How Does Alcohol Dissolve the Complex of DNA with a Cationic Surfactant?

Vladimir G. Sergeyev,*,† Sergey V. Mikhailenko,†,‡ Olga A. Pyshkina,† Igor V. Yaminsky,† and Kenichi Yoshikawa*,§

Contribution from the Department of Polymer Science, Faculty of Chemistry, Moscow State University, Moscow 119899, Russia, Graduate School of Human Informatics, Nagoya University, Nagoya 464-8601, Japan, and Department of Physics, Faculty of Science, Kyoto University, Kyoto 606-8502, Japan

Received May 15, 1998

Abstract: The single-chain observation of isolated giant DNAs complexed with a cationic surfactant, CTAB, was performed using fluorescence microscopy. The DNA−CTAB complex exhibits a re-entrant transition, collapsed globule → elongated coil → collapsed globule, with an increase in the alcohol concentration. The existence of DNA in its coil state at an intermediate concentration of alcohol implies that this environment is a good solvent for the DNA chains. On the other hand, the presence of the globule state at both low and high alcohol concentrations indicates that this is a poor solvent for the complex. *Regardless of this fact, the globule generated at a high alcohol concentration is unexpectedly soluble; i.e., this is a good solvent for the complex with respect to the solvability, but it is a bad solvent with respect to the polymer conformation.* This unique property of the complex is attributable to the effect of micelle formation, where surfactant molecules cover the entire globule and lower the surface energy of the collapsed state. This conclusion is supported by additional experiments on the conformational change in DNA with alcohol in the absence of CTAB and on observations with CD and UV spectroscopy for the complex with different alcohol concentrations.

Introduction

The interactions between polymers and surfactants in aqueous solutions have been the subject of intense investigation because of their widespread application and because of the fundamental interest in complex fluids on a mesoscopic scale.[1−5] Among the various polymer−surfactant systems, the interaction between DNA and cationic surfactants holds special interest because of its significance in biomedical applications and because of the need for a better understanding of DNA behavior in living cells.

DNA−surfactant complexes are generally insoluble in water, and the precipitation of various DNAs by cationic surfactants has been applied to DNA extraction, concentration, and counting.[6−9] As described in well-established protocols in molecular biology, DNA is recovered from the DNA−surfactant complexes in a purified state after being washed with mixed organic solvents,[8,9] which suggests that such complexes are insoluble in hydrophobic solvents.

On the other hand, it has recently been shown that DNA−surfactant complexes are soluble in low-polarity organic solvents, such as chloroform, hexane, cyclohexane, and benzene, as opposed to being insoluble in aqueous solutions.[10−14] Similarly, it has also been shown that, like those of DNA, stoichiometric complexes of polyelectrolytes and oppositely charged surfactants are soluble in low-polarity organic solvent. It has also recently been discovered, on the basis of direct observation of individual DNA molecules using fluorescence microscopy,[15−18] that isolated long DNA molecules exhibit an all-or-none transition from an elongated coil to a compact globule state upon the formation of complexes with cationic surfactants. It has been shown that 2-propanol induces a large discrete transition in isolated DNA chains.[19] These studies have

* To whom all correspondence and reprint requests should be addressed.
† Moscow State University.
‡ Nagoya University.
§ Kyoto University.

(1) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *Molecular Biology of the Cell*; Garland Publishing: New York & London, 1989.
(2) Lindman, B.; Thalberg, K. Polymer-Surfactant Interactions-Recent Developments. In *Interactions of Surfactants with Polymers and Proteins*; Goddard, E. D., Ananthapadmanabhan, K. P., Eds.; CRC Press: Boca Raton, Florida, 1993; pp 203−276.
(3) Blow, J. J.; Sleeman, A. M. *J. Cell Biol.* **1990**, *95*, 383−391.
(4) Leno, G. H.; Lasky, R. A. *J. Cell Biol.* **1991**, *112*, 557−566.
(5) Gelbart, W. M.; Ben-Shaul, A. *J. Phys. Chem.* **1996**, *100*, 13169−13189.
(6) Sibatani, A. *Anal. Biochem.* **1970**, *33*, 279−285.
(7) Trewavas, A. *Anal. Biochem.* **1967**, *21*, 324−329.
(8) Pontius, B. W.; Berg, P. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8237−8241.
(9) Geck, P.; Nasz, I. *Anal. Biochem.* **1983**, *135*, 264−268.
(10) Sergeyev, V. G.; Pyshkina, O. A.; Zezin, A. B.; Kabanov, V. A. *Polymer Science, Ser. A.* **1997**, *39*, 12−16.
(11) Pyshkina, O. A.; Sergeyev, V. G.; Zezin, A. B.; Kabanov, V. A. *Dok. Chem.* **1996**, *348*, 138−140.
(12) Pyshkina, O. A.; Sergeyev, V. G.; Lezov, A. V.; Mel'nikov, A. B.; Ryumtsev, E. I.; Zezin, A. B.; Kabanov, V. A. *Dokl. Phys. Chem.* **1996**, *349*, 207−210.
(13) Ijiro, K.; Okahata, Y. *J. Chem. Soc., Chem. Commun.* **1992**, *18*, 1339−1341.
(14) Tanaka, K.; Okahata, Y. *J. Am. Chem. Soc.* **1996**, *118*, 10679−10683.
(15) Mel'nikov, S. M.; Sergeyev, V. G.; Yoshikawa, K. *J. Am. Chem. Soc.* **1995**, *117*, 2401−2408.
(16) Mel'nikov, S. M.; Sergeyev, V. G.; Yoshikawa, K. *J. Am. Chem. Soc.* **1995**, *117*, 9951−9956.
(17) Mel'nikov, S. M.; Sergeyev, V. G.; Mel'nikova, Yu. S.; Yoshikawa, K. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 283−288.
(18) Bloomfield, V. A. *Curr. Opin. Struct. Biol.* **1996**, *6*, 334−341.
(19) Ueda, M.; Yoshikawa, K. *Phys. Rev. Lett.* **1996**, *77*, 2133-2136.

