Discrete Coil−Globule Transition of Large DNA Induced by Cationic Surfactant

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Abstract: It becomes clear that large DNA molecules exhibit discrete conformational change between the coil and globule states with the addition of a very small amount (with the order of 10^{-5} M) of cationic surfactant, cetyltrimethylammonium bromide (CTAB). We use fluorescence microscopy as a tool of single molecular observation of double-stranded T4DNA in an aqueous environment. When the concentration of CTAB is less than 9.4×10^{-6} M, all DNA molecules exhibit the extended coil state. Whereas, when the CTAB concentration is higher than 2.0×10^{-5} M, only compacted DNA molecules in the globular state are observed. In the region between these two critical concentrations, the coil and globule states coexist in the solution. A small but apparent increase of the size of the DNA globule is noticed at the CTAB concentration higher than 10^{-3} M, due to the penetration of CTAB molecules into the DNA globule. To study the dynamical aspect of coil−globule transition, the process of the structural change from the coil into the globule state is observed under the spatial gradient of the CTAB concentration. The formation of aggregates from two or more globules is noticed at high concentrations of surfactant above 1.6×10^{-4} M. Below this concentration, the globules do not coalesce into an aggregate even if they collide with each other. The translational diffusion constant D of DNA molecules is measured from the time series of video frames of the fluorescence image. The hydrodynamic gyration radius ξ_H is evaluated from the D and the viscosity of the bulk aqueous solutions. The increase of the globule size at the higher CTAB concentrations above 10^{-3} M is confirmed by the increase of ξ_H values.

Introduction

It is well-established[1−3] that in a living cell, the information stored as the base sequence along the DNA chain is expressed as the production of a rich variety of proteins in a self-regulatory manner. Thus, it is expected that the interaction of DNA with various kinds of chemical components in a cell, such as proteins, lipids, and inorganic ions, is essentially important in the regulation of a living state. Interactions between ionic surfactants and polyions with the opposite charge lead to the formation of polymer−colloid complexes,[4−8] in which the polyelectrolyte chain binds with surfactant molecules through Coulomb attractive interaction, and the hydrophobic moieties of the surfactant molecules may stabilize the complexes due to the hydrophobic interactions in aqueous solution. The formation of polymer−colloid complexes induces a conformational change in the polymer molecules. In relation to this, the DNA in bacterial nucleotides and chromosomes was found to be in a highly condensed state in comparison with the free DNA in the solution.[9−12] Cationic lipids are known as efficient nonviral reagents to transfect outer DNA into animal cells in vitro.[13] Although cationic surfactants are widely used as effective germicidal chemicals,[14] physicochemical studies on the interaction of cationic surfactants with DNA seem to have been rather few.

In this paper, we use fluorescence microscopy for the study of the conformational change of the double-stranded DNA molecules induced by surfactant ions. During the past decade, fluorescence microscopy has been applied both for the study of the conformational change of DNA molecules in the presence of synthetic hydrophilic polymers[15,16] and various types of fluorescent dyes[17,18] and for the analysis of relaxation[19] and tube-like motion[20] of single DNA molecules. In these studies, it is shown that long DNA macromolecules with the sizes

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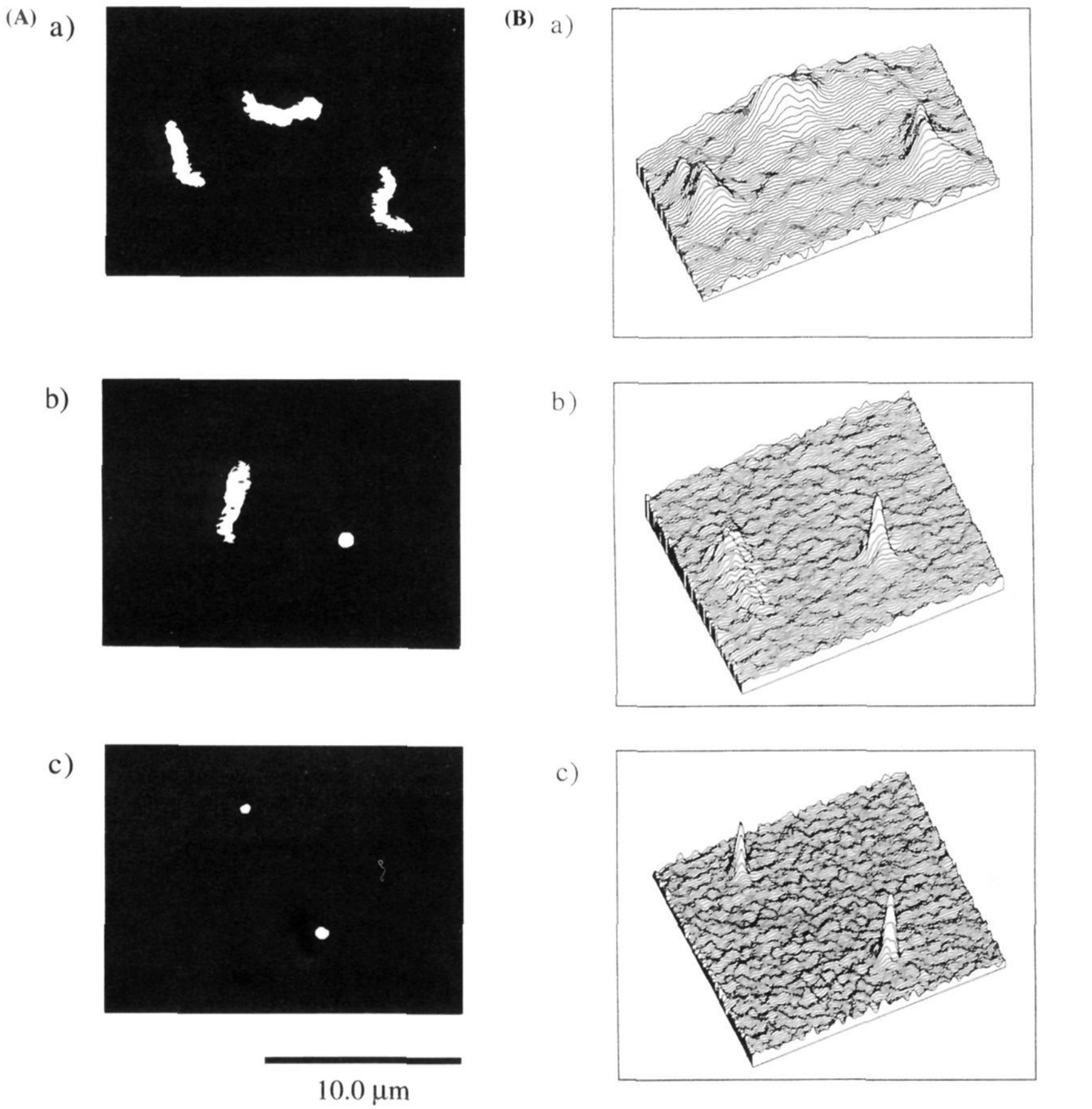

