Cooperativity or phase transition? Unfolding transition of DNA cationic surfactant complex

Sergey M. Mel'nikov[a)]
Division of Informatics for Natural Sciences, Graduate School of Human Informatics, Nagoya University, Nagoya 464-01, Japan

Vladimir G. Sergeyev
Department of Polymer Science, Faculty of Chemistry, Moscow State University, Moscow 119899, Russia

Kenichi Yoshikawa[b)]
Division of Informatics for Natural Sciences, Graduate School of Human Informatics, Nagoya University, Nagoya 464-01, Japan

Hiroshi Takahashi and Ichiro Hatta
Department of Applied Physics, Nagoya University, Nagoya 464-01, Japan

(Received 21 May 1997; accepted 23 July 1997)

We recently reported that single duplex DNA, with the size above the order of several tens kilobase pairs, undergoes a large discrete transition from an elongated coil into a collapsed globule with the addition of a cationic surfactant. In the present article, we describe the manner of the unfolding transition of compact long DNA, or globule DNA, complexed with cationic surfactants, cetyltrimethylammonium bromide (CTAB) and distearyldimethylammonium bromide (D_{18}DAB), as is induced by the addition of sodium bromide. The conformational dynamics of individual single duplex T4DNA molecules was directly observed with the use of fluorescence microscopy. We found that on the level of individual DNAs, the salt-induced unfolding transition of the globules is largely discrete, or first-order phase transition for the both complexes with CTAB and D_{18}DAB. On the other hand, for the ensemble average of the DNAs, the transition is discrete with CTAB but is continuous (sigmoidal) with D_{18}DAB. The discreteness for the coil-globule transition in the ensemble of DNAs complexed with CTAB is attributed to the existence of the phase transition in whole over the bulk solution: the sphere-rod transition in surfactant micelles. On the other hand, for D_{18}DAB such phase transition on the micelle structure in the bulk solution seems to be absent. In correspondence to such a large difference on the manner of the transition, x-ray diffraction analysis indicates marked difference on the structure of DNA complexes with CTAB and with D_{18}DAB.
 [S0021-9606(97)50541-1]

I. INTRODUCTION

Increasing interest has been directed to aqueous systems containing polyelectrolytes and oppositely charged surfactants, both for technological and biomedical importance.[1–3] Interaction between DNA polyion and oppositely charged surfactants results in ''complex'' formation, which exhibits considerable importance not only in the field of physical chemistry, but also for gene transfection.[4–8] The binding of surfactant ions to DNA macromolecules is highly cooperative due to hydrophobic effect and has been often interpreted with the term of phase transition.[9–11] However, all of the past studies on the surfactant binding have shown a *continuous transition* between free and completely bound polyelectrolyte.[12–14]

Contrary to this, we have recently found that by the addition of cationic surfactants even with the concentrations far below the critical micelle concentration (CMC) individual long DNAs exhibit large discrete first-order phase transition between elongated coil and collapsed globule, by use of the experimental technique of single DNA observation with fluorescence microscopy (FM).[15–17] It has become clear that *the ensemble average of DNA chains* exhibits steep but *continuos character* of the transition. In other words, although the transition is markedly *discrete on the level of single chains*, the change on the level of ensemble looks like a typical *cooperative transition* without any discrete character due to the presence of a considerably wide coexistence region of coil and globule states.

To obtain a better understanding on the conformational change in single DNA macromolecules both on the level of single chain and on their ensemble average, we have focused our work on the effect of low-molecular-weight electrolyte on the unfolding transition of long DNA chains complexed with monoalkyl cationic surfactant, cetyltrimethylammonium bromide (CTAB), and dialkyl cationic surfactant, distearyldimethylammonium bromide (D_{18}DAB).

II. EXPERIMENT

A. Materials

T4DNA, 166 kilobase pairs (contour length 57 μm),[18] was purchased from Nippon Gene. Monodispersity of

[a)]Also at Department of Polymer Science, Faculty of Chemistry, Moscow State University, Moscow 119899, Russia.
[b)]Author to whom correspondence should be addressed.

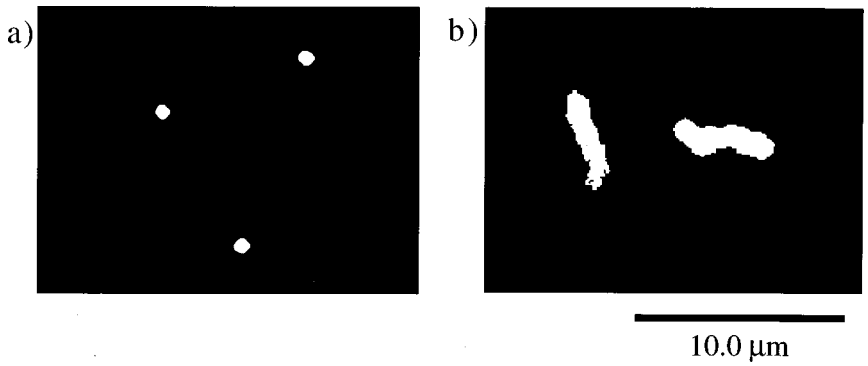

FIG. 1. Fluorescence images of typical globule and coil DNAs. (a) With $[CTAB]=1\times10^{-4}$ M and (b) addition of 0.5 M NaBr to (a).