10.1021/ja981683+ CCC: $18.00 © 1999 American Chemical Society
Published on Web 02/11/1999

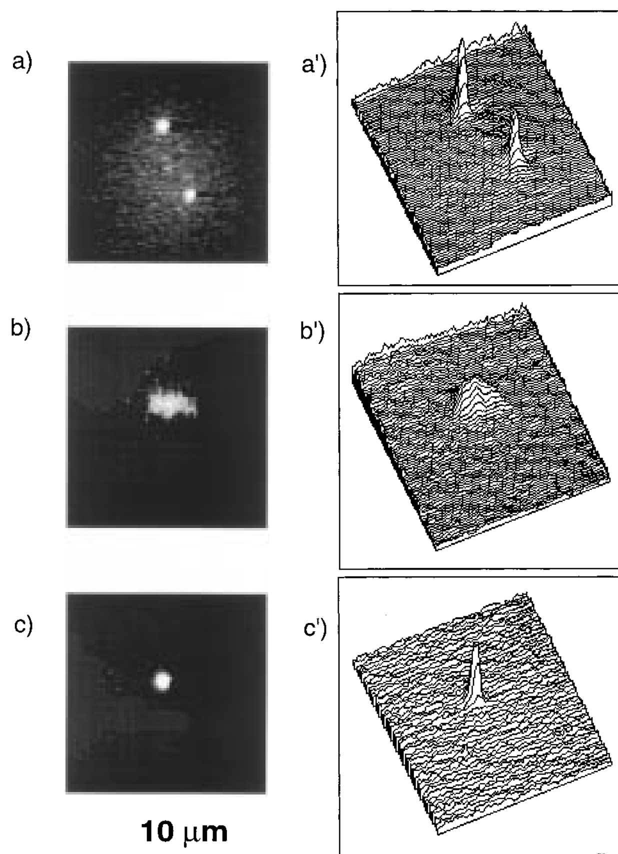


Figure 1. Fluorescent images of T4DNA−CTAB complex at various 2-propanol concentrations: (a) globule state at 20% (v/v) observed at the glass surface, (b) coil state at 40% (v/v) in the bulk solution, and (c) globule state at 90% (v/v) in the bulk solution. Figures on the right show the fluorescence intensity distribution on the corresponding photographs.

revealed that, although the transition of individual DNA molecules is largely discrete and can be considered a kind of first-order phase transition, the transition of the ensemble of DNA molecules follows a typical sigmoidal curve and does not correspond to any kind of phase transition.[20]

To clarify the physicochemical characteristics of DNA−surfactant complexes in solutions, in the present study we examined the effect of alcohol−water solvents on conformational changes in DNA molecules in the absence and presence of a cationic surfactant. By varying the relative composition of the alcohol−water solution, the DNA conformation over a wide range of polarity was investigated using different experimental techniques; i.e., fluorescence microscopy for single-molecule observation, circular dichroism (CD), and UV spectroscopy.

Results

Unfolding and Folding of DNA with Alcohol in the Presence of CTAB. Figure 1 shows typical fluorescence images of the T4DNA molecules in the presence of 1.0×10^{-4} M CTAB at various concentrations of 2-propanol. At a low alcohol content, the T4DNA−CTAB complex exists as small compact particles, i.e., globules, which are observed as precipitants on the glass surface (Figure 1a), indicating that the DNA−CTAB complex is insoluble in high-polarity media, whereas with an intermediate alcohol content, DNA molecules exhibit an elongated coil conformation in the bulk solution and show random fluctuating movement (Figure 1b). A further increase in the alcohol concentration restores the compact conformation of the DNA−CTAB complex, but the compact globules exhibit thermal fluctuation with Brownian motion and avoid adsorption or precipitation onto the glass surface (Figure 1c).

(20) Yoshikawa, K.; Takahashi, M.; Vasilevskaya, V. V.; Khokhlov, A. R. *Phys. Rev. Lett.* **1996**, *76*, 3029−3031.

