacteriophage T4dC DNA (166 kilobase pairs, contour length 55 μm[13]) was purchased from Nippon Gene. CTAB, obtained from Wako Pure Chemical Industries Ltd. and Tokyo Kasei Kogyo Co., was recrystallized twice from acetone and dried overnight at 25 °C under vacuum. Fluorescent dye, 4,6′-diamidino-2-phenylindole (DAPI), and antioxidant, 2-mercaptoethanol (ME), were obtained from Wako Pure Chemical Industries Ltd. Poly(vinyl chloride) (PVC, degree of polymerization 1020), tetrahydrofuran, and bis(2-ethylhexyl) phthalate were obtained from Kishida Chemical Co. and used to prepare the surfactant ion-selective membrane without further purification. Polyacrylic acid (PAA, M_w ca. 240 000) was purchased from Aldrich Chemical Co.

Potentiometric Titration. The cell for potentiometric titration was assembled as follows:

Reference electrode (Ag/AgCl) | 1 M NH_4NO_3 agar bridge | reference solution (CTAB in 0.5TBE buffer, 10^{-4} M) | PVC ion-selective membrane | sample solution | 1 M NH_4NO_3 agar bridge | reference electrode (Ag/AgCl).

The PVC ion-selective membrane was prepared as previously described.[3,14−16] Briefly, 0.5 g of PVC powder was mixed with 2 mL of bis(2-ethylhexyl) phthalate and 2 mL of 10^{-4} M CTAB solution in THF. This mixture was homogenized under gentle heating and stirring and then poured onto a glass Petri dish (10 cm diameter) with a loose cover. After gradual evaporation of THF over 2 days, the resulting membrane was cut into pieces and placed into a PVC tube (length 100 mm, internal diameter 9 mm) using a viscous PVC solution in THF as an adhesive. The electrode was then dried for 24 h at room temperature before the experiment.

The electrode was placed in standard CTAB solutions (from 3.0×10^{-6} M to 1.0×10^{-4} M) for 3 min, and a calibration curve was constructed from the millivolt readings for each standard. In general, electrode calibration was highly reproducible. A typical surfactant calibration curve is shown by the filled circles in Figure 1. As can be seen, the calibration curve is linear over the CTAB concentrations examined, indicating a Nernstian response with a slope of 58 mV per decade in the buffer. Binding isotherms were obtained by titration of T4DNA or PAA solutions in 0.5TBE buffer with standard CTAB solutions in a glass cell with a thermostating water jacket (NESLAB Exacal EX200 and NESLAB Endocal 350) at 20.0 ± 0.1 °C. The electromotive force of the cell was measured with a digital multimeter (Takeda Riken TR6844) with an accuracy of ±0.1 mV. The concentration of free surfactant was estimated using the above calibration curve.

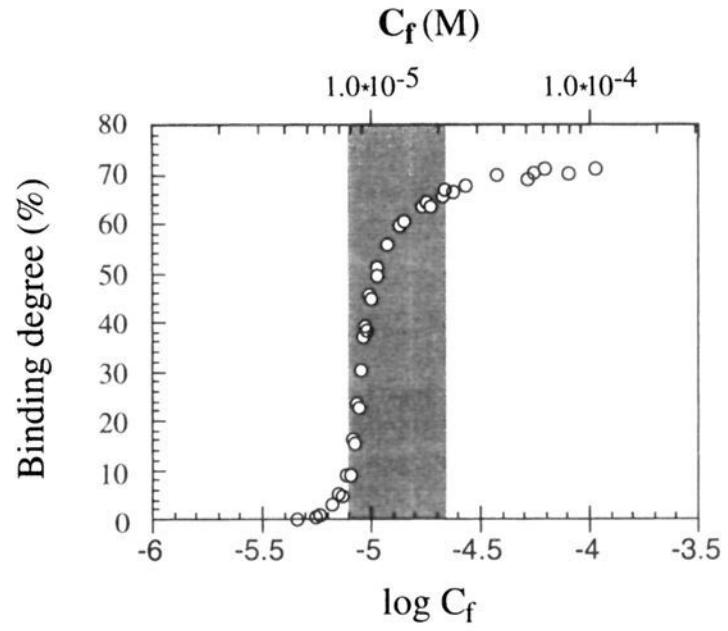

Figure 2. Binding isotherm of T4DNA−CTAB system in 0.5TBE buffer at 20 °C. The shaded region corresponds to the coexistence region, observed with fluorescence microscopy (see Figure 5).