T4DNA was checked by gel electrophoresis in 0.5×TBE buffer (45 mM tris, 45 mM borate, 1 mM EDTA (ethylenediaminetetraacetic acid), pH 8.0). The concentration of T4DNA was determined spectrophotometrically; the molar extinction coefficient for DNA bases was 6600 M^{-1} cm^{-1} at 260 nm. The ratio of the absorbance of T4DNA stock solution at 260 nm to that at 280 nm was 1.8. The cationic surfactants, CTAB and D_{18}DAB were obtained from Tokyo Kasei. CTAB was recrystallized twice from acetone and dried overnight under a vacuum at 25 °C. The fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) and the antioxidant 2-mercaptoethanol (ME) were obtained from Wako and used without further purification. ME, a free radical scavenger, was used to reduce fluorescence fading and light-induced damage of DNA molecules. Sodium bromide (Wako) was dried before use.

B. Methods

Fluorescence microscopic measurements were performed as follows. T4DNA molecules were diluted in 0.5 ×TBE buffer solution containing 4% (v/v) ME and DAPI. The final concentrations of DNA and DAPI were 0.6 μM in nucleotide units and 0.6 μM, respectively. Under these conditions, the number of DAPI molecules bound per base pair is estimated to be 0.05,[19] and the persistence length is expected to remain nearly the same as in the absence of DAPI.[18,20] 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× oil-immersed objective lens. The time series of the DNA images were recorded on S-VHS videotapes through a high-sensitivity Hamamatsu SIT TV camera. 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 0.3–0.5 μm.[15] Sample solutions, microscope slides, and coverslips were prepared as previously described.[15–17] It has been ascertained that the reported results do not depend on the timing of the mixing of the reagents.

Viscosity was measured by a Tokimec Visconic ELD viscometer at 35.0± 0.1 °C. Viscometer was calibrated with JS 2.5 calibration liquid (Showa Shell). For fluorescence microscopy and viscometry, the temperature was kept constant at the desired temperature with the precision of ± 0.1° using a water jacket, connected to a Neslab RTE-111 thermostating system. Fluorescence spectra were recorded on a Shimadzu UV-2200 spectrophotometer.

The samples for the x-ray diffraction experiments were prepared as follows. DNA was dissolved in 0.5×TBE buffer at 6.0×10^{-5} M in nucleotide units. Under gentle stirring, an aqueous solution of CTAB was added to the DNA solution in an equimolar ratio. The resulting precipitate was centrifuged for about 15 min at 3000 rpm using a KS-5000P centrifuge (Kubota, Japan). The resulting wet precipitate was used for x-ray diffraction measurements, which were performed using a double-mirror focusing camera with nickel-filtered Cu $K\propto$ radiation from a rotating anode RU200BEH x-ray generator (Rigaku, Tokyo, Japan) with a power of 1.2 kW. The sample-to-detector distance was about 220 mm. The typical exposure time was 15 h. X-ray diffraction patterns were recorded on imaging plates that measured 20 ×25 cm^2 (BAS-III, Fuji Photo Film Co. Ltd, Tokyo, Japan). The data on the imaging plates were digitized on a BAS2000 system (Fuji Photo Film Co. Ltd., Tokyo, Japan). Two-dimensional diffraction patterns on the imaging plates were transformed into one-dimensional data as described elsewhere.[21] This process was performed on a FACOM M-780 computer at the Computation Center of Nagoya University.

III. RESULTS

Figure 1 shows typical fluorescence micrographs on (a) collapsed globules of T4 DNA complexed with CTAB, and (b) elongated coiled T4DNA unfolded from the state of (a) by the addition of small electrolyte, [NaBr]=0.5 M. With the conditions that $[CTAB]=1.0\times10^{-4}$ M and very low DNA concentration (0.6 μM in nucleotide) in the absence of NaBr, all of the T4DNA molecules were found to be in the collapsed globule state, without aggregation between DNA chains, as was confirmed with the time-sequenced observation with FM. Figure 2(a) shows the change in the distribution of the long-axis length **L** of T4DNA at $[CTAB]=1.0\times10^{-4}$ M, depending on the NaBr concentration. Figure 2(b) shows the ensemble average and the width of the distribution as the mean square deviation for the results given in Fig. 2(a). It is obvious from Fig. 2 that when