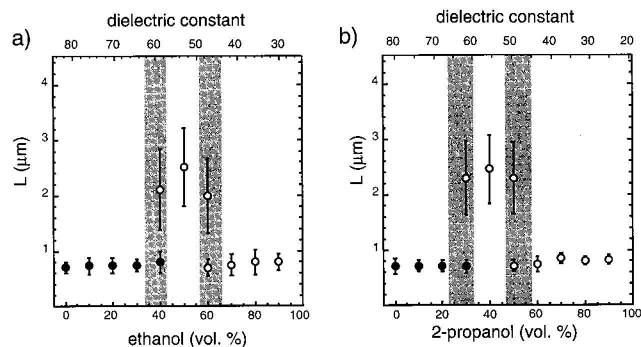


Figure 2. Dependence of the long-axis length, **L**, of T4DNA, complexed with CTAB, on (a) ethanol and (b) 2-propanol concentrations and the corresponding dielectric constant. The open and closed circles indicate the DNAs dissolved in solution and adsorbed on the glass surface, respectively. At least 50 DNA molecules were analyzed for each alcohol concentration. Error bars indicate the standard deviation in the distribution. The shaded region corresponds to the coexistence of molecules in coil and globule states. The DNA concentration was 0.6 μM in bp, corresponding to 0.2 μg/mL, and the CTAB concentration was fixed to be 1.0×10^{-4} M.

To characterize the conformational change in the DNA−CTAB complex in a quantitative manner, a series of fluorescence microscopic measurements were carried out by changing the alcohol concentration from 10 to 90% (v/v). In Figure 2, the apparent long-axis length **L** of T4DNA calibrated from the video image is summarized as a function of the concentration of (a) ethanol or (b) 2-propanol. In both cases, at low concentrations of alcohol, less than 40% (v/v) for ethanol or 30% (v/v) for 2-propanol, all of the DNAs are observed on the glass surface in the collapsed state, with an apparent diameter of around 0.8 μm in the fluorescent image. At intermediate alcohol concentrations (40−60% (v/v) for ethanol and 30−50% (v/v) for 2-propanol), DNA molecules exist in the solution as elongated coils with **L** distributed around 3 μm. With a high alcohol content (more than 70% (v/v) for ethanol and 60% (v/v) for 2-propanol), in the bulk solution only collapsed globules of T4DNA−CTAB complexes with an apparent average size of 0.8 μm and a narrow distribution are observed. Thus, it is clear that the T4DNA−CTAB complex, with an increase in the alcohol concentration, undergoes a re-entrant globule−coil−globule transition and that dissolution of the complex is accompanied by the globule to coil transition, whereas the globule DNAs remain in the solution after the re-entrant transition at a high alcohol content. The change in the distribution of the length of T4DNA molecules with an increase in the alcohol concentration is shown in Figure 3.

On the basis of above results, the effect of the alcohol content in the solvent on the DNA−CTAB complex is summarized in Table 1. In relation to the complex of the elongated DNA with CTAB, we have recently found that the sedimentation behavior of DNA molecules differs markedly in the presence and absence of CTAB (unpublished results), suggesting that the elongated DNAs are interacting with CTAB at the intermediate concentrations of alcohol.

To gain further insight into the conformational transition of DNA molecules together with the mechanisms of solvation and precipitation, we measured the amount of dissolved DNA

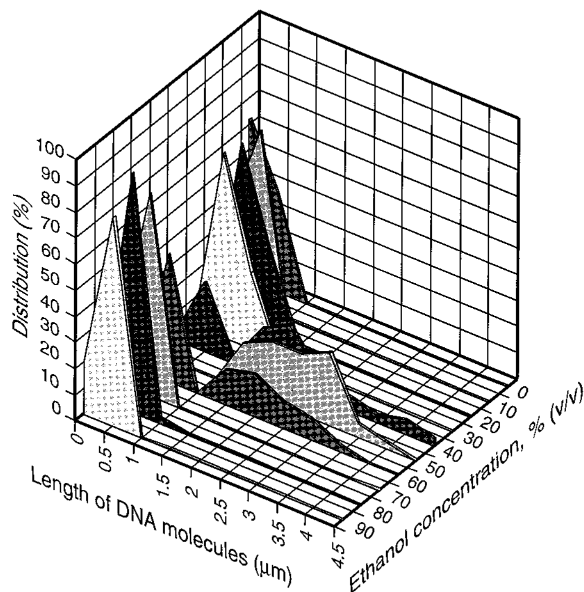


Figure 3. Change in the distribution of the long-axis lengths of T4DNA molecules at different composition of water-ethanol solution. The concentration of CTAB was fixed to be 1.0×10^{-4} M. The number of analyzed DNA molecules at each alcohol concentration was at least 50, and the area of histograms is normalized to be equal.

Table 1. Solvent Quality with Respect to the Solvability and the Conformation of DNA

alcohol content	DNA with CTAB		native DNA	
	solvability	conformation	solvability	conformation
low	poor	collapsed	good	elongated
medium	good	elongated	good	shrunken coil, intrachain segregation
high	good	collapsed	poor	collapsed

