DNA conformational dynamics in the presence of catanionic mixtures

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Abstract DNA conformational behavior in the presence of non-stoichiometric mixtures of two oppositely charged surfactants, cetyltrimethylammonium bromide and sodium octyl sulfate, was directly visualized in an aqueous solution with the use of a fluorescence microscopy technique. It was found that in the presence of cationic-rich catanionic mixtures, DNA molecules exhibit a conformational transition from elongated coil to compact globule states. Moreover, if the catanionic mixtures form positively charged vesicles, DNA is adsorbed onto the surface of the vesicles in a collapsed globular form. When anionic-rich catanionic mixtures are present in the solution, no change in the DNA conformational behavior was detected. Cryogenic transmission electron microscopy, as well as measurements of translational diffusion coefficients of individual DNA chains, supported our optical microscopy observations.
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Key words: DNA; Catanionic mixture; Coil-globule transition; Fluorescence microscopy; Cryogenic transmission electron microscopy

1. Introduction

The interaction between DNA and amphiphilic molecules, which gained preliminary interest mainly in the studies on colloid and physical polymer chemistry [1–3], received much attention from biophysics, biochemistry and, consequently, pharmaceutical sciences in recent years [4]. Among other amphiphilic systems, liposomes consisting of positively charged and zwitterionic lipids were found to be the best candidates for medical applications, i.e. in vitro and in vivo delivery of genetic material [5]. Several physico-chemical methods were used for the examination of DNA-amphiphile interactions in solutions [6]. They provide us the important information on the interaction, but do not answer the question on the behavior of individual DNAs during the interaction. At the same time, microscopic methods (transmission electron or atomic force microscopies), allowing single-molecule observation of DNA, often operate with the dried DNA, deposited onto metal grids, mica plates, etc. Therefore, monomolecular DNA behavior in the solution in the presence of amphiphiles remained uncertain until the mid-90s.

Recently, the interaction between single large DNA in an aqueous environment and amphiphilic molecules has been studied with the use of a fluorescence microscopy technique [7]. The conformational behavior of individual DNAs stained with a fluorescence marker has been detected with respect to the activity of amphiphilic molecules, i.e. cationic and neutral lipids and synthetic surfactants. It has been found that isolated DNA chains undergo a discrete coil-globule transition upon the increase of the concentration of amphiphiles in the solution [8]. This process starts at very low concentrations of a positively charged surfactant, i.e. circa two orders of magnitude lower than the critical micellization concentration (cmc) of individual surfactant in polyelectrolyte-free solution [9]. If non-ionic surfactant is added to the DNA solution, collapse occurs at much higher surfactant concentrations due to the increase of the osmotic pressure of surfactant molecules [10].

Despite numerous studies of DNA interaction with oppositely charged amphiphiles, the conformational behavior of DNA in the presence of mixtures of two oppositely charged surfactants has never been studied. Nevertheless, this system presents certain interest for the pharmaceutical applications, since variation of the molar ratio between oppositely charged amphiphiles may strongly affect linear dimensions and the charge of individual DNAs and, consequently, influence the process of a transmembrane DNA delivery.

A 'catanionic surfactant' term was defined in the end of the last decade as an equimolar mixture of two oppositely charged surfactants, from which the inorganic counterions are completely removed [11]. Thus, the catanionic surfactant is uncharged and one amphiphilic ion acts as a counterion for another. However, this report is devoted to the behavior of non-equimolar cationic and anionic surfactant mixtures, where the counterions are present. This mixtures will be referred to as 'catanionic mixtures' [12]. These pseudoternary aqueous mixtures exhibit a rich phase behavior and interfacial properties, in comparison with those of the individual surfactants, namely lowering of the critical aggregation concentration and an enhanced surface activity, adsorption and detergency, with relevance to application-oriented goals.