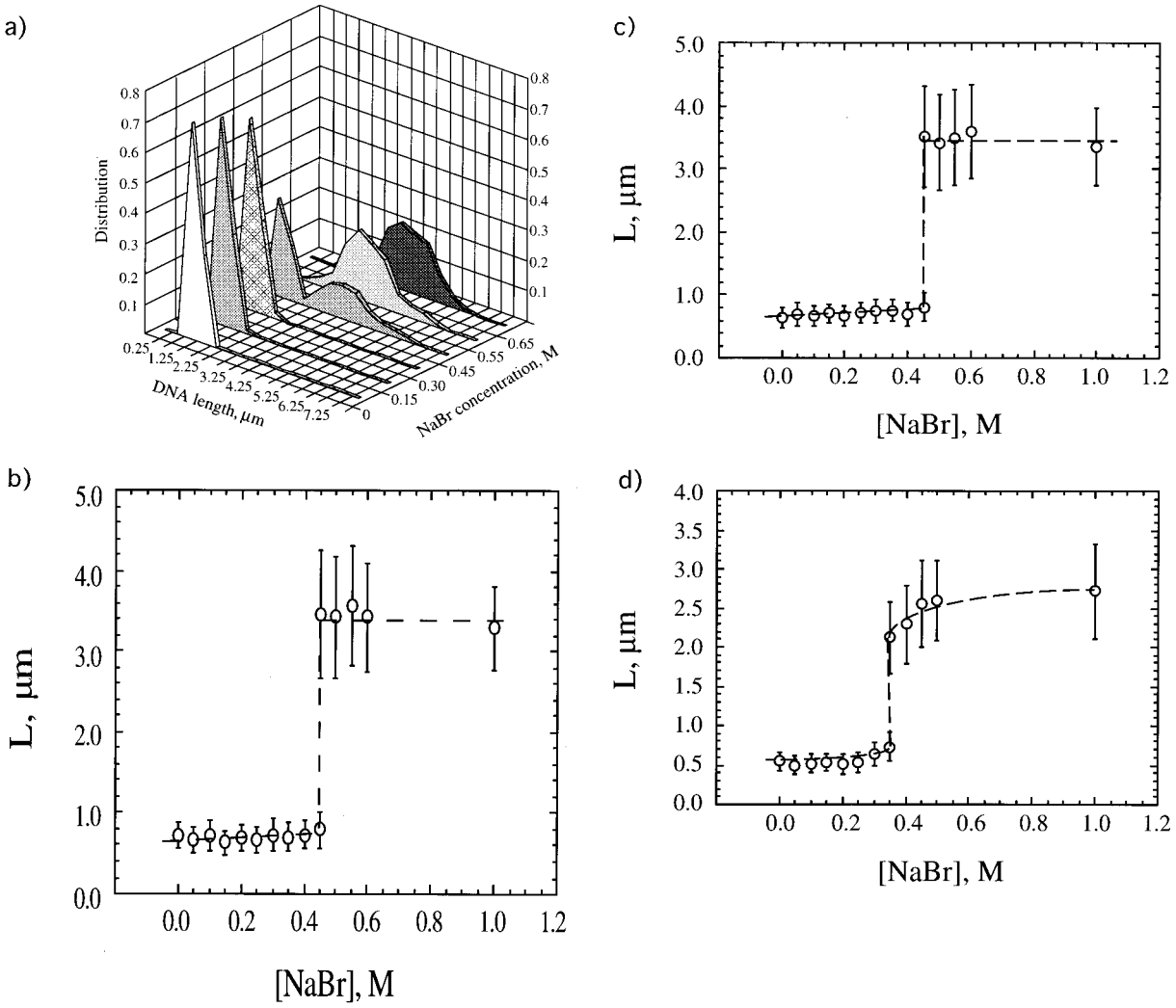

FIG. 2. (a) Distribution of long-axis lengths **L** of T4DNA vs NaBr concentration at $[CTAB]=1.0\times10^{-4}$ M. 200 DNA molecules were analyzed for each NaBr concentration. The final concentrations were as follows: DNA in nucleotide, 0.6 μM; DAPI, 0.6 μM; ME, 4% (v/v). (b) Long-axis lengths **L** of T4DNA vs NaBr concentration at constant $[CTAB]=1.0\times10^{-4}$ M. Error bars indicate the statistical error in the distribution [experimental conditions are the same as in Fig. 2(a)]. (c) Long-axis lengths **L** of T4DNA vs NaBr concentration at constant $[CTAB]=1.0\times10^{-3}$ M. 200 DNA molecules were analyzed for each NaBr concentration. Error bars indicate the statistical error in the distribution. The final concentrations were as follows DNA in nucleotide, 0.6 μM; DAPI, 0.6 μM ME 4% (v/v). (d) Long-axis lengths **L** of T4DNA vs NaBr concentration at constant $[CTAB]=0.137$ M. 200 DNA molecules were analyzed for each NaBr concentration. Error bars indicate the statistical error in the distribution. The final concentrations were as follows: DNA in nucleotide, 0.6 μM; DAPI, 0.6 μM; ME, 4% (v/v).

the NaBr concentration is less than 0.40 M, all of the T4DNAs exhibited a compact globular conformation with almost constant **L** values. These globule DNAs show rather fast thermal fluctuations in aqueous buffer solution due to small hydrodynamic radii on the order of 0.1 μm.[15] However, at the threshold salt concentration, 0.45 M, elongated DNAs appear to coexist with globular DNAs, i.e., coiled and globular chains with substantially different fluorescent intensities[15–17] are observed simultaneously in the solution. At $[NaBr]\geqslant0.50$ M, globular DNAs with high fluorescence intensity disappear; DNAs exist only in the unfolded coil conformation. Figures 2(a) and 2(b) indicate that, after the unfolding of T4DNA globules, DNA macromolecules are about 3.5 μm in size, i.e., similar to that before compaction at low CTAB concentration.[15]

Next, we examined the behavior of DNA–CTAB complex in globule conformation at a CTAB concentration higher than that in the above experiment. Figure 2(c) shows the effect of NaBr on the unfolding transition of T4DNA in the presence of $[CTAB]=10\times10^{-3}$ M, being ten times larger than that in Figs. 2(a) and 2(b). Interestingly, the threshold value of [NaBr] for the unfolding of DNA globules was the same (0.45 M) at both $[CTAB]=10\times10^{-3}$ M and $[CTAB]=1.0\times10^{-4}$ M. Figure 2(d) presents data on the change in the long-axis length **L** of single DNA molecules in the T4DNA–CTAB system at a much higher surfactant concentration (0.137 M) while varying the NaBr concentration, at 35 °C. As with the lower CTAB concentrations [Figs. 2(b) and 2(c)], only collapsed globules present up to $[NaBr]=0.3$ M. Above $[NaBr]\geqslant0.40$ M, the globular DNAs disappear and all of the DNA molecules exhibit an elongated conformation. The threshold concentration at [NaBr]