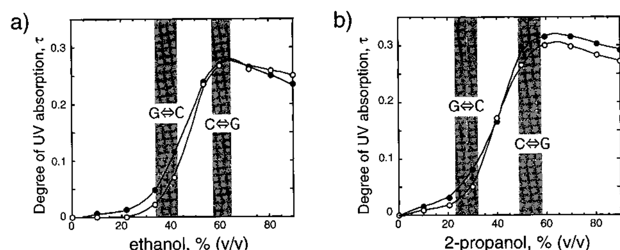


Figure 4. Degree of absorption of 260 nm UV light, τ, in DNA–CTAB complexes based on salmon sperm DNA (open circle) and chicken erythrocyte DNA (closed circle) as a function of (a) ethanol and (b) 2-propanol concentrations. $\tau = -\ln(I/I_0)$, where I and I_0 are the light intensities for the sample and for the control, respectively. The optical length was 1 cm, and the DNA concentration was 16.5 μg/mL. The regions of the transitions found from the single-chain observation (see Figure 2) are indicated with the mark of G ⇔ C and C ⇔ G, where G and C denote globule and coil, respectively.

molecules in the bulk solution by UV absorption spectroscopy (260 nm) for the CTAB complex of DNA from salmon sperm and chicken erythrocytes. Figure 4 shows the change in UV absorption τ for the complex with the addition of alcohols. The steep increase in τ at around 35% (v/v) for ethanol (a) and around 25% (v/v) for 2-propanol (b) indicates that the precipitated DNA–CTAB complex dissolves into the solution at these concentrations. This trend corresponds to the results of the fluorescence measurements (Figure 2). τ values reach a maximum at 60% (v/v) for ethanol and 50% (v/v) for 2-propanol, suggesting that all of the DNA molecules are dissolved in the solution. With the further addition of alcohol, τ values decrease only slightly. Interestingly, the region of the steep increase in τ values corresponds well with that of the globule–coil transition observed in isolated long T4DNAs (e.g., Figure 2). Thus, the effect of alcohol on short DNAs complexed with CTAB is very similar to its effect on the globule–coil transition in complexes of giant DNAs.

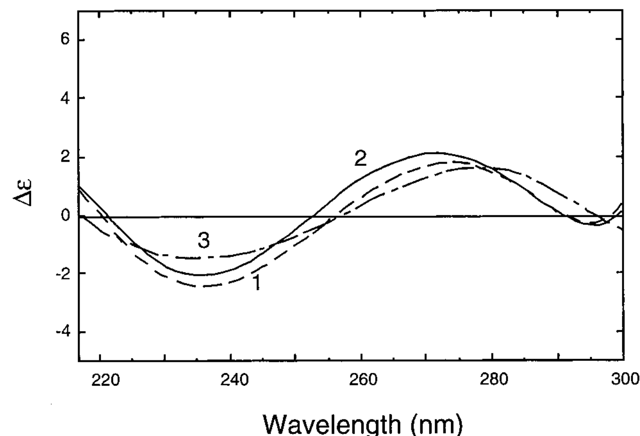


Figure 5. CD spectra of DNA and DNA–CTAB complexes: (1) DNA in 0.5 × TBE buffer solution; (2) DNA–CTAB complex in 35% (v/v) 2-propanol; (3) DNA–CTAB complex in 90% (v/v) 2-propanol. Salmon sperm DNA was used in all cases.

To gain insight into the change in the secondary structure of DNA in relation to the folding- and unfolding-transition of DNA,[21–23] we performed CD spectroscopic measurements of the DNA–CTAB complex. Figure 5 shows the CD spectra of the complex of salmon sperm DNA with CTAB at 35 and 90% (v/v) 2-propanol in comparison with that of the initial DNA in buffer solution. The CD spectrum of the DNA–CTAB complex at 35% (v/v) 2-propanol (curve 2) resembles that of the DNA in buffer solution (curve 1) where DNA molecules are known to be in the B-form. In contrast, the CD spectrum of DNA at 90% (v/v) 2-propanol (curve 3) differs from that of native DNA and displays an A-like structure, which is characterized by a positive band around 270 nm, a negative band around 240 nm, and a crossover at 252 nm.[24] Similar CD spectra have been found for double-stranded DNAs in particles of medium-sized bacteriophage.[25] The CD spectra of DNA–CTAB complexes at 40% (v/v) and 90% (v/v) are essentially the same in both ethanol and 2-propanol.

Folding of DNA with Alcohol in the Absence of CTAB. Next, we studied the conformational change in T4DNAs at different alcohol concentrations in the absence of CTAB. Figure 6 shows typical fluorescence images of DNA molecules at several concentrations of 2-propanol. At a low alcohol content, 20% (v/v) in Figure 6a, individual T4DNA molecules exist as elongated coils. The Brownian motion of individual coils in a solution with a low alcohol content is shown in Figure 7. At an intermediate concentration of 2-propanol, 50% (v/v) in Figure 6b, the elongated coil and compact globule states coexist. At alcohol concentrations above 50% (v/v), only globular DNAs are observed in the solution (80% (v/v) in Figure 6c).

The dependence of the long-axis length, **L**, of T4DNA on the concentration of (a) ethanol and (b) 2-propanol is shown in Figure 8. At low concentrations for both ethanol and 2-propanol,

(21) Widom, J.; Baldwin, R. L. *J. Mol. Biol.* **1980**, *144*, 431–453.
(22) Widom, J.; Baldwin, R. L. *Biopolymers* **1983**, *22*, 1595–1620.
(23) Marx, K.; Ruben, G. *Int. J. Biol. Macromol.* **1986**, *11*, 241–248.
(24) Ivanov, V. I.; Minchenkova, L. E.; Schyolkina, A. K.; Poletayev, A. I. *Biopolymers* **1973**, *12*, 89–110.
(25) Karasev, A. V.; Dobrov, E. N. *Int. J. Biol. Macromol.* **1988**, *10*, 227–237.