The system used in this work is the aqueous mixture of cetyltrimethylammonium bromide (CTAB) and sodium octyl sulfate (SOS), studied by Kaler et al [13,14]. The formation of microstructures observed in the phase diagram of the mixture is largely the result of the electrostatic interactions between charged head groups of two amphiphiles. By varying the mixture composition, those, along with hydrophobic interactions between surfactant tails, can be tuned to produce aggregates with different charges and geometries, ranging from spherical to cylindrical and planar. The main interest in the catanionic mixtures is the formation of vesicle phases which are not observed when only one of the surfactants is present in the aqueous solution [14]. Catanionic vesicles are believed to be thermodynamically stable systems since they form spontane-

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ously and reversibly and remain stable for a long period of time [15]. These systems are used in several pharmacological and personal care applications [4,16,17].

In the present report, we used a fluorescence microscopy technique to observe large individual DNA molecules in the presence of both positively and negatively charged catanionic mixtures. The conformational behavior of fluorescently labelled DNA molecules at different molar ratios between cationic and anionic surfactants in the mixture was directly visualized in an aqueous solution. To better understand the mechanism of DNA interaction with catanionic vesicles, the diffusion of individual DNA molecules was quantified for different compositions of catanionic mixtures. Dynamic light scattering and cryo-transmission electron microscopy experiments were carried out to support the results of fluorescence microscopy.

2. Materials and methods

2.1. Materials

Coliphage T4 DNA ($M = 1.1 \times 10^8$ D, circa 167 kbp) was supplied by Sigma. Synthetic surfactants, CTAB and SOS were obtained from Sigma and Merck, respectively, CTAB was re-crystallized twice from acetone, SOS was used as received. The DNA concentration was determined spectrophotometrically, considering the molar extinction coefficient of DNA bases to be equal to 6600 M^{-1} cm^{-1} [18], the ratio of the absorbance of a DNA stock solution at 260 nm to that at 280 nm was found to be 1.9. The fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and the antioxidant, 2-mercaptoethanol (ME), were from Sigma. Bidistilled water was purified with a Millipore filter (pore size 22 μm).

2.2. Sample preparation

Solutions of catanionic mixtures were prepared by making stock solutions of both surfactants, mixing the stock solutions at desired concentrations and adding water. Then, they were gently vortexed and left without further mechanical agitation for several days to equilibrate at 25°C. The sample compositions are reported in Table 1. DNA stock solutions were prepared as follows. DNA molecules were diluted with the 10 mM Tris-Cl buffer (pH = 7.6) containing 4% (v/v) ME, a free radical scavenger and a fluorescent dye. The resulting solution was gently mixed with the solutions of catanionic mixtures and was then kept for 2 h. The final concentrations were as follows: DNA in nucleotide units 0.5 μM, DAPI 0.5 μM. Under these conditions, the binding number of DAPI per 1 bp DNA in an aqueous buffer solution is estimated to be equal to 0.05 and the persistence length of the DNA chain is expected to remain nearly the same as in the absence of DAPI [19,20].

2.3. Fluorescence microscopy

A fluorescence microscopy study was performed as follows. The samples were illuminated with a UV-mercury lamp, the fluorescence images of single DNA molecules were observed using a Zeiss Axioplan microscope, equipped with a 100× oil-immersed objective lens, and digitized on a personal computer through a high-sensitive SIT C-video camera and an image processor, Argus-20 (Hamamatsu Photonics, Japan). The apparent long-axis length of the DNA molecules, L, was defined as the longest distance in the outline of the fluorescence image of single DNA. Images of the dynamic motion of single DNA-lipid complexes in organic solvents were taped with a conventional S-VHS video recorder. The observations were carried out at 25°C. Special care was taken to clean the microscope glasses (No. 0, Chance Propper, England) thoroughly before the observation to prevent DNA degradation, as well as precipitation to the glass surface [21].

2.4. Cryogenic transmission electron microscopy (cryo-TEM)

Specimens for electron microscopy were prepared in a controlled environment vitrification system (CEVS) to ensure a fixed temperature and to avoid water losses from the solution during sample preparation [22]. The vitrified specimens were stored under liquid nitrogen and transferred to the electron microscope (Philips CM 120 BioTWIN) equipped with a post-column energy filter, using an Oxford CT3500 cryoholder and its workstation. The acceleration voltage was 120 kV and the defocus was circa 1 μm. A magnification of 55 000 allowed a pixel width of 5 Å. Images were collected under low dose conditions, with the dose being less than 0.1 electron per nm^2, and recorded digitally with a CCD camera (Gatan MSC791).