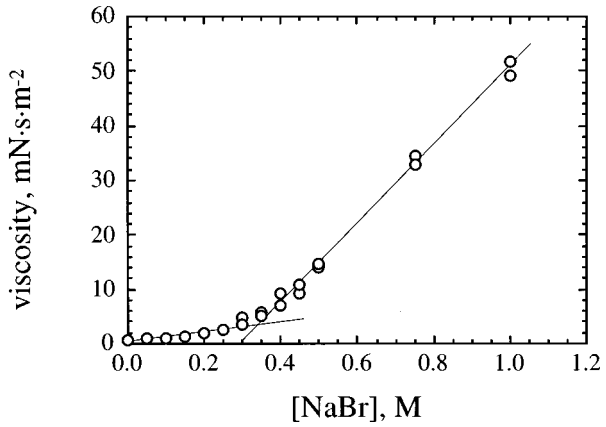

FIG. 3. Dependence of the viscosity of CTAB–T4DNA–NaBr solutions at [CTAB]=0.137 M on the NaBr concentration. The concentrations of CTAB, T4DNA, and NaBr are analogous to that used for FM observation at [CTAB]=0.137 M, $T=35\,^{\circ}\mathrm{C}$.

=0.35 M is a little bit lower compared to the results with less CTAB concentration [Figs. 2(b) and 2(c)]. By careful observation of the conformation at [NaBr]=0.35 M, we have noticed that some DNAs exhibits intrachain segregation: coil and globule parts coexist along a single chains. It is also noted that the discreteness of the transition at the transition point is softened a little bit. The transition curve with the less discrete character is thus partly attributable to the existence of the intrachain segregated state.

In order to clarify the mechanism of the transition of DNA chains in relation to the state of the surfactant molecules, we measured the solution viscosity. Figure 3 gives the change in the viscosity of the DNA solution at [CTAB]=0.137 M. At low concentrations of NaBr, the viscosity stays nearly constant, and then increases slightly, which is attributed to the formation of ellipsoidal micelles in the surfactant solution.[22] At higher concentrations of NaBr, the viscosity tends to increase rather steeply. Figure 3 shows an inflection point at [NaBr]≈0.35 M, indicating that the inflection point in the viscosity coincides with the transition point in the conformation of T4DNA as observed by FM [Fig. 2(d)]. The sharp increase in viscosity at a high concentration of NaBr is most probably due to the sphere-rod transition of surfactant micelles in the surrounding aqueous solution.[23–26]

Then, let us describe the manner of the unfolding transition of DNA induced by D_{18}DAB. The chemical structure of the hydrophilic group is essentially the same for CTAB and D_{18}DAB, while the hydrophobicity and geometrical shape of the alkyl tails are quite different. Therefore, D_{18}DAB forms vesicles, rather than rodlike micelles in solution.[27] Figures 4(a) and (b) show the change in the long-axis length **L** of T4DNA in the presence of a D_{18}DAB at $[D_{18}DAB]=4.0\times10^{-6}$ M with a change in the NaBr concentration. The region of the coexistence of coil and globule DNA is very wide, between 0.10 and 0.35 M in contrast to the transition in T4DNA–CTAB systems. In relation to the unfolding transition, recently it has been reported that the collapsing transition of DNA caused by the addition of

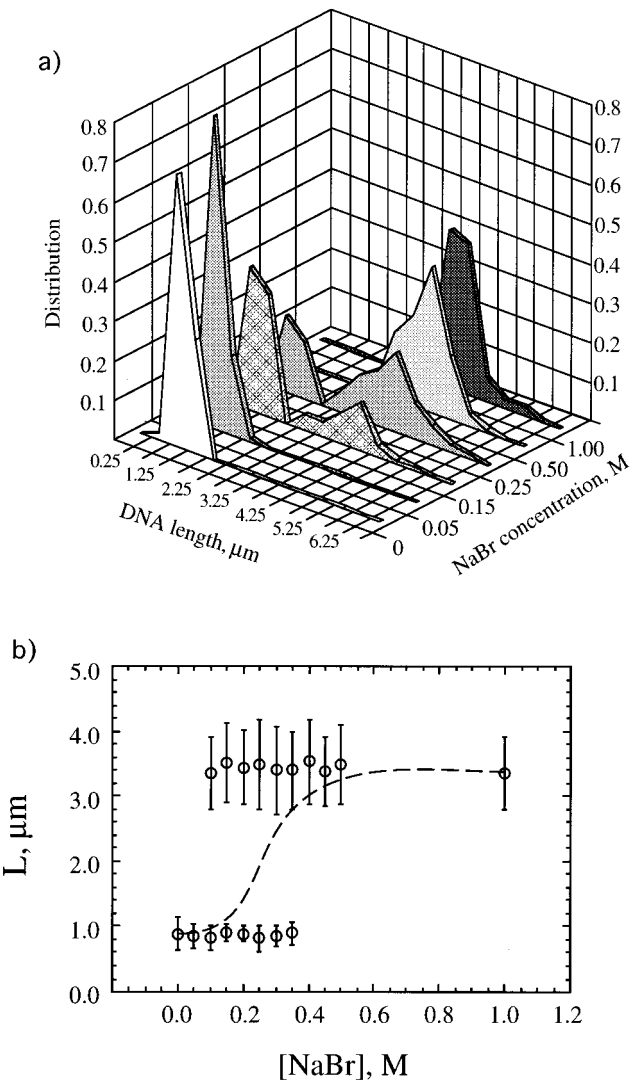

FIG. 4. (a) Distribution of long-axis lengths **L** of T4DNA vs NaBr concentration at $[D_{18}DAB]=4.0\times10^{-6}$ M. 200 DNA molecules were analyzed for each NaBr concentration. The final concentrations were as follows: DNA in nucleotide, 0.6 μM; DAPI, 0.6 μM; ME, 4% (v/v). (b) Long-axis lengths **L** of T4DNA vs NaBr concentration at constant $[D_{18}DAB]=4.0\times10^{-6}$ M. Error bars indicate the statistical error in the distribution [experimental conditions are the same as in Fig. 4(a)].