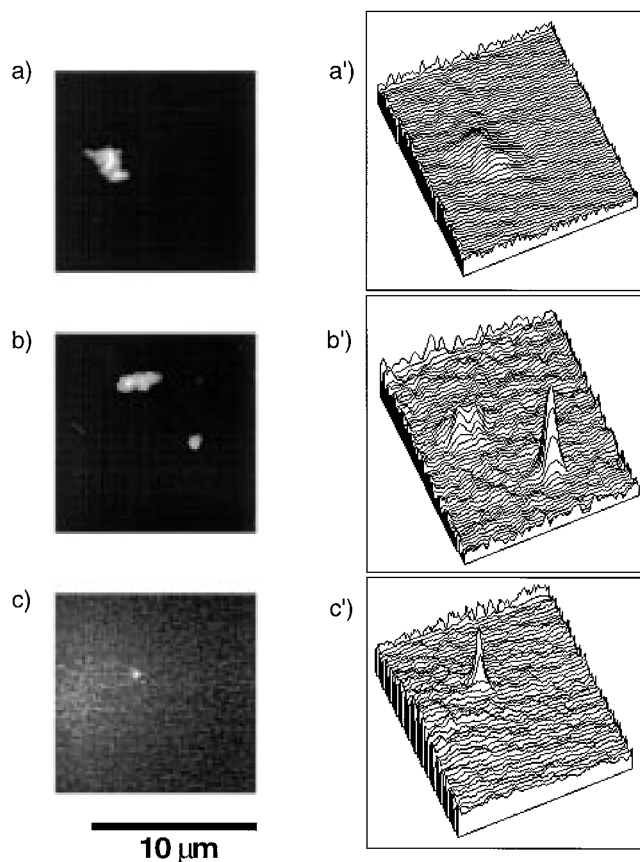


Figure 6. The fluorescent images of individual T4DNA molecules at various 2-propanol concentrations: (a) coiled state at 40% (v/v), (b) coexistence of coil and globule at 50% (v/v), and (c) precipitated onto the glass surface globules at 80% (v/v). Figures on the right show the fluorescence intensity distribution on the corresponding photographs.

DNA molecules remain in the elongated coil state; the size distribution has a maximum value of around 3.4 μm and a standard deviation of 0.5−0.7 μm, indicating fluctuation of the conformation. At intermediate concentrations (between 30% (v/v) and 50% (v/v) for 2-propanol (Figure 8a) and between 30% (v/v) and 60% (v/v) for ethanol (Figure 8b)), the coiled DNA gradually shrinks with an increase in the alcohol content. This experimental trend is attributable mainly to the occurrence of intrachain segregation within individual single DNA molecules and partly to enhanced counterion condensation in low-polarity medium.[19] When the alcohol concentration is increased to 60% (v/v) for 2-propanol or 70% (v/v) for ethanol, all of the DNAs exhibit a compact globule structure. Above these concentrations, DNA globules tend to be adsorbed on the glass surface, indicating that an aqueous solution with a high alcohol content is a poor solvent for DNA molecules, unlike the globules of the DNA−CTAB complex at high alcohol concentrations. The distribution of T4DNA length for different ethanol-in-water concentrations is shown in Figure 9.

Using AFM, we tried to observe the DNA conformation at intermediate concentrations of alcohol. Figure 10 shows the preliminary results of AFM measurement of T4DNA in 80% (v/v) 2-propanol solution, indicating a partial intrachain segregated structure. This structure corresponds to the morphology of the DNA at intermediate alcohol concentrations, as observed by fluorescence microscopy.[19] Extensive AFM measurements are now in progress in our laboratories not only on free DNA in alcohol but also on the DNA−surfactant complex.