Figure 1. (A, left) Fluorescence images of T4DNA molecules moving freely in the buffer solution under the different concentrations of CTAB. Existence of coil conformation of T4DNA molecules ([CTAB] = 1.0×10^{-6} M, photo (a)), globule conformation ([CTAB] = 1.6×10^{-4} M, photo (c)), and coexistence of coil state and globule state ([CTAB] = 9.4×10^{-6} M, photo (b)) is shown. (B, right) Light intensity distribution on the photographs of (A) are shown. (Abbreviations are the same as in (A)).

reaching several tens of micrometers (or several hundreds of kilobase pairs) can be visualized[21,22] with fluorescence microscopy in a quite dilute solution of DNA. Using fluorescence microscopy, we find in the present study that *single DNA molecules exhibit discrete phase transition* in their higher order structure between the elongated coil state and the compacted globule state by the addition of cationic surfactant, cetyltrimethylammonium bromide (CTAB). From the video frames of the fluorescence images, we carry out the quantitative analysis on various physical values, such as the long axis length, the translational diffusion constant, and the hydrodynamic radius of gyration of DNA macromolecules.

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Results and Discussion

Direct Observation of the Conformational Changes of Single DNA. Figure 1A(a) shows the fluorescence images of T4DNA molecules, exhibiting translational and intramolecular Brownian motion in TBE buffer solution without surfactant. Here, the double-stranded DNA molecules exist in a rather extended conformation, i.e., coil state, as is depicted in a schematic manner in Figure 2. With the addition of CTAB until the concentration reaches 9.4×10^{-6} M, DNA molecules remain in the coil state and display random intramolecular fluctuation movement. A further increase of the CTAB concentration induces the collapse of individual T4DNA molecules to small compact particles, i.e., globules ((c) in Figure1 A). In the region of intermediate CTAB concentration, the coil and globule states coexist as is shown in Figure 1A(b). Figure 1B shows the quasi-

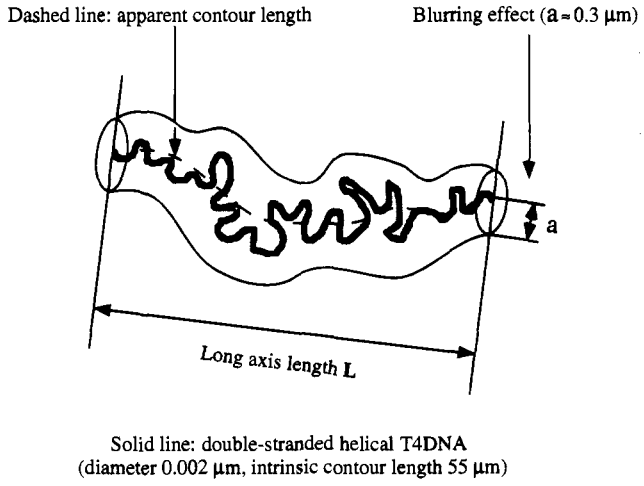

Figure 2. Schematic illustration of the fluorescent image of a double-stranded T4DNA molecule.

three-dimensional representation with the fluorescent light intensity in the vertical axis, for the same video pictures as in Figure 1A. It is clear that individual DNA molecules show marked conformational transformation between the coil and globule states through the interaction with the surfactant molecules.

In order to characterize the conformational transition of DNA induced by CTAB molecules in a quantitative manner, a series of measurements have been carried out by changing the surfactant concentration from 1.0×10^{-6} to 2.5×10^{-3} M. As a parameter on the effective size, the long axis length L of DNA macromolecules (see Figure 2) has been measured directly from the video images. The result is summarized in Figure 3, showing the distribution of long axis length L values at different CTAB concentrations (at least 100 DNA molecules were measured at each concentration). This figure indicates that, when the concentration of CTAB is lower than 9.4×10^{-6} M, the maximum of the L distribution is around 3 μm and the distribution is rather wide; i.e., DNA is in the coil state. On the other hand, when the CTAB concentration is more than 2.0×10^{-5} M, the maximum becomes less than 1 μm and the distribution becomes narrower; i.e., DNA is in the globule state. Between these limits lies the intermediate case where the bimodality is noticed in the long axis length distribution, indicating the coexistence of the coil and globule states.