D_{18}DAB also exhibits rather wide coexisting region, two orders of magnitude on the surfactant concentration.[17] Figure 4(b) clearly shows that on the level of the ensemble average of the DNA molecules, the transition curve looks like a typical sigmoidal line, i.e., *the transition is not any kind of phase transition but a cooperative one*. On the contrary, *the all or none character on the level of individual DNA molecules, is the same both for CTAB and* D_{18}DAB.

From the measurements with fluorescence microscopy we have obtained the unfolding transition in the DNA complexes with ''course graining,'' or with low resolution. To clarify the microscopic structure in the DNA complexes, we performed x-ray diffraction measurements of DNA-surfactant complexes. Figures 5 and 6 present x-ray diffraction profiles for the DNA complexes with CTAB and

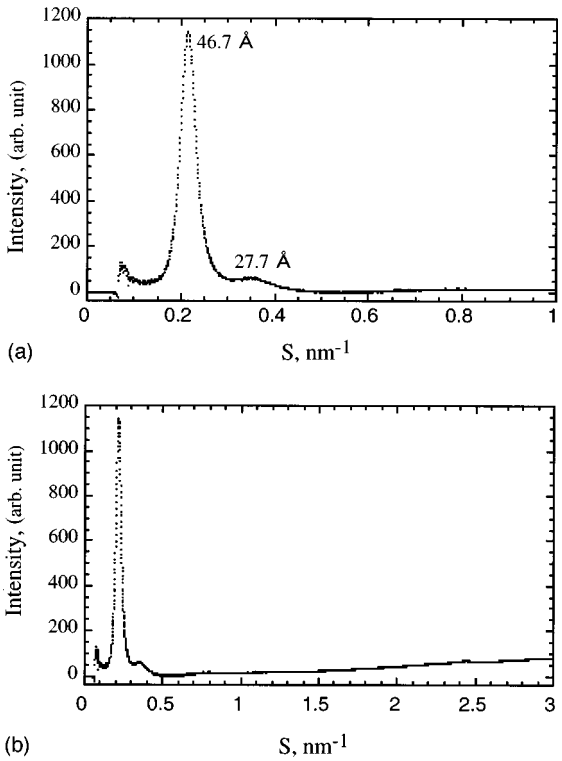

FIG. 5. X-ray diffraction patterns in the (a) small-angle and (b) whole region for the DNA–CTAB complex.

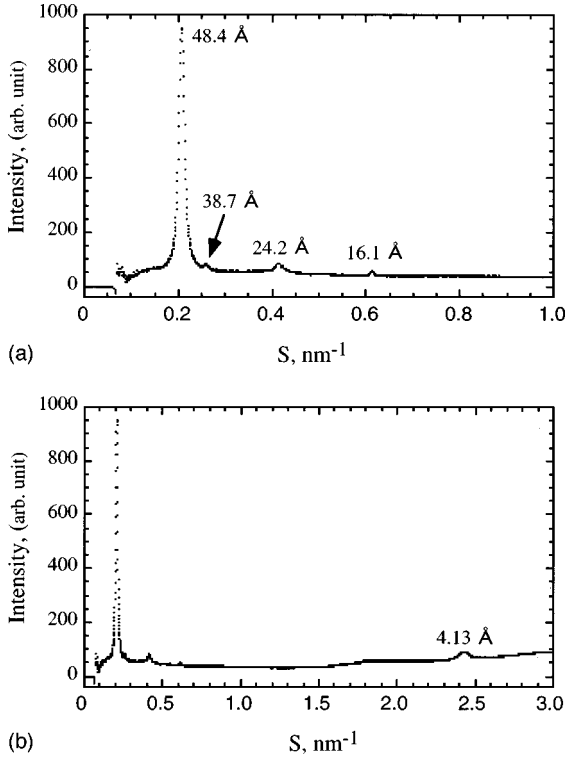

FIG. 6. X-ray diffraction patterns in the (a) small-angle and (b) whole region for the DNA–D_{18}DAB complex.

D_{18}DAB, respectively. In Fig. 5, the DNA–CTAB complex shows two broad diffraction peaks in the small-angle region. As the ratio in the reciprocal spacing for the two diffraction peaks is $1/\sqrt{3}$, the packing structure with somewhat irregular hexagonal lattice is expected in the DNA–CTAB complex. Such a hexagonal structure of the complex has been already found in a recent research with short DNA chains.[28] On the other hand, for D_{18}DAB complex four sharp reflection peaks appear in the small-angle region [Fig. 6(a)]. The small peak observed on the wider-angle side of the highest peak has a spacing of ca. 39 Å, which is consistent with previously published results on the crystal structure of dialkyldimethylammonium halides.[29,30] This suggests that a trace amount of anhydrated D_{18}DAB molecules is left in the sample, due to the low solubility of D_{18}DAB. The other three small-angle diffraction peaks give a reciprocal spacing ratio of 1:2:3. This fact clearly indicates that the DNA–D_{18}DAB complex forms a highly ordered multilamellar structure. The lamellar spacing is ca. 48.4 Å. In addition to these lamellar diffraction peaks, a small diffraction peak with a spacing of 4.13 Å is observed in the wide-angle region [Fig. 6(b)]. From the symmetric shape of the diffraction peak it becomes clear that the alkyl tails of D_{18}DAB are packed in a hexagonal lattice.[31]

Thus, it has become clear that the complex structures are markedly different from each other with CTAB and D_{18}DAB. Although the complex is highly concentrated for the measurement with x-ray diffraction compared to the experimental conditions with fluorescence microscopy measurement, the large difference of the complex structure is expected to be connected with the difference in the unfolding transition on the level of the ensemble of DNA molecules.