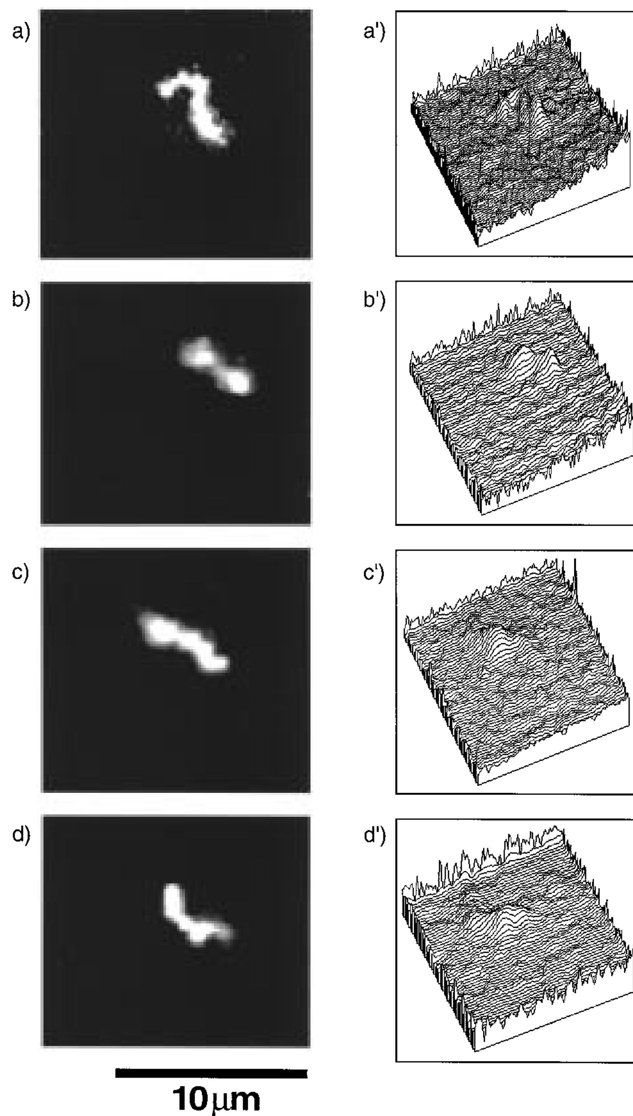


Figure 7. The fluorescent images of T4DNA molecules moving freely in the solution with low content of 2-propanol (10% (v/v)) in the absence of CTAB. The time interval between conformations shown on consequential photographs is about 0.1 s. The figures on the right show the light intensity distribution on the according photographs.

Discussion

Among the results described above, the most interesting finding is that, in a solution with high alcohol content, the collapsed globule is soluble and avoids the precipitation or adsorption on the solid surface; i.e., the solution with high alcohol content is a good solvent for the DNA−surfactant complex with respect to its solvability. According to the general understanding in standard polymer science, the globule state is generated in poor solvent; i.e., the globule should exhibit a tendency to be expelled from the solution and to be precipitated or adsorbed onto the vessel surface. Thus, in this section we will focus on this curious behavior of globular DNA complexed with CTAB.

It has been previously established that T4DNA molecules exhibit a discrete conformational transition from an extended coil to a compact globule state upon the formation of micelles with cationic surfactants.[15−17] Actually, it has been confirmed that, in the presence of T4DNA, micelles are generated at very low CTAB concentrations, i.e., 2 orders of magnitude smaller than the usual CMC in the absence of polyanion. These

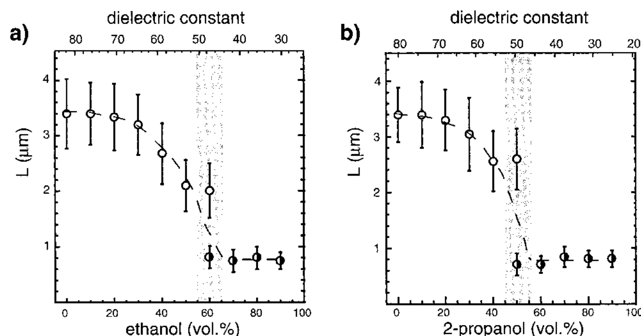


Figure 8. Dependence of the long-axis length, **L**, of T4DNA on alcohol concentration. The open circle indicates the DNAs dissolved in the solution. The half-closed circle indicates the DNAs present both in the solution and on the glass surface, and the relative ratio of the adsorbed species increases gradually with the time over the course of several hours: (a) water-ethanol solution, (b) water-2-propanol solution. At least 50 DNA molecules were analyzed for each alcohol concentration. Error bars indicate the standard deviation in the distribution. The shaded part corresponds to the coexistence region of coil and globule DNAs. The broken line indicates the change in the ensemble average of **L**. The DNA concentration was 0.6 μM in bp, corresponding to 0.2 μg/mL.

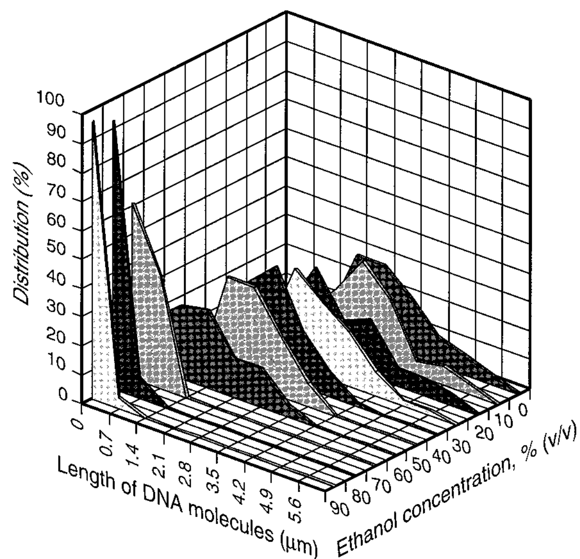
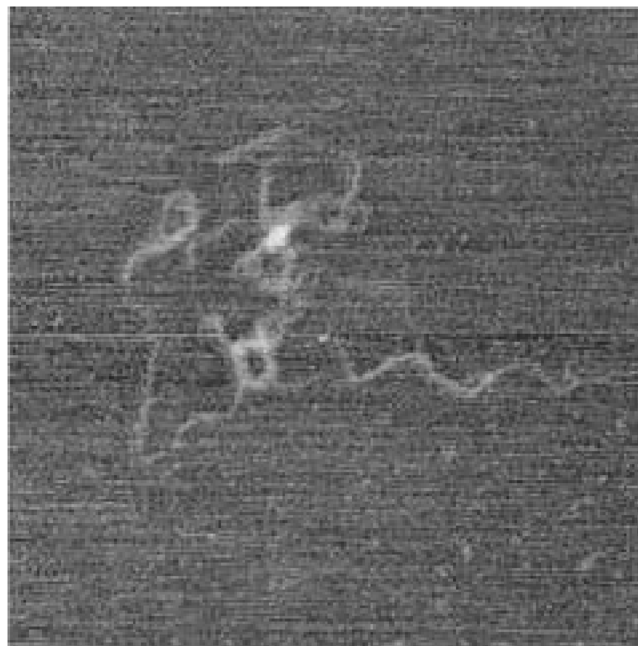