Fluorescence Microscopy. A fluorescence microscopic study[7,8] was performed as follows: the samples were illuminated with 365-nm UV light, and fluorescence images of DNA molecules were observed using a Zeiss Axiovert 135 TV microscope equipped with a 100^x oil-immersed objective lens and recorded on S-VHS videotape through a high-sensitivity Hamamatsu SIT TV camera. The observations were carried out at room temperature, ca. 20 °C. The apparent length of the long axis L, which was defined as the longest distance in the outline of the DNA image, was evaluated with an image processor (Argus 10, Hamamatsu Photonics). Due to the blurring effect, L is estimated to be larger than the actual size by about 0.3 μm.[4] Sample solutions were prepared according to the previously described procedure.[4] Special care was taken to clean the glass microscope slides and coverslips thoroughly before the observations. They were soaked in hydrogen peroxide for more than 1 h, washed repeatedly with distilled water, and then immersed in ethanol for 1 h. Finally, they were dried at 35 °C for 30 min.

Results and Discussion

Potentiometric Study of the Binding Equilibrium of CTAB to T4DNA. The concentration of free surfactant ions in the presence of DNA was measured with an ion-selective membrane electrode. The plot of the electromotive force (EMF) vs the logarithm of the total surfactant concentration (C_t) is shown in Figure 1. In the presence of T4DNA, the calibration curve deviated from a linear relationship when the surfactant concentration exceeded 3.0×10^{-6} M. This deviation is believed to be due to the decrease in the free surfactant ion concentration caused by binding to oppositely charged DNA macroions. The amount of CTAB bound to the DNA chains, ΔC, is evaluated from the following relationship

$$\Delta C = C_t - C_f \quad (1)$$

where C_t is the total concentration of the added surfactant, and C_f is the free concentration of surfactant in the test solution, estimated from the calibration curve.

A binding isotherm (Figure 2) was derived from the potentiometric curve in Figure 1. The shaded region in Figure 2 corresponds to the "coexistence region", which has been confirmed by the measurement with fluorescence microscopy (see Figure 5). As for the significance of the "coexistence region", we will discuss it in the following section. The binding data are given in terms of binding degree, parameter β

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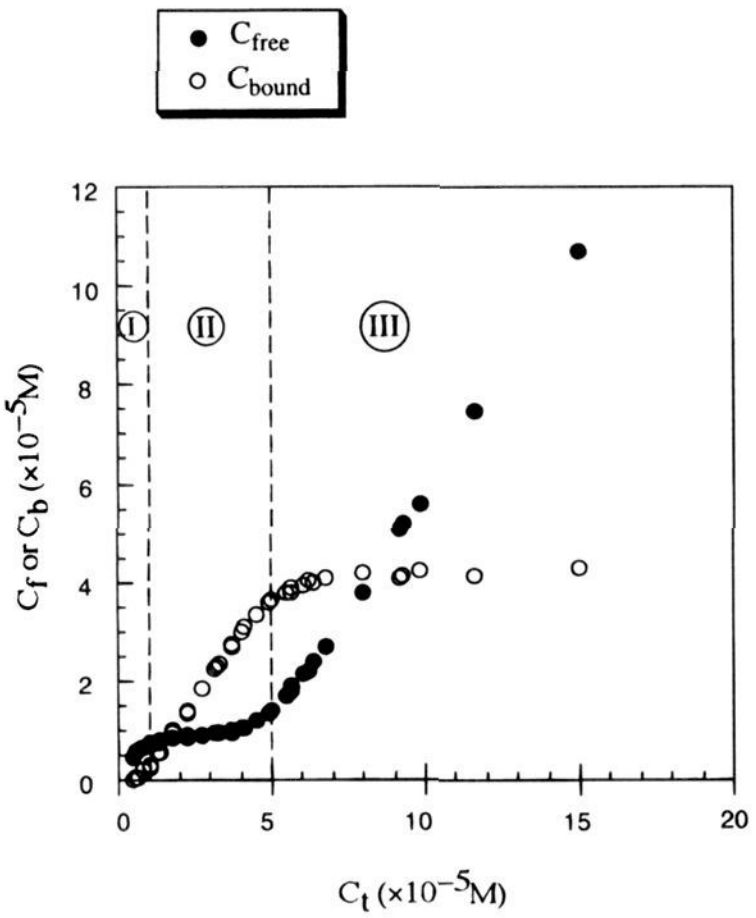