IV. DISCUSSION

A. Phase transition in a finite system is dominated by that in a large system

On the basis of the above results, the difference in the nature in the unfolding transition of DNA with CTAB and D_{18}DAB is depicted in schematic manner in Fig. 7. Such a difference is considered to be due to the competition among various types of physicochemical processes; conformation of DNA; aggregation of the micelle, the condensation and release of small ions, interaction of DNA with the surfactant, etc. Although our experimental system is rather complicated, in the following we would like to try to grasp the essential feature on the unique behavior of the transition of DNA chain interplaying with the process of micelle formation.

Let us consider a polymer chain which contains N segments with persistence length L and width D. The free energy per single chain is expressed as a function of swelling coefficient $\alpha(=R/R_0)$, where R and R_0 are the gyration radius for a real polymer chain and an ideal Gaussian chain, respectively.[32]

$$F/kT \approx \frac{3}{2}\left(\frac{1}{\alpha^2}+\alpha^2\right)+\frac{BN^{1/2}}{\alpha^3 l^3}+\frac{C}{\alpha^6 l^3}. \tag{1}$$

Here the first term describes elastic free energy, and the second and third terms are the free energy of interaction. B and C are the second and third virial coefficients for the interac-

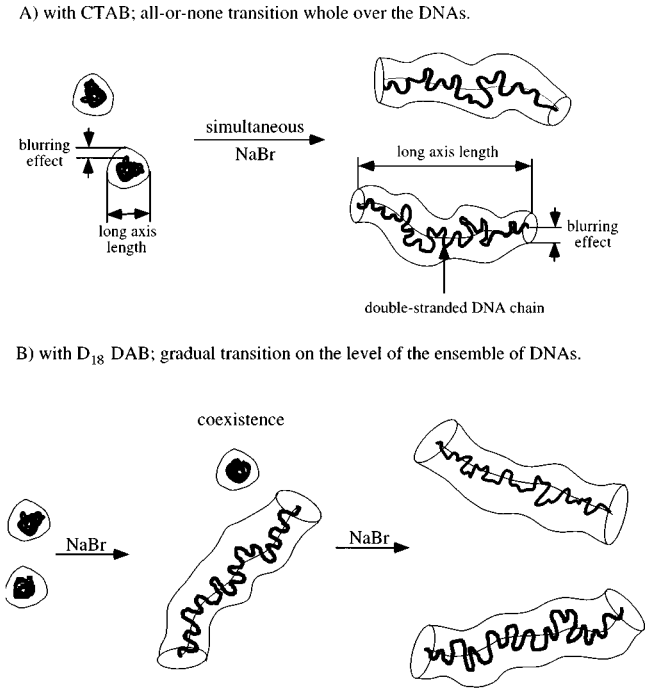

FIG. 7. Schematic representation on the difference on the nature of coil - globule transition in DNA, with (A) CTAB and (B) D_{18}DAB. It is noted that on the level of the individual DNAs the transition is always all or none.

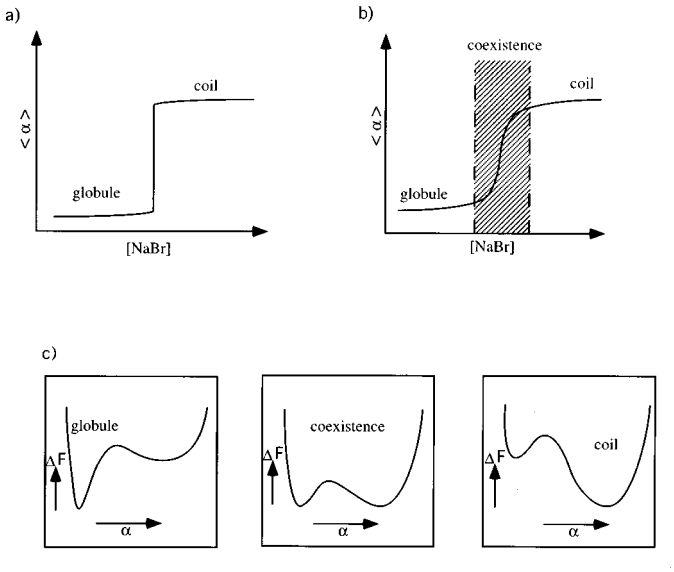

FIG. 8. Schematic diagram of the transition curve on the ensemble average $\langle\alpha\rangle$ of the DNA swelling coefficient, for the case with (a) CTAB and (b) D_{18}DAB. (c) The change in the free energy profile. In the case of CTAB, all DNA molecules undergo the coil-globule transition at a certain threshold NaBr concentration. In other words, the free energy difference between the coil and globule states changes abruptly accompanied with the phase transition of the micelles in the buffer solution. In the case of D_{18}DAB the free energy profile changes gradually from left to right through the coexistence state with finite width.

tion of the segments. The dependence of the equilibrium swelling coefficient of the polymer chain in the solvent can be found by minimizing free energy F over α. As a result, we obtain the following relationship,

$$\alpha^5-\alpha-\frac{2y}{\alpha^3}=\chi\frac{d}{l}, \tag{2}$$

where $\chi=BN^{1/2}/dl^{1/2}$, $y=C/l^6$.