Figure 9. Change in the distribution of the long-axis lengths of T4DNA molecules at different composition of water−ethanol solution. The number of analyzed DNA molecules at each alcohol concentration was at least 50, and the area of histograms is normalized to be equal.



1 µm

Figure 10. Example of the morphology of the partially segregated T4DNA in 80% (v/v) 2-propanol, as is observed by AFM measurement. The observation was carried out using a Nanoscope III (Digital Instruments, Santa Barbara, CA) equipped with commercial AFM cantilevers with silicon tips. The liquid cell (Digital Instruments), used for AFM measurements, was flushed with about 5 mL of 40% (v/v) 2-propanol solution before the initial imaging. After that the chamber was filled with 40% (v/v) 2-propanol solution containing T4DNA with concentration 0.6 μm in nucleotides, and then the alcohol content was gradually increased to 80% (v/v) for the observation.

complexes are practically insoluble in aqueous solutions because the phosphate groups of DNA molecules are screened by the positive charge of the headgroups and also by hydrophobic moieties in the surfactants. On the other hand, it is well-known[26] that the addition of low-molecular-weight alcohols to an aqueous solution weakens hydrophobic interaction. The driving force to induce the change in conformation from a globule to a coil is mainly attributed to the decrease in attractive interaction between the surfactant molecules, i.e., to the destabilization of micelles complexed with DNA. Due to the destabilization effect, the DNA−CTAB complex is destroyed, which results in the adoption of the elongated conformation by the DNA molecules.

Another important factor in the decollapsing transition with an intermediate alcohol content is that a solvent with rather low polarity or hydrophilicity is still a good solvent for negatively charged DNA. The experimental results given in Figure 8 indicate that, with up to 50% (v/v) ethanol and 45% (v/v) 2-propanol, the solvent remains a good one for the DNA chain in the absence of CTAB. The elongated DNA generated by the decollapsing transition is expected to recover negative charges by releasing at least some of the bound cationic surfactant, induced by the decrease of the attractive interaction between the surfactant radicals with the increase of the alcohol content.

With both ethanol and 2-propanol, the alcohol concentration needed to induce the coil−globule transition in a re-entrant process (Figure 2) almost coincides with the concentration needed to induce a coil−globule transition in the absence of CTAB (Figure 8). Thus, we expect that the coil−globule transitions in the absence and presence of CTAB have a similar origin, i.e., the change in solvent quality from good to poor with an increase in the alcohol concentration. The appearance of the globule state means that this is a poor solvent for DNA. We should clarify why the globule in the absence of CTAB tends to be precipitated while that in the presence of CTAB is soluble in a solution with a high alcohol content. This difference in the property of the globule is attributable to the effect of CTAB; i.e., the globule in the absence of CTAB is repelled by the solvent. If the surface of the globule is covered with CTAB, where the hydrophobic moieties are oriented toward the bulk solution, the surface energy may be greatly decreased. Due to this effect, the globule becomes soluble in solution with a high alcohol content in the presence of CTAB (Figure 11).

On the basis of CD measurements, it is clear that the re-entrant transition of DNA complexed with CTAB does not lead

(26) Franks, F.; Ives, D. J. G. *Q. Rev. Chem. Soc. (London)* **1966**, *20*, 1−44.

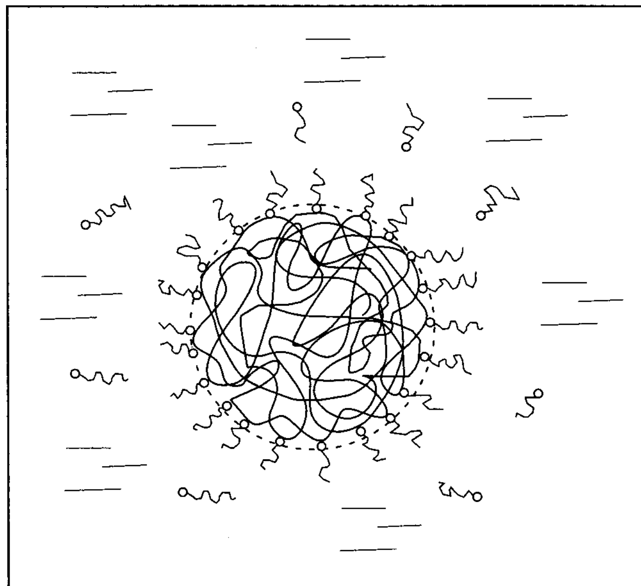


Figure 11. Plausible structure of the solvable DNA−CTAB complex in the solution with high alcohol content. For the simplicity, only the CTAB molecules located on the surface of the globule are shown.