3. Results and discussion

3.1. DNA behavior in the presence of negatively charged catanionic mixtures

Upon the addition of DNA stock solution to the samples I–III, corresponding to the negatively charged catanionic vesicles, no change in the conformational behavior of individual DNA molecules was detected. This result seems to be plausible, taking into account the general viewpoint in colloid and polymer chemistry that polyelectrolyte and a similarly charged amphiphilic molecule should not interact with each other due to the strong electrostatic repulsion between a polyion and amphiphile [23]. However, we suspected that since both cationic and anionic surfactants are present in the catanionic mixture, DNA might interact with cationic surfactant with the formation of a globular complex. Since we have not seen any globular DNA in the presence of anionic-rich catanionic vesicles, we decided to check the stability of DNA globules formed in the presence of cationic surfactant, upon the addition of anionic surfactant from a corresponding catanionic mixture.

In our previous reports, it was found that DNA-surfactant complexes in highly diluted solutions, when monomolecular collapse of DNA occurs accompanied by the formation of single DNA-surfactant globules, may be disintegrated by the addition of various co-solutes [7]. For instance, it was shown that the addition of an aqueous buffer solution [9], simple monovalent salts [24], synthetic polyacid [9] and neutral liposomes [25] induces the unfolding globule-coil transition in single linear DNA molecules. It was also presumed that the addition of anionic surfactant to the DNA-cationic surfactant globules will lead to DNA unfolding due to the formation of complexes between oppositely charged surfactants. However, the actual experiment has never been done. In the context of our present studies, this experiment seems to be quite important, since it might explain the absence of interaction between anionic-rich catanionic mixtures and DNA.

To study the effect of SOS on the stability of DNA-CTAB globules, the latter were prepared at [CTAB] = 3.3×10^{-4} M. It is known, both from our present experiment and from previously reported data, that under those conditions, DNA macromolecules exhibit compact globular conformation [7], distribution of DNA long-axis lengths is unimodal and the average L value for single DNAs is less than 1.0 μm. The

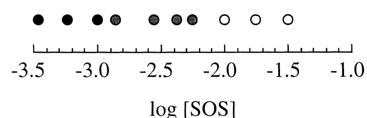

Fig. 1. Dependence of the conformational behavior of single T4 DNA macromolecules in aqueous buffer solution at constant [CTAB] = 3.3×10^{-4} M on the total SOS concentration in the solution. Filled circles correspond to the globular DNA conformation, shaded circles to the co-existence between elongated coiled and compact globular DNAs, whereas opened circles correspond to the coiled conformational state of DNA. $T = 25$°C.

aqueous SOS solution was added to the sample with DNA-CTAB globules stepwise and the conformational behavior of DNA was monitored with the fluorescence microscopy technique. The results of these observations are summarized in Fig. 1.

Firstly, when the SOS concentration in the solution was less than 1.0×10^{-3} M, no visible effect on DNA globules was detected in the solution. DNA molecules remained in a highly compact conformation with a relatively high fluorescence intensity. Most of the DNA globules gradually precipitated onto a microscope glass surface, while others underwent Brownian motion in the bulk of the sample. As soon as the SOS concentration reached 1.3×10^{-3} M, unfolded DNA coils were found in the sample solution together with the compact DNA-CTAB globules. It is necessary to mention that the coexistence between coils and globules remained visible even after 24 h after the sample preparation, that proves that the appearance of unfolded DNA molecules is not due to the kinetic effects after addition of SOS. A further increase of the SOS concentration in the solution resulted in the complete unfolding of DNA-CTAB globules at $[SOS] = 1.0 \times 10^{-2}$ M. All DNA molecules, even those previously precipitated to the glass surface, were fluctuating in the solution, exhibiting a relatively slow worm-like motion. Therefore, it became clear from our experiment that DNA-CTAB complexes undergo a reverse discrete globule-coil conformational transition upon the addition of anionic surfactant, SOS. This result is quite important for the understanding of single DNA behavior in the presence of anionic-rich catanionic mixtures. In our experiments, it was demonstrated that DNA molecules remain in an extended coiled state being introduced to the aqueous solution of an anionic-rich catanionic mixture. Based on the obtained results, we can conclude that since the equilibrium interaction constant of CTAB-SOS association is higher than that of CTAB-DNA association, DNA is unable to remove cationic surfactant from catanionic vesicles and remains in a coiled state, as in buffer solution in the absence of other cosolutes. Since the net charge of catanionic vesicles in the conditions I–III is negative, the strong electrostatic repulsion between DNA and vesicles also opposes their association. Concluding, the presence of anionic-rich catanionic vesicles in aqueous DNA solutions does not lead to any associative interaction and does not affect the DNA conformational behavior.