Figure 3. Free CTAB concentration (solid circles) and concentration of surfactant bound to T4DNA (open circles) *vs* the total surfactant concentration in the system at 20 °C. [T4DNA] = 6.0×10^{-5} M in nucleotide.

$$\beta = \frac{\Delta C}{C_a} \tag{2}$$

where C_a is the concentration of the ionic phosphoric group in T4DNA, or the concentration of DNA in nucleotide. In the actual experiment to obtain the binding isotherm, we started the measurement with a 6.0×10^{-5} M DNA solution and performed the potentiometric measurement with the successive addition of CTAB solution. The effect of the gradual decrease in T4DNA concentration with the addition of CTAB solution has been taken into account to obtain the correct β values.

A concentration of T4DNA of up to 6.0×10^{-5} M is 100 times higher than that for the measurement with fluorescent microscopy. Together with the results reported by Hayakawa et al.,[1] our present results demonstrate that the binding isotherm of alkyltrimethylammonium bromide to DNA is almost independent of the polyacid concentration at concentrations below [DNA] = 10^{-3} M. The isotherm has a typical sigmoidal shape, indicating that the binding is highly "cooperative". The onset of surfactant binding is recognized at around [CTAB] = 8.0×10^{-6} M, which corresponds to the so-called critical aggregation concentration (CAC).[17] It is interesting to note that the CAC is about 2 orders of magnitude smaller than the critical micelle concentration (CMC) of the CTAB solution without DNA.[18] Note that the β value does not reach unity and remains essentially constant around 0.7 for C_f values above 2.0×10^{-5} M. This behavior can be attributed to the combination of two different effects: Manning's counterion condensation effect[19,20] and a change in the higher order structure of DNA chains induced by surfactant binding. According to Manning's theory of polyelectrolyte dilute solutions[21] for double-stranded DNA, 76% of the polyion charge is compensated by the presence of condensed counterions. This means that binding is accompanied