Figure 4 shows the dependence of the long axis length L on the CTAB concentration. The shaded region corresponds to the bimodal distribution, and the maximums of each peak are given by opened circles. At the CTAB concentrations less than 9.4×10^{-6} M, all DNA molecules are found to be in the coil state, accompanied by the gradual decrease of the apparent size with the increase of CTAB concentration. Whereas, at the concentrations of surfactant higher than 2.0×10^{-5} M, only globular DNA molecules are observed. In this region, the globular molecules tend to be excluded from the bulk aqueous phase and forced onto the glass surface, suggesting that the solvent is poor[23] for DNA molecules. At CTAB concentrations higher than 10^{-3} M, a small increase in the L values is noticed. In this condition, DNA molecules fluctuate with Brownian motion in the aqueous phase, almost without adsorption on the glass surface. This phenomenon may be caused by the penetration of CTAB molecules inside the DNA globules, due to the attractive interaction of surfactant molecules with the negative charges of the DNA chain. The aggregation between CTAB molecules is enhanced in the presence of DNA, which results in the increase of the size of a globule.

In the coexistence region, it was found that the coil state is metastable and transformed spontaneously into the globule state within the period of several tens of minutes. The rate of the transformation from the coil into the globule tends to be slower, accompanied with the decrease of CTAB concentration within the coexistence region. For example, we noticed that some of the DNA molecules remain in the coil state for the order of a day at the [CTAB] = 1.0×10^{-5} M. Such an experimental trend indicates that the real phase transition point situates at, or near, the lower limit of the shaded region in Figure 4. Further study on the kinetics of the transformation may be important to obtain the full understanding of the mechanism of the phase transition of a single DNA chain. Nevertheless, it is clear that the coil molecules in the coexistence region are, thus, considered to be similar to the "supersaturated" or "supercooled" state. The fact that the coil–globule transition is observed far below the surfactant's critical micelle concentration (CMC) indicates that DNA molecules have the effect of inducing aggregation with the oppositely charged surfactant molecules. In relation to this, it has been reported[5] that the reaction resulting in the formation of DNA–colloid complexes proceeds in a cooperative manner in aqueous solution of DNA and surfactant, the surfactant concentrations being lower than the CMC. Such a kind of cooperative character seems to be rather general for other complexes between polyelectrolyte and oppositely charged surfactant.[24,25]

In order to confirm definitely that the individual globules are formed from single DNA molecules, we have tried to observe the dynamic process of globular formation from a coil molecule. To trace the conformational transition of the DNA chains upon interaction with the surfactant molecules, the observation was performed under the spatial gradient of CTAB concentration. The sample without surfactant was placed in the middle of a glass slide, and a drop of CTAB solution with a concentration equal to 1.0×10^{-4} M was placed on the border of the cover slip. The formation of a globular molecule under the diffusional flow of the surfactant is shown on the photographs in Figure 5A(a–e) and Figure 5B(a–e). It is clear that a single DNA coil shrinks with the increase of CTAB concentration. The molecular mechanism on the process of the conformational change from coil to globule may be described as follows. In the first step, the positively charged head groups of surfactant molecules interact with negative charges of the phosphate groups through the attractive Coulomb interaction. Accompanied by the increase of CTAB concentration, surfactant molecules tend to be accumulated onto the anionic DNA chain. Hydrophobic interactions between the alkyl moieties in amphiphilic surfactant ions cause a cooperative effect on the accumulation of the surfactant molecules. The driving force for the conformational transition from coil to globule should be incompatibility with solvent;[26,27] that is, the interaction between the hydrophobic segments of DNA molecules and solvent becomes disadvantageous with respect to the free energy.

It should be noted that one can observe the formation of multimolecular aggregates from the globules only at relatively high concentrations of CTAB (above 1.6×10^{-4} M), where the DNA macromolecules exist only in the globular state. The