The parameter χ characterizes the solvent quality and y is related to the flexibility of the chain. From Eq. (2), it becomes clear that, for chains with sufficient stiffness ($l/d \gg 1$), α exhibits three real positive roots at a fixed χ value, where two roots (α_c and α_g; $\alpha_c \gg \alpha_g$) are stable and one is unstable with respect to a small perturbation. This has the physical meaning that elongated coil α_c and collapsed globule α_g states coexist with a finite parameter width ($0<\chi_l<\chi<\chi_h$, where χ_l and χ_h are the lower and higher limits of the coexistence). It is predicted that the width of the coexistence, $\Delta\chi=\chi_h-\chi_l$, decreases with the increase of the segment number N. In other words, the finiteness of the coexistence region is attributed to the finiteness of the number of elements, N. It is to be noted that in usual first-order phase transition the coexistence is found only at the phase-transition point with respect to an intensive variable, such as temperature, pressure, or chemical potential in the environment. Such a trend is well known as the central limit theorem in statistical physics,[33] which is due to the infiniteness of the number of elements in a system. As a next step, let us discuss the effect of the finiteness in the number of elements in relation to our experimental results.

As for DNA in usual aqueous environment, the persistence length is ca. 50 nm. Since the segment length L is double the persistence length, $l=100$ nm. In the case of T4DNA with the counter length of 57 mm, the total number of segments N is, thus, ca. 600. Because of this finiteness of N, the phase transition between elongated coil and collapsed globule in the level of individual chains appears continuous in the ensemble, that is, there is no discreteness in the transition curve of the ensemble average including its derivative. As the change in the solvent conditions (environment) corresponds to the change of the interaction parameters, B and C, it may be allowed to consider that Eq. (1) is applicable to the interpretation of the transition of DNA chain.

In usual experiments to change the solvent conditions, the terms B and C will change in a continuous manner accompanied with the change in the concentration of chemical species. Thus, the result schematically depicted as in Fig. 8(b) will be obtained for $\langle\alpha\rangle$, the ensemble average of the DNA size. In contrast to this, if the state parameter in the environment will change in an abrupt manner as in the case of sphere-rod transition of surfactant micelles, the interaction terms, B and C, will also change in a discrete manner. Then, such a discrete change will cause the discrete transition on the ensemble of the DNA chains as in Fig. 8(a). As for the transition of the surfactant micelles, the transition should be discrete according to the central limit theorem, that is, the number of the surfactant molecules in the bulk solution is ''infinitely'' large. Along this line of the theoretical consideration, the marked different character on the transition,

whether cooperative or discrete is explained as shown in Fig. 8.

B. Phase transition in the bulk solution

In a previous study we have shown that CTAB induces collapse transition on DNA molecules at a very low surfactant concentration of about 10^{-5} M, below the CMC (ca. 10^{-3} M).[15] Such a result has been discussed in relation to the formation of micellelike aggregates with the help of polyanion.[16] These complexes are formed through interaction of the negatively charged DNA phosphate groups with surfactant counterions and stabilized by hydrophobic interaction of the hydrocarbon tails of the surfactant molecules. The surfactant ions, which form micellelike aggregates in a globular T4DNA–CTAB complex and micelles in solution are in dynamic equilibrium.

Then, with the addition of NaBr up to a critical threshold concentration, rodlike surfactant micelles are formed in the bulk solution. It has been well established that the sphere-rod conversion of ionic micelles appears as an abrupt transition with respect to the salt concentration.[34] In other words, the chemical potential of the surfactant, together with that of the coexisting monovalent ions, changes in a discrete manner at the sphere-rod transition. With regard to the free energy of the DNA-surfactant complex, the experimental trend as in Fig. 2 means that the free energy of ''coiled DNA/rodlike micelle'' systems suddenly becomes lower than that of a ''DNA-surfactant complex/rodlike micelle'' system at high concentration of NaBr. As a result, the transition from the globule to coil states is induced simultaneously in all of the DNA chains; the ensemble of DNAs exhibits discrete transition.

As has been discussed in the preceding section, when the size of DNA molecules is large enough (more then several kilo base pairs), the coil-globule transition is discrete at the level of individual DNA chains, whereas the transition is continuous at the level of the ensemble of DNA chains.[16] Thus, the markedly discrete character for the unfolding, transition on the ensemble of the DNA–CTAB complexes shown in Figs. 2(a)–2(d), indicates that the existence of some kind of discrete transition whole over the solution which is most probably attributable to the sphere-rod transition of CTAB micelles.

The expectation that the transition from the globule to coil states in DNA chains is driven by the sphere-rod transition of CTAB is supported by the existence of the inflection point in the solution viscosity as a function of [NaBr], for the case [CTAB]$=0.137$ M (Fig. 3). Unfortunately, at lower CTAB concentrations, we have failed to detect the inflection point in the dependency of the solution viscosity on [NaBr]. Such a failure may be simply related to the technical difficulty to detect the change at low surfactant concentration. The similar behavior on the manner of the unfolding transition at higher surfactant concentration [CTAB]$=1.0\times10^{-4}$ M and [CTAB]$=10\times10^{-3}$ M may simply be explained by the existence of the sphere-rod transition in the bulk solution even at low surfactant concentration. This consideration is supported by the markedly different profile of the transition of DNA from coil to globule states induced by the dialkyl cationic surfactant D_{18}DAB (see Fig. 6), which shows a continuous character of the unfolding transition on the DNA ensemble. The continuous transition for the ensemble of DNAs (i.e., the finite width of the transition region) is described by the gradual change in the chemical potential of D_{18}DAB in the system.