to degradation of the double-helical structure of DNA molecules. However, we should be cautious in drawing conclusions regarding the A-conformation of DNAs in 90% (v/v) 2-propanol, since scattering from such compact particles fluctuating in the solution could mask the true spectrum.[27]

Conclusions

We have established the conditions for the dissociation−formation of DNA−CTAB complexes in organic solvents of different polarity. Fluorescence microscopy and UV and CD spectroscopy indicate that DNA−CTAB complexes dissociate into their initial components at concentrations of 40−60% (v/v) for ethanol or 30−50% (v/v) for 2-propanol, while conserving the double-stranded structure of native DNA. A further increase in the alcohol concentration leads to restoration of the complex, which is soluble under these conditions, i.e., in low-polarity organic media. DNA−CTAB complexes with different DNAs behave almost identically in alcohol solutions. Moreover, we have demonstrated the alcohol-induced re-entrant globule-coil-globule transition in single T4DNAs in the presence of CTAB. The conformational state of DNA−surfactant or DNA−lipid complexes in organic solutions of various polarities may be useful in cell transfection and gene therapy because the living cellular environment is generally composed of regions of different polarity or hydrophobicity.

Experimental Section

Materials and Methods. Bacteriophage T4dC DNA (166 kilobase pairs, contour length 57 μm[28]) was purchased from Nippon Gene and was mainly used in single-molecule observations by fluorescence microscopy. On the other hand, DNA from chicken erythrocytes (1500 bp) (Soyuzkhimreactive, Russia) and salmon sperm DNA (300−500 bp) (GosNIIOKhT, Russia) were used in spectroscopic measurements to obtain reliable data with a sufficient amount of the sample. The cationic surfactant, cetyltrimethylammonium bromide (CTAB) (Tokyo Kasei Kogyo Co.), was recrystallized twice from acetone and dried overnight at 25 °C under vacuum. The fluorescent dye 4,6′-diamidino-2-phenylindole (DAPI), the antioxidant 2-mercaptoethanol (ME), ethanol, and 2-propanol (spectral grade) were obtained from Wako Pure Chemical Industries Ltd. ME was used as a free-radical scavenger to reduce fluorescent fading and light-induced damage of DNA molecules.

Fluorescence microscopic measurement 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 × oil-immersed lens and recorded on S-VHS videotape through a high-sensitivity Hamamatsu SIT TV camera. The observations were carried out at 25 °C. The apparent length of the long axis **L**, which was defined as the longest distance in the outline of the DNA image, was calibrated with an Argus 10 image processor (Hamamatsu Photonics). The blurring effect was estimated to be on the order of 0.3 μm, and the data for **L** are given in the text without correction. The sample solutions, microscope slides, and coverslips were carefully prepared as in previous studies.[15,16] DNA molecules were diluted with 0.5 × TBE buffer solution containing 4% (v/v) ME. The surfactant solution was added into the DNA solution containing DAPI and ME, mixed in a gentle manner, and kept for 15 min before the addition of alcohol. The final concentration of DNA in nucleotides was 0.6 μM, [DAPI]; 0.6 μM, [CTAB]=1.0 × 10^{-4} M. According to our previous results[16] and the data reported by Hayakawa et al.,[29] the binding isotherms of CTAB to DNA are almost independent of the polyacid concentration at DNA concentrations below 10^{-3} M.

Absorption spectra were obtained with a Specord M-40 spectrophotometer. The concentration of DNA in the solution was calculated by assuming that the molar extinction coefficient for native DNA in water is 6500 cm^2/mol. The CD spectra were recorded with a Jasco J-500C spectropolarimeter. Cells with an optical path of 1 cm were used for the UV and CD measurements. $\Delta\epsilon$ values are given in moles of nucleotide. All measurements were carried out at 25 °C.

Solvent mixtures were prepared on a volume basis. Alcohol was added dropwise to a DNA−CTAB solution, which was stirred continuously and then stored for at least 24 h before observation.

Acknowledgment. This work was partly supported by Grant No. 96-03-33522a from the Russian Foundation for Basic Research and the Sasakawa Scientific Research Grant from the Japan Scientific Society. V.S.G. is also grateful to the Japan Society for the Promotion of Science, which provided the opportunity for his work in Japan. S.V.M. acknowledges the Ministry of Education, Science and Culture of Japan for the scholarship. We wish to thank Mr. M. O. Gallyamov, Physical Department of Moscow State University, for technical assistance in AFM experiments.

JA981683+

(27) Girod, J. D.; Johnson, W. C.; Huntington, S. K.; Maestre, M. F. *Biochemistry* **1973**, *12*, 5092−5096.

(28) Yoshikawa, K.; Matsuzawa, Y.; Minagawa, K.; Doi, M.; Matsumoto, M. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 1274−1279.

(29) Hayakawa, K.; Santerre, J. P.; Kwak, J. C. T. *Biophys. Chem.* **1983**, *17*, 175−181.