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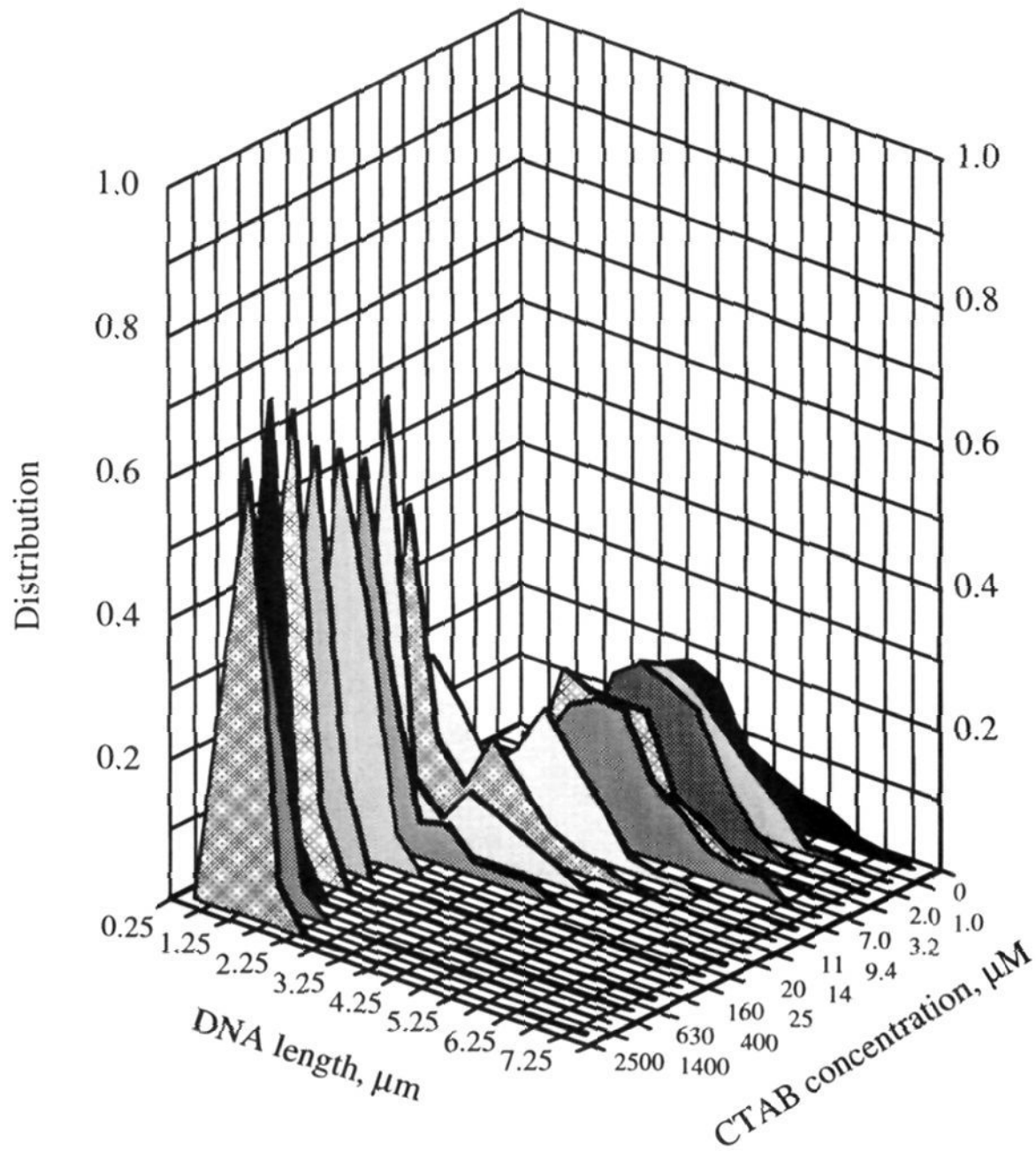

Figure 3. Distribution of the long axis lengths of T4DNA molecules at various concentrations of CTAB. The number of the analyzed T4DNA molecules was 100 for the regions of existence of only coil and only globule states. In the coexistence region, the number of the molecules analyzed was 200. Each area of the histograms is normalized to be equal.

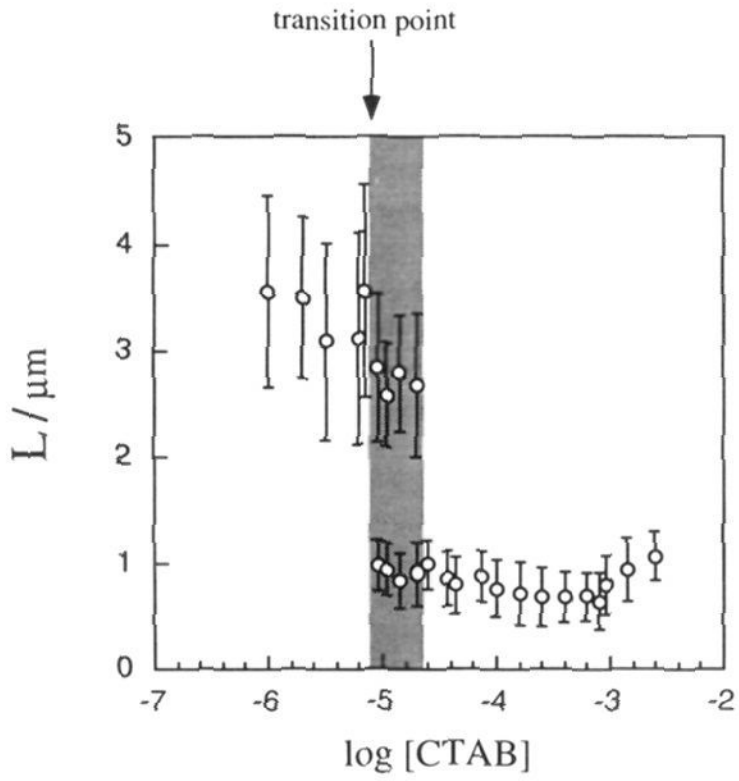

Figure 4. Long axis lengths of T4DNA molecules vs logarithm of CTAB concentration (the opened circles indicate the maximums of the DNA lengths' distribution). The statistical error in the distribution is given as the standard deviation.

process of the aggregate formation is exemplified in Figure 6A-(a−f) and Figure 6B(a−f). For the measurement of the long axis lengths and the translational diffusion constant of globules, we only accounted for the globules formed from a single DNA. Actually, under the conditions in the present study, the probability of aggregate formation is quite small because of the low concentration of DNA.