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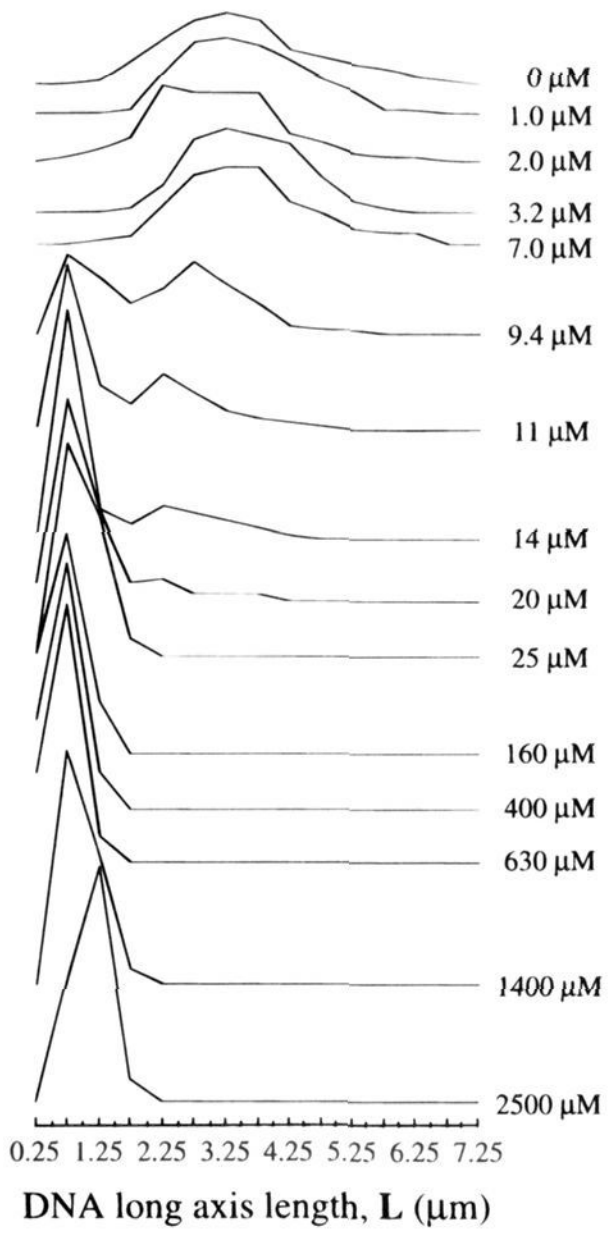

Figure 4. Distribution of long-axis lengths of T4DNA molecules at various CTAB concentrations. For the regions which showed only the coil or only the globule state, 100 T4DNA molecules were analyzed. In the coexistence region, 200 molecules were analyzed. The area of each histogram has been normalized to be equal. The experimental conditions are the same as in ref 4.

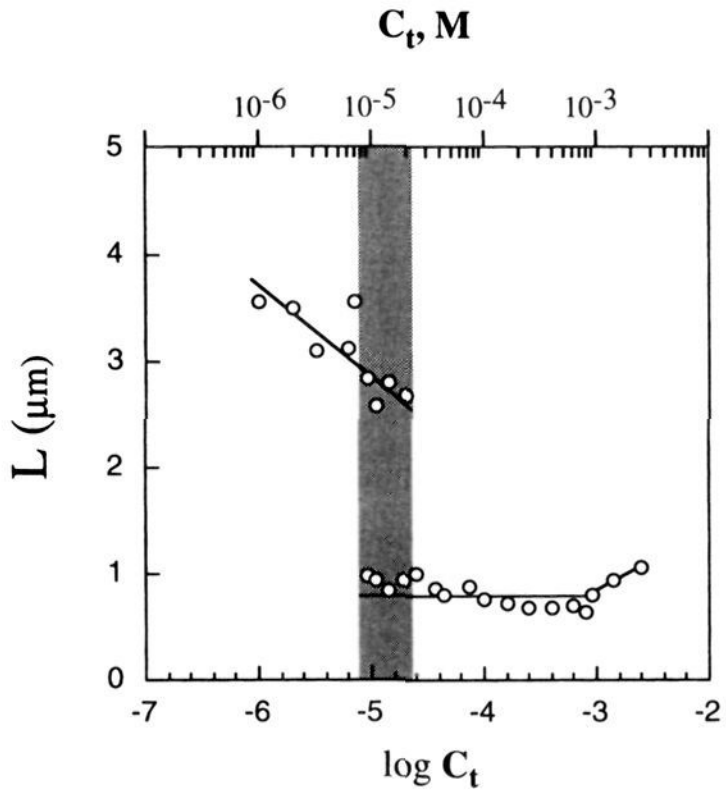

Figure 5. Long-axis lengths of T4DNA molecules *vs* the logarithm of the total CTAB concentration (the open circles indicate the maxima of the DNA-length distribution). The experimental conditions are the same as in ref 4.