Further detailed study on the manner of interaction between DNA and cationic surfactant is necessary to make clear the whole story of the transition, including the direct observation in the change of the micelle structure together with that of the DNA conformation. Especially, the observation on conformation of the rodlike micelle at very low CTAB concentration as in Fig. 2 is an important target as the next study.

In the present article, we have proposed a new concept that *the phase transition in a finite system is dominated by that in a large system.* Future studies based on this concept are expected to open a novel area in chemical physics.

ACKNOWLEDGMENTS

We gratefully acknowledge the High Intensity X-Ray Diffraction Laboratory Execution Committee of Nagoya University for their approval to use the BAS2000 system and the technical support of T. Hikage. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan to H.T., I.H., and K.Y. V.S. is grateful to the Japan Society for the Promotion of Science, which provided the opportunity for his work at Nagoya University.

[1] B. Lindman and K. Thalberg, *Interactions of Surfactants with Polymers and Proteins*, edited by E. D. Goddard and K. P. Ananthapadmanabhan (CRC, Boca Raton, Florida, 1993).
[2] E. D. Goddard, Colloids Surf. **19**, 301 (1986).
[3] K. Hayakawa and J. C. T. Kwak, *Cationic Surfactants. Physical Chemistry*, edited by D. N. Rubingh and P. M. Holland (Marcel Dekker, New York, 1991).
[4] V. G. Sergeyev, O. A. Pyshkina, A. B. Zezin, and V. A. Kabanov, Polymer Sci. **39**, 12 (1997).
[5] J.-S. Remy, C. Sirlin, P. Vierling and J.-P. Behr, Bioconjugate Chem. **5**, 647 (1994).
[6] B. Sternberg, F. L. Sorgi, and L. Huang, FEBS Lett. **356**, 361 (1994).
[7] X. Zhou and L. Huang, Biochim. Biophys. Acta **1189**, 195 (1994).
[8] D. L. Reimer, Y.-P. Zhang, S. Kong, J. J. Wheeler, R. W. Graham, and M. B. Bally, Biochemistry **34**, 12 877 (1995).
[9] K. Hayakawa, J. P. Santerre, and J. C. T. Kwak, Biophys. Chem. **17**, 175 (1983).
[10] K. Shirahama, T. Masaki, and K. Takashima, *Microdomains in Polymer Solutions*, edited by P. Dubin (Plenum, New York, 1985).
[11] K. Shirahama, K. Takashima, and N. Takisawa, Bull. Chem. Soc. Jpn. **60**, 43 (1987).
[12] G. Schwarz, Eur. J. Biochem. **12**, 442 (1970).
[13] K. S. Schmitz and J. M. Schurr, Biopolymers **9**, 697 (1970).
[14] I. Satake, and J. T. Yang, Biopolymers **15**, 2263 (1976).
[15] S. M. Mel'nikov, V. G. Sergeyev, and K. Yoshikawa, J. Am. Chem. Soc. **117**, 2401 (1995).
[16] S. M. Mel'nikov, V. G. Sergeyev, and K. Yoshikawa, J. Am. Chem. Soc. **117**, 9951 (1995).
[17] S. M. Mel'nikov, V. G. Sergeyev, Y. S. Mel'nikova, and K. Yoshikawa, J. Chem. Soc., Faraday Trans. **93**, 283 (1997).
[18] K. Yoshikawa, Y. Matsuzawa, K. Minagawa, M. Doi, and M. Matsumoto, Biochem. Biophys. Res. Commun. **188**, 1274 (1992).

[19] Y. Matsuzawa and K. Yoshikawa, Nucleosides Nucleotides **13**, 1415 (1994).
[20] Y. Matsuzawa, K. Minagawa, K. Yoshikawa, M. Matsumoto, and M. Doi, Nucleic Acids Symp. Ser. **25**, 131 (1991).
[21] T. Takahashi, S. Matuoka, S. Kato, K. Ohki, and I. Hatta, Biochim. Biophys. Acta **1069**, 229 (1991).
[22] H. V. Tartar, J. Phys. Chem. **59**, 1195 (1955).
[23] P. Debye and E. W. Anacker, J. Phys. Colloid. Chem. **55**, 644 (1951).
[24] S. Ikeda, S. Ozeki, and M. Tsunoda, J. Colloid Interface Sci. **73**, 27 (1980).
[25] T. Imae and S. Ikeda, J. Phys. Chem. **90**, 5216 (1986).
[26] T. Imae and S. Ikeda, *Surfactant in Solution*, edited by K. L. Mittal (Plenum, New York, 1989).
[27] D. F. Evans and H. Wennerström, *The Colloidal Domain: Where Physics, Chemistry, Biology and Technology Meet* (VCH, New York, 1994).
[28] R. Ghirlando, E. J. Wachtel, T. Arad, and A. Minsky, Biochemistry **31**, 7110 (1992).
[29] E. J. R. Sudholter, J. B. F. N. Engberts, and W. H. de Jeu, J. Phys. Chem. **86**, 1908 (1982).
[30] K. Fontell, Coll. Polymer Sci. **268**, 264 (1990).
[31] T. Adachi, H. Takahashi, K. Ohki, and I. Hátta, Biophys. J. **68**, 1850 (1995).
[32] A. Yu. Grosberg and A. R. Khokhlov, *Statistical Physics of Macromolecules* (AIP, New York, 1994).
[33] L. D. Landau and E. M. Lifshits, *Statistical Physics* (Elseiver, New York, 1994).
[34] S. Ozeki and S. Ikeda, J. Colloid Interface Sci. **87**, 425 (1982).