Actual Size of the DNA Molecules in the Presence of CTAB. There remains uncertainty about the actual size of a globule, due to the resolution limit originated from the wavelength of the fluorescent light and also from the blooming effect on the side of the high-sensitive TV camera. Thus, we have tried to estimate the actual size of the DNA molecules from the measurement of the diffusional constant for the individual fluorescent obstacles. The translational diffusion constant D was obtained from the time t dependence of the mean square displacement (MSD) of the center of mass in DNA macromolecules. During the observation, spontaneous convective flow in the sample solution was recognized, probably due to the illumination effect. As the bulk flow rate was almost constant (at least, for the observation area), we can eliminate the effect of the convection from the diffusional movement, on the basis of following equation:[28]

$$\langle (R_G(t) - R_G(0))^2 \rangle = 4Dt + At^2 \qquad (1)$$

where $R_G = (R_x, R_y)$ and A is a numerical constant as a scale of the magnitude in the convective flow. From the least-square fitting to the second-order polynomial of t for the various time intervals observed, we have evaluated the actual diffusion

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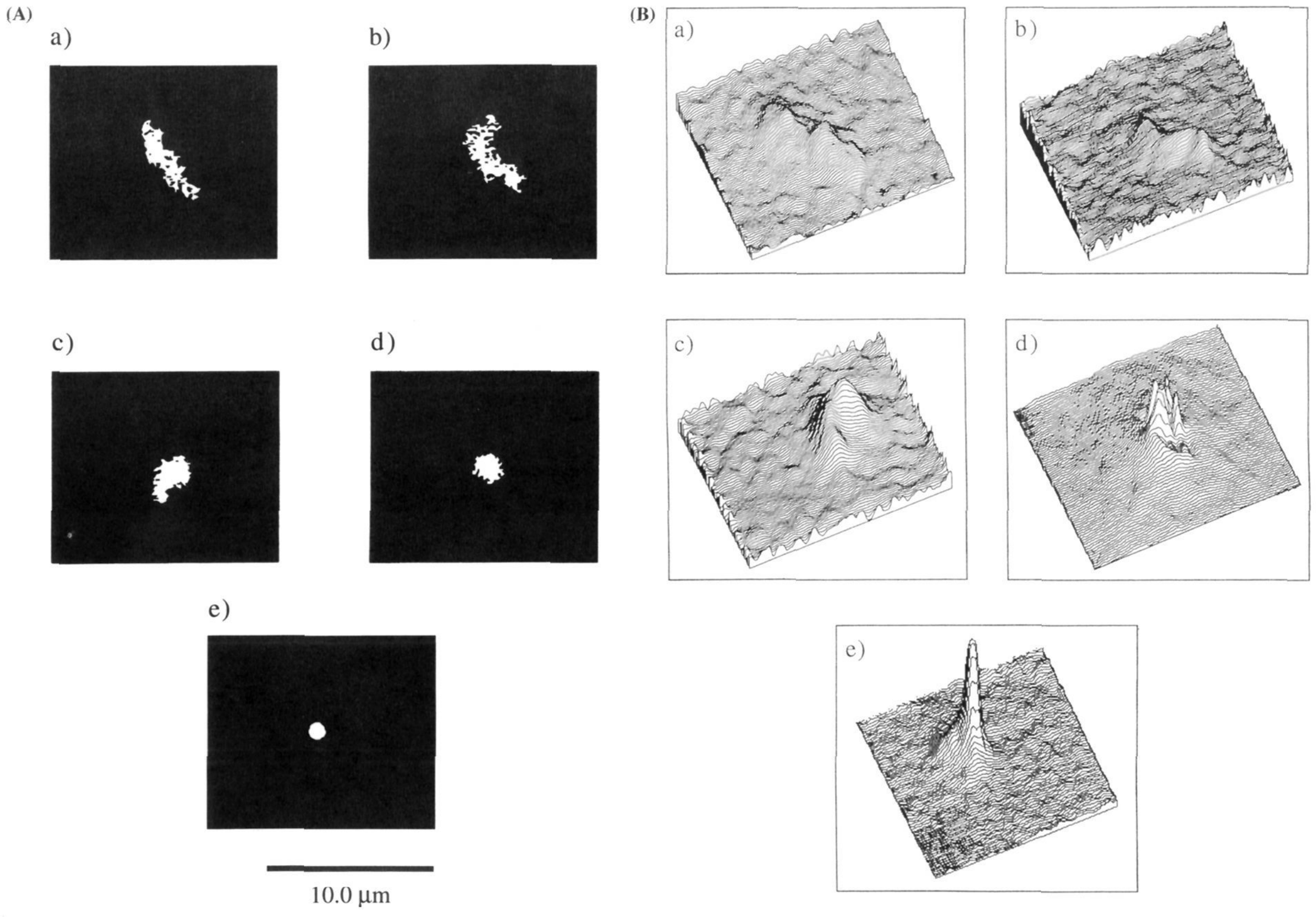

Figure 5. (A, left) Process of globule formation from the coil state of T4DNA in buffer solution under the concentration gradient of the surfactant. The concentration of CTAB placed on the border of the cover slip was 1.0×10^{-4} M. The time interval between the states shown at photos (a) and (e) is 3 s. (B, right) Light intensity distributions on the photographs of the globule formation from the coil state of T4DNA in buffer solution under the concentration gradient of CTAB are shown (abbreviations are the same as in (A)).