Table 1. Average Long-Axis Length L of T4DNA Chains and the Standard Deviation As Determined from Fluorescence Microscopy

condn	coil state, without CTAB	globule state in the CTAB soln[a]	8:1 molar excess of PAA to CTAB[a]	4:1 molar excess of PAA to CTAB[a]	2:1 molar excess of PAA to CTAB[a]	50 times dilution of the CTAB soln[a]	100 times dilution the CTAB soln[a]
L, μm	3.53	0.82	3.48	3.42	3.56	3.40	3.50
standard devn, μm	0.61	0.24	0.66	0.68	0.74	0.56	0.64

[a] [CTAB] = 5.0×10^{-5} M.

by an exchange reaction with the small counterion, which in the present study is Na^+. Figure 2 shows that the β value is very close to 0.76, as expected from the Manning's theory. The condition of $\beta = 1$ corresponds to the 1:1 binding of CTAB with ionized phosphate groups of DNA molecules. However, a 1:1 complex of CTAB with DNA may not be attained in actual experiments due to geometric restriction arising from the steric size of the detergent head group. The head size of the alkyltrimethylammonium ion is estimated to be 6.9 Å from CPK space-filling models.[1] On the other hand, the nearest charge–charge distance is about 5 Å for the B-form of double-stranded DNA.[22] Since the size of the ionic head group of CTAB is larger than the charge–charge distance in DNA, steric hindrance may play an important role in limiting the degree of binding.

Figure 3 shows the change in the concentration of free and bound CTA ions with a change in the total CTAB concentration at a fixed DNA concentration (6.0×10^{-5} M in nucleotide). The most significant aspect of Figure 3 is the existence of an intermediate region, II, where the bound fraction increases linearly with an increase in the total concentration, C_t, of CTAB. In contrast, the free fraction shows only weak dependence on C_t. In surfactant solutions, the fraction of surfactant ions generally remains fairly constant above CMC. The result in Figure 3 suggests that in region II the behavior of the surfactant ions is similar to that above CMC in normal surfactant solutions. Thus, there may be a type of critical micelle concentration in the space occupied by the long DNA chain;[1] the contour length of T4DNA is ca. 55 μm.[13] However, since the bound fraction, or the surfactant ions bound to DNA, has an upper limit due to the finite number of negatively charged binding sites in diluted DNA solution, C_f increases in region III, accompanying the increase in C_t. Again, the critical concentration, CAC, between regions I and II is 2 orders of magnitude less than the CMC of CTAB without the polyanion.

Transition of the Higher Order Structure in T4DNA. Let us compare these data with the results obtained by fluorescence microscopy. As we have recently reported,[4] T4DNA molecules exhibit conformational transition from a coiled to a globule state with the addition of CTAB. In Figure 4, the distribution of the long-axis length L of T4DNA chains is shown with different CTAB concentrations. At a CTAB concentration of less than 9.4×10^{-6} M, all of the T4DNA molecules exhibit the elongated coil state, whereas only collapsed DNAs in the globule state are found when the surfactant concentration is more than 2.0×10^{-5} M. Between these two CTAB concentrations, both the coil and globule states coexist, as shown in Figure 4. Figure 5 shows the maxima in the distribution dependent on C_t. Individual DNA chains exhibit either the coil state or the globule state in the coexistence region (shaded region in Figure 5). In other words, individual chains show a discrete transition, which is typical of a first-order phase transition. However, if, instead of Figure 5, a graph is made for the ensemble average, the transition curve becomes rather sigmoidal, indicating typical "cooperative" behavior. It now becomes possible to evaluate the number of surfactant ions bound to a single T4DNA under