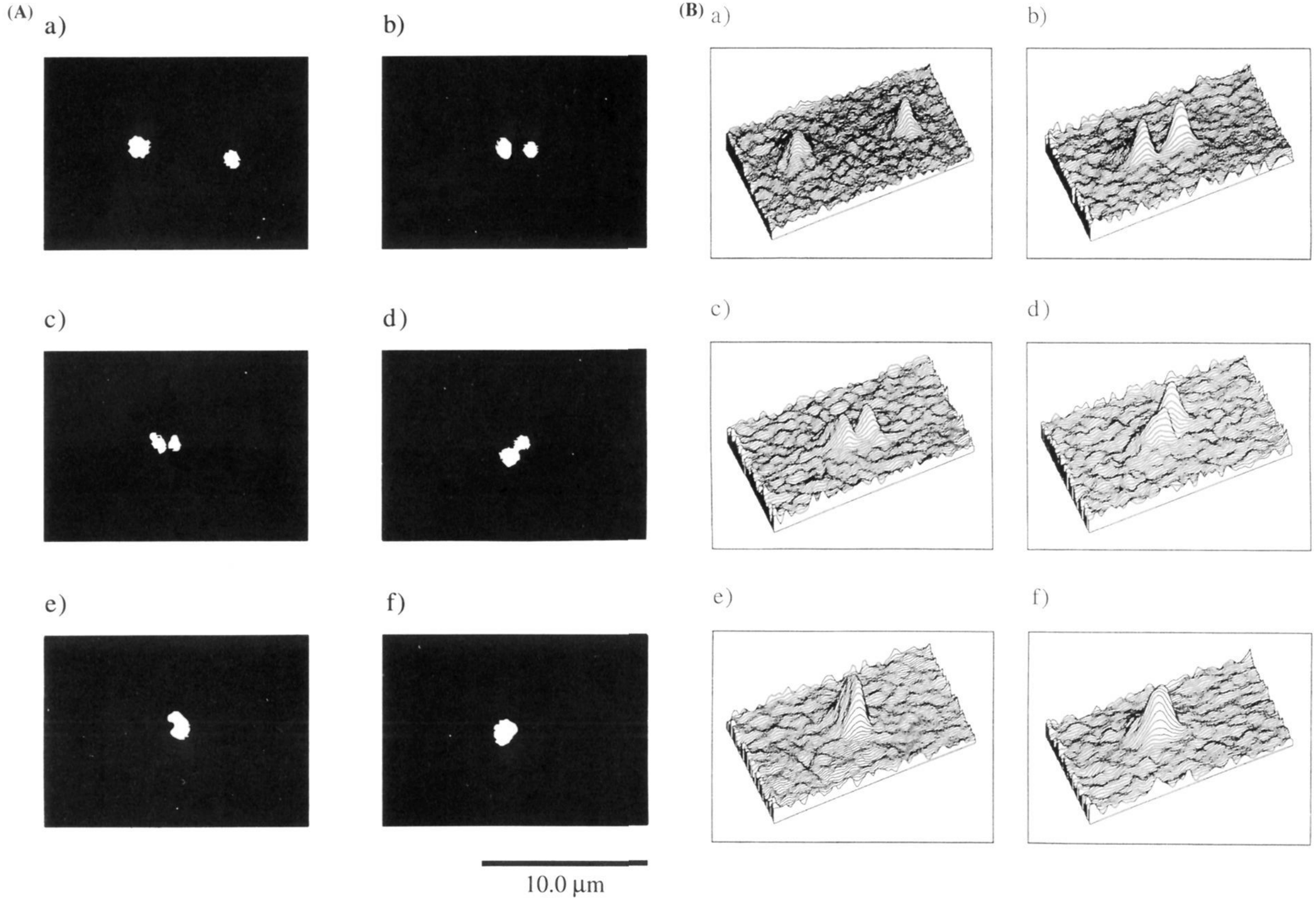

Figure 6. (A, left) Process of the formation of an aggregate between T4DNA globules observed at the CTAB concentration equal to 6.3×10^{-4} M. The time interval between the states shown at photos (a) and (f) is 6 s. (B, right) Light intensity distributions on the photographs of (A) are shown.

Table 1. The Viscosity η_S of the DNA Solutions, the Translational Diffusion Constant D, the Hydrodynamic Gyration Ratius ξ_H, and the Long Axis Length L of T4DNA Macromolecules at Various Concentrations of CTAB

c(CTAB), μM	2.0	3.2	160	630	1400	2500
η_S, mPa s[a]	1.098	1.099	1.103	1.112	1.120	1.124
D, $\mu m^2/s$[b]	0.19	0.22	1.11	0.87	0.64	0.54
ξ_H, μm[b]	1.03	0.89	0.18	0.21	0.30	0.36
L, μm[c]	3.50	3.10	0.73	0.70	0.94	1.07

[a] The experimental error is less than ±2%. [b] The experimental error of the mean D values is estimated to be in the order of ±10%. Accordingly, the estimated error of ξ_H is in the order of ±10%. [c] The blurring effect of the order 0.3–0.4 μm is not eliminated in these values.

constant D. The hydrodynamic gyration radius ξ_H was calculated from D on the basis of the Stokes–Einstein relation according to the Zimm model as follows:[29,30]

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

where k_B is the Boltzmann constant and η_S is the viscosity of the solvent.

The experimental data are summarized in Table 1. The hydrodynamic radius ξ_H for the coil state is found to be in good correspondence with the previously reported data.[28,31,32] Thus, we have confirmed that ξ_H changes abruptly by about 5 times in magnitude through the coil–globule phase transition; see ξ_H = 0.89 μm at c = 3.2 μM and ξ = 0.18 μm at c = 160 μM (Table 1). The change in magnitude of the long axis length L before and after transition is also 4 or 5 times. However, such an excellent correspondence of the change obtained by the different experimental measures seems to be somewhat accidental. The apparent value of L is larger than the actual value by the order of 0.3–0.4 μm due to the blurring effect of the fluorescent image. On the other hand, as the spatial density in the coil state is quite low, ξ_H should be somewhat smaller than the actual extent of the coil. This is because eq 2 holds only for nearly spherical particles with relatively high density. In addition to this, it is noted that L should be larger than ξ_H in general. Nonetheless, it is obvious that large DNA exhibits discrete phase transition, although further study is necessary in order to evaluate precisely the real change in the size. The increase of the size of the globule at high (more than 10^{-3} M) concentrations of CTAB is also recognized again from the measurement of the diffusion constant. Thus, the general trend on the change of the conformation dependent on CTAB concentration observed from the shape in the fluorescent microscopy has been reproduced from the measurement of the diffusion constant for the individual molecules.

Peculiarity of Coil–Globule Transition in Large DNA. Let us make a brief theoretical explanation on the single molecular phase transition between the coil and globule states. According to the standard treatment in the statistical physics of linear polymers,[33,34] the free energy is given in virial expansion:

$$\frac{G(n)}{kT} = \frac{3R^2}{8N\lambda^2} + NBn + Ncn^2 + \bar{o}(n^3) \tag{3}$$

where $G(n)$ is the free energy of a polymer molecule, N is the number of links, R is the maximum length between links in the same molecule, λ is the persistent length of the polymer, and n is the density of links per unit volume. B and C are the second and third virial coefficients, respectively.

When $B > 0$, the linear polymer exists in a random coil state, due to the repulsive interaction between the links with respect to the free energy. In this case, neglecting the third term, from the condition of $\partial G/\partial R = 0$, we expect the following scaling law, with the approximation that $n \approx N/R^3$:

$$\xi_{coil} \propto N^{3/5} \tag{4}$$

When $B < 0$, the third term becomes important in eq 3, due to the attractive interaction between the links. Then, by neglecting the first term in eq 3, we expect the following scaling law:

$$\xi_{glob} \propto N^{1/3} \tag{5}$$

According to the above scaling law, the difference in size between the coil and globule becomes larger with the increase of N. When N becomes large enough, discrete phase transition may be expected. Thus, not only large DNA but also usual synthetic or natural polymers with enough chain lengths may exhibit discrete phase transition between the coil and globule. For example, it is quite possible that the steep but continuos change in the size of polyacrylamide,[35] observed with the technique of light scattering, may reflect the intrinsic nature of the discrete coil–globule transition. Studies with the single molecular observation on various linear polymers are awaited to make clear the possibility of the discrete phase transition.

Our research is performed with the large T4DNA macromolecules with relatively large N value. The contour length L_c of the single T4DNA macromolecule is known to be about 55 μm.[17] The persistent length λ of a T4DNA molecule, which was measured at the mole ratio of fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and T4DNA equal to 1:1 (see Experimental Section), is found to be 0.079 μm[36] with the assumption of Gaussian chain. Thus, the number of Kuhn segments N_K of the T4DNA macromolecule[34] in our conditions is

$$N_K = \frac{L_c}{2\lambda} \approx 350 \tag{6}$$

For such a giant DNA molecule, we can expect a significant difference between the size of the coil and globule states. In fact, as is seen in the Table 1, the ratio between the hydrodynamic gyration radii of DNA molecules in the coil (2.0×10^{-6} and 3.2×10^{-6} M of CTAB) and globule (1.6×10^{-4} and 6.3×10^{-4} M of CTAB) states lies between 4.2 and 5.7. It is necessary to mention that our above theoretical consideration is rather preliminary, because it doesn't touch upon, for example, the conclusions of renormalization theory[33] or polyelectrolyte effect.[37] Further study on the details of the coil–globule transition of a single DNA molecule is awaited, together with