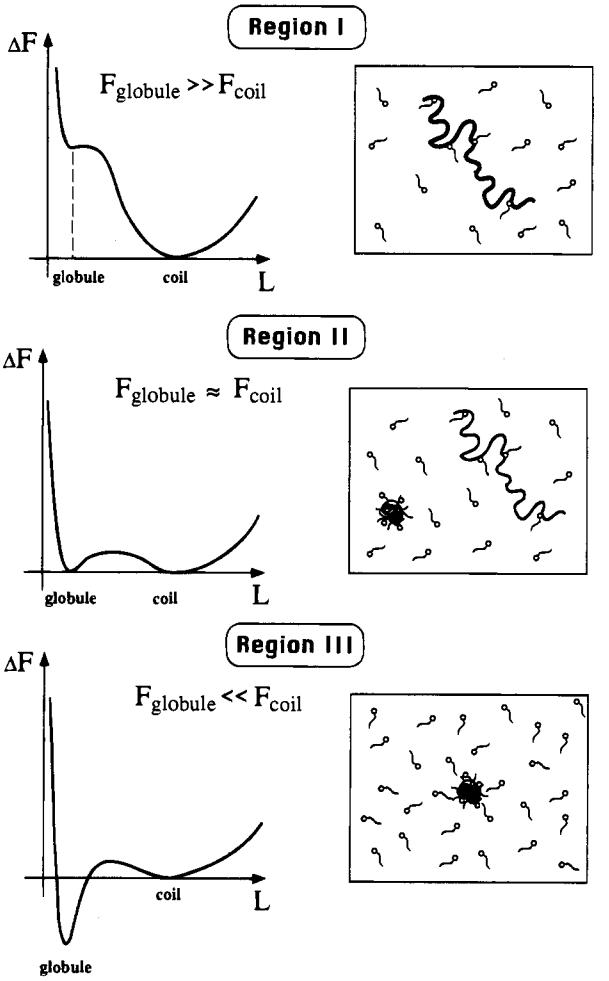

Figure 6. Schematic representation of T4DNA coils (region I), globules (region III), and the coexistence of coil and globule states (region II) with respect to the free energy (ΔF) of single T4DNA chains in the CTAB solution. F is the energy of individual DNA chain, and ΔF is given by $\Delta F = F_{globule} - F_{coil}$.

conditions of saturated binding. In the globule state, about 240 000 surfactant ions are estimated to bind to a single T4DNA.

Bimodality in the Distribution and the "Cooperativity". The coexistence region of the coil and globule states (C_t in Figure 5) almost coincides with the region of "cooperative" binding in the binding isotherm (C_f in Figure 2), despite the difference in the notation of the concentration: C_t or C_f. This may be due to the fact that under the experimental conditions for the measurement with fluorescent microscopy, the bound fraction of CTAB was negligible because the DNA concentration was quite small (6.0×10^{-7} M in nucleotide).

If we compare the results in Figures 3 and 5, the essential nature of the "cooperativity" becomes more obvious. Region II in Figure 3 corresponds to the coexistence region in Figure 5, indicating that the structural change from coil to globule induced by binding with CTAB is discrete for individual DNA chains. On the other hand, since there is a rather wide region for the bimodality of the distribution, or the coexistence of coil and globule, the concentration of the bound surfactant ions

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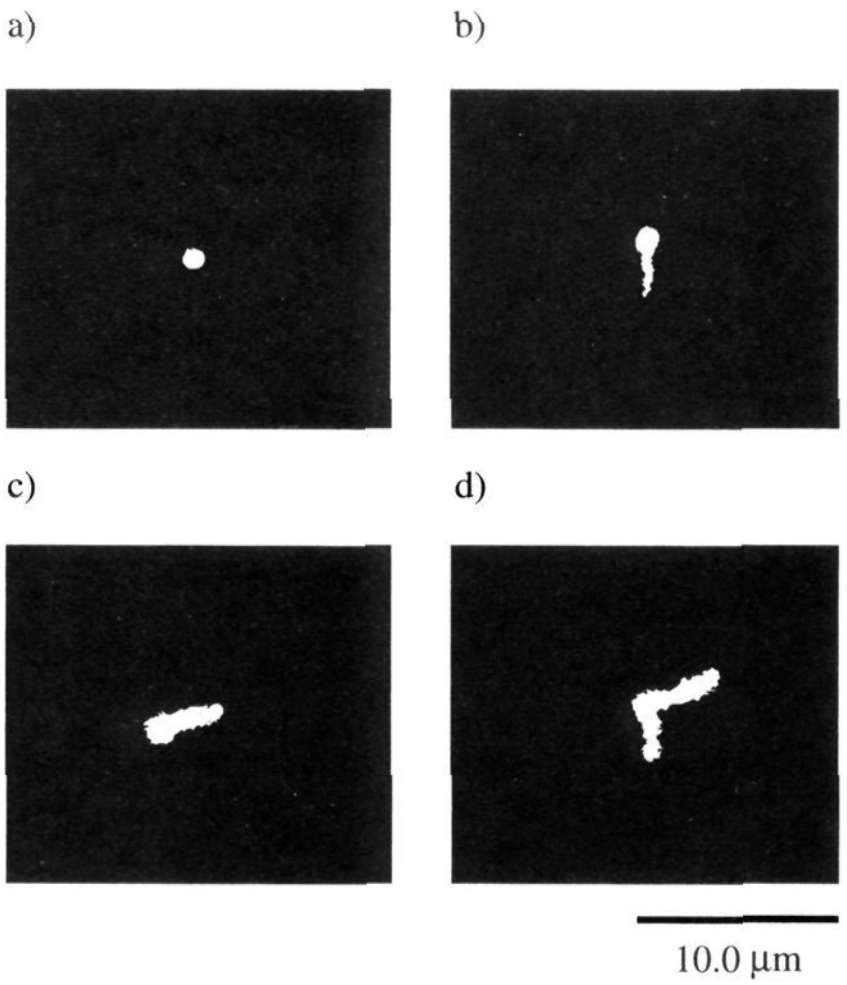

Figure 7. (Top panels, a–d) coil formation from a single globular T4DNA in buffer solution at [CTAB] = 5.0×10^{-5} M under a concentration gradient of polyacrylic acid. The concentration of PAA solution on the border of the coverslip was 1.0×10^{-2} M. (Bottom panels, a–d) light intensity distribution in photographs of coil formation from the globule state of T4DNA in buffer solution at [CTAB] = 5.0×10^{-5} M under a concentration gradient of PAA (panels a–d coincide with a–d in the top panel).

appears to reflect a "cooperative", continuous transition. A similar continuous change with a sigmoidal curve in the interaction between DNA and cationic surfactant has been reported using potentiometric titration.[1–3]

On the basis of the above results and discussion, the change in free energy, F, can be schematically presented as shown in Figure 6. In region I, with low surfactant concentrations, the free energy of the DNA coil is sufficiently lower than that of the DNA globule. Thus, only the coil state is stable. As the CTAB concentration increases, the difference in free energy between the coil and globular states of DNA becomes on the same order as the thermal energy kT (region II). Here, we can expect the coexistence of the coil and globule states, as is actually observed in Figures 4 and 5. In region III, the free energy of globular DNA is much smaller than that of coiled DNA, and only the globule state can exist.

Reversibility of the Coil–Globule Transition. Next, we address whether the structural change between coil and globule is reversible. We prepared a sample with T4DNA in a globular state ([CTAB] = 5.0×10^{-5} M) and diluted it 50- and 100-fold with 0.5TBE buffer, containing 4% (v/v) ME. With this dilution procedure, a change from the globule to the coil is expected if the transition is reversible (see Figures 2 and 5). The long-axis length L of 100 T4DNA molecules for each condition was measured using fluorescence microscopy. Table 1 shows that the L value for the coil recovered after dilution is essentially the same as that for coil chains prepared in the control solution of T4DNA without CTAB. This result suggests that the cationic surfactant does not induce irreversible denaturation of the DNA molecules. This result may be important, since cationic surfactants are frequently used[9–12] as precipitation agents for DNA in order to purify DNA samples.