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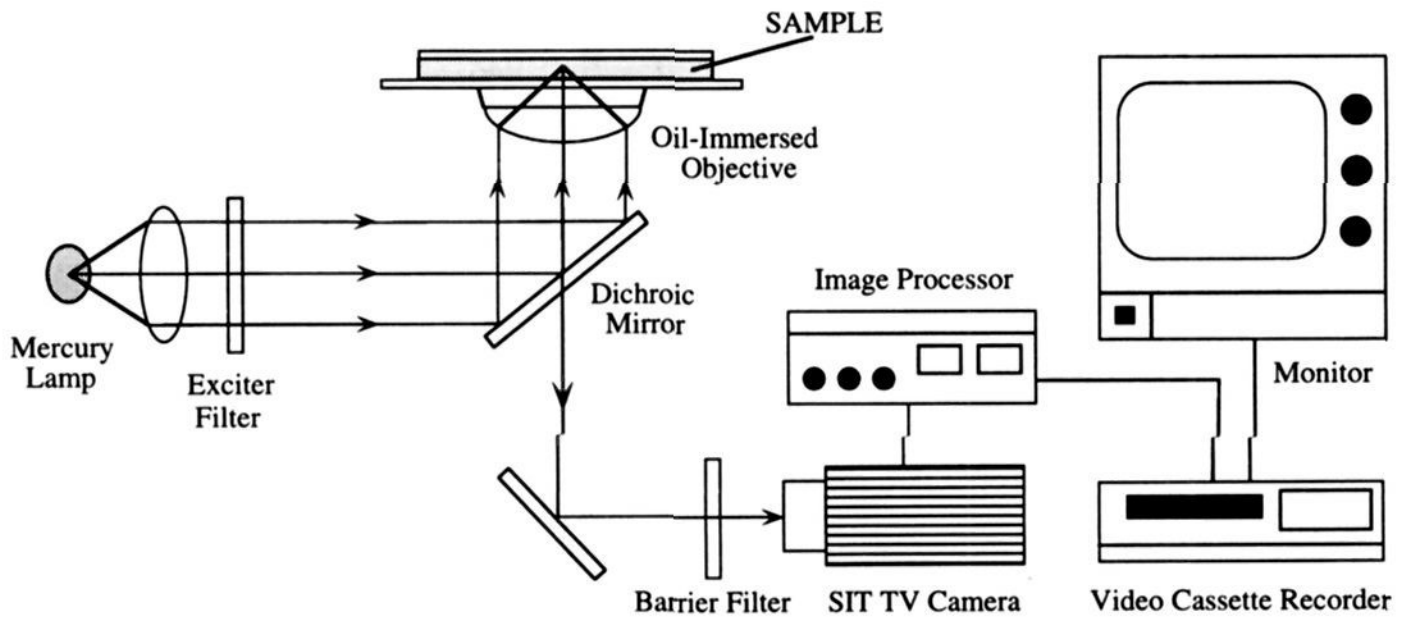

Figure 7. Schematic diagram of the experimental apparatus.

the measurement of the binding number of the surfactant molecules onto a single DNA.

It is known that several chemicals, including cationic surfactant,[38-40] can be used to purify DNA molecules through the process of collapse, or precipitation. It is also noted that reagents such as PEG,[41-43] CTAB,[44] and hexamine cobalt[45] modulate the reaction kinetics of renaturation and ligation of DNA. However, the past knowledge on the higher order structure of collapsed DNA has been insufficient, espesially for the problem whether single molecular compaction or multimolecular aggregation occurs. In this paper, we have shown that a single large DNA in aqueous solution can form a globular structure without the aggregation between DNA molecules. This conclusion may be useful for biochemists and molecular biologists to shed light on the mechanism of DNA collapse.

Conclusion

In summary, we have found that individual DNA molecules exhibit a discrete transition between the coil and globule states by binding with cationic surfactant. It becomes clear that fluorescence microscopy is quite useful for studying the conformational change in the higher order structure of a single DNA in an aqueous environment.

Experimental Section

Materials and Methods. T4DNA, 166 kilobase pairs (contour length 55 μm),[17] was purchased from Nippon Gene. Monodispersity of T4DNA was checked by gel electrophoresis in 0.5 × TBE buffer. The absence of fragmented DNA was also confirmed from the observation with fluorescence microscopy. 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 absorbancy of T4DNA stock solution at 260 nm to that at 280 nm was 1.8. Cationic surfactant, cetyltrimethylammonium bromide (CTAB), was purchased from Wako Pure Chemical Industries Ltd. and recrystallized twice from acetone. Fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and antioxidant, 2-mercaptoethanol (ME), were purchased from Wako Pure Chemical Industries Ltd. and were used without further purification. ME, a free radical scavenger, was used to reduce fluorescence fading and light-induced damage of DNA molecules.

The observation of DNA with fluorescence microscopy has been carried out with experimental conditions similar to those previously reported.[21,22,31] The experimental system is schematically shown in Figure 7. The samples were illuminated with 365 nm UV light. Fluorescence images of DNA molecules were observed by use of a Zeiss Axiovert 135 TV microscope equipped with a 100× oil-immersed objective lens and were recorded on videotapes with the sampling rate of 30 frames/s through the high-sensitive 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), for the individual video frames.

The viscosity was measured by TOKIMEC Visconic ELD viscometer at 20.0 ± 0.1 °C. The viscometer was calibrated with JS 2.5 calibration liquid (Showa Shell Co., Ltd., Tokyo). The temperature was kept constant using a water jacket connected to the thermostating system NESLAB Exacal EX200/NESLAB Endocal 350. No apparent non-Newtonian behavior was recognized within the conditions of our measurement.

Fluorescence spectra were measured on a SHIMADZU UV-2200 recording Spectrophotometer.

Sample Preparation. The sample solutions were prepared according to the following procedure. DNA molecules were diluted with 0.5 × TBE buffer solution (45 mM Tris, 45 mM borate, 1mM EDTA) containing 4% (v/v) ME and fluorescent dye. The DNA solution, containing DAPI and ME, was mixed with the surfactant solution in a gentle manner and then was kept for 15 min before the observation. The final concentrations were as follows: DNA in nucleotide, 0.6 μM and DAPI, 0.6 μM. The concentrations were kept constant throughout the experiment. In this condition, the binding number of DAPI per one base pair is estimated to be equal to 0.05,[18] and the persistent length is expected to remain nearly the same as in the absence of DAPI.[36]

Microscope Glasses. Special care was taken to clean the glass microscope slides and cover slips 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.

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