It is well known[23] that various anionic polyelectrolytes affect processes in the cell nucleus. Therefore, we examined the effect of PAA on the complex formed between DNA and oppositely charged surfactant. A sample of globular T4DNA with [CTAB] = 5.0×10^{-5} M was prepared, and a drop of 1.0×10^{-2} M PAA solution in 0.5TBE buffer was placed on the border of the coverslip. Under these conditions, the transition from globule to coil can be observed. An example of the globule–coil transition of a single T4DNA is shown in Figure 7 (top panels). Figure 7 (bottom panels) shows the spatial distribution of the fluorescence intensity for the DNA chain. It is clear that PAA induces the transition from globule to coil for T4DNA chains.

Table 1 shows the long-axis length L for coiled DNA chains recovered under different conditions, i.e., with the molar ratio between T4DNA and PAA at a fixed concentration of T4DNA (0.6 μM in nucleotide). Under these conditions, the size of the coil DNA is almost the same as above.

Description of the Binding Equilibrium with a "Cooperative" Scheme. It has been demonstrated above that the sigmoidal curve for the transition, reported previously, should be interpreted in terms of the intrinsic properties of the coil–globule transition, i.e., discrete for individual chains and continuous for the ensemble average. In this section, we will analyze our present results based on the "classical model" for a "cooperative transition". It will be shown that the binding isotherm obtained in the present study can be interpreted rather well using the classical idea of "cooperative binding", although the real characteristics are quite different from the "classical model" as has been discussed in the previous sections.

According to the Ising model,[24] a linear biopolymer is considered as a linear lattice of equivalent binding sites, vacant or occupied, with nearest neighbor interaction. The partition function Z in this case can be expressed as

$$Z = (1,1)\begin{pmatrix} 1 & 1 \\ s & \\ \overline{u} & s \end{pmatrix}^n \begin{pmatrix} 1 \\ 0 \end{pmatrix} \tag{3}$$

$$s = KuC_f \tag{4}$$

where u is an equilibrium constant of interaction between the

bound surfactant molecules, n is the number of binding sites on the biopolymer molecule (in our case DNA), and K is an equilibrium constant of the binding of a single surfactant molecule to isolated binding sites of the biopolymer. From such a theoretical model, we can expect cooperative behavior for the transition, with a sharp but continuous transition curve. In fact, we can obtain the following thermodynamic relationship[25,26] regarding the surfactant−DNA interaction

$$Ku = \frac{1}{(C_f)_{\beta=0.5}} \tag{5}$$

$$\left(\frac{d\beta}{d \ln C_f}\right)_{\beta=0.5} = \frac{\sqrt{u}}{4} \tag{6}$$

where $(C_f)_{\beta=0.5}$ is the free surfactant concentration at the half-bound point, estimated from the binding isotherm. Equation 6 was also proposed by Schmitz and Schurr[27] in their theoretical consideration of cooperative binding of adenosine by polyuridic acid. From eq 5, it is possible to evaluate the Ku value. In our case, the Ku(T4DNA) value was found to be 9.3×10^4 M^{-1} L. u can be estimated by fitting the binding isotherm to eq 6. Following Manning's notion[19,20] that the electrostatic potential on the surface of polyelectrolyte remains relatively constant until the surface-charge density reaches a certain level, we use the lower part of the binding isotherm (Figure 2), with actual binding of less than 40%. With this limitation, the u value was 80 with a standard deviation of about ±20%. With the same order of precision, K(T4DNA) was calculated to be 1.2×10^3 M^{-1} L. These values are consistent with the previously reported results.[1]

Using a similar procedure, the Ku(PAA) value in our system was 2.7×10^5 M^{-1} L. This indicates that the attractive interaction of surfactant ions with PAA is more favorable than that with T4DNA molecules.

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