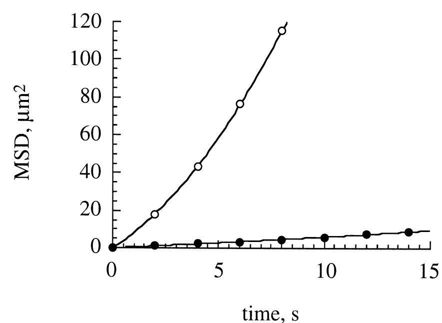

Fig. 2. MSD of the center of the mass of single DNA globules in the experimental conditions IV (opened circles) and V (filled circles).

3.2. DNA behavior in the presence of positively charged catanionic vesicles

Contrary to the results obtained for anionic-rich catanionic mixtures, the addition of oppositely charged mixtures to DNA

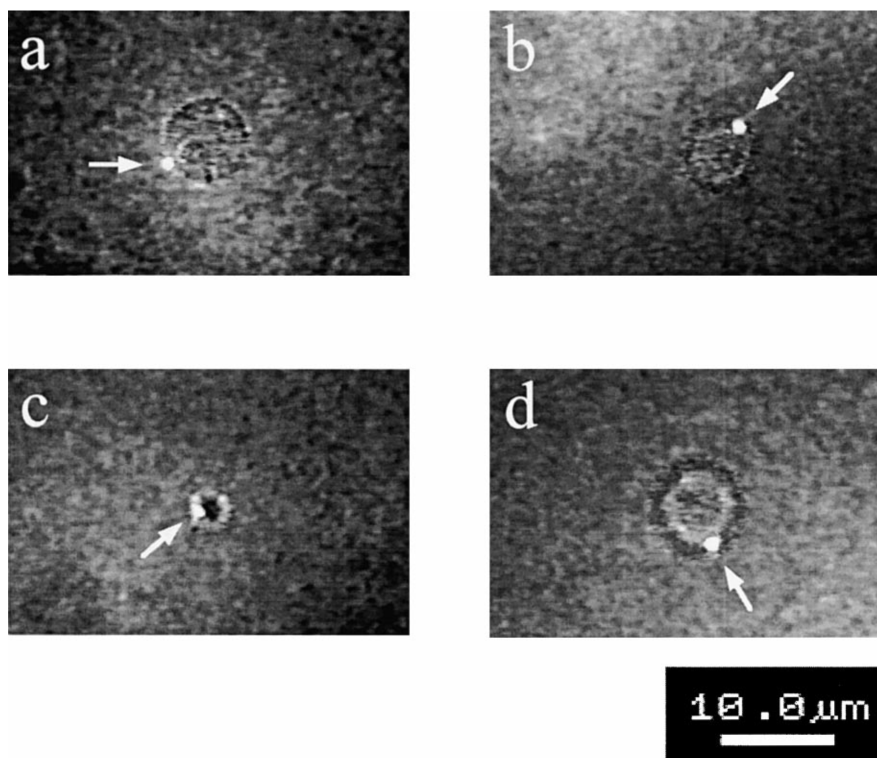

Fig. 3. Optical microscopy images of single T4 DNA globules adsorbed onto the surface of positively charged catanionic vesicles in sample V. Arrowheads indicate single DNA globules.

Table 1
Experimental conditions for the formation of catanionic vesicles used in the present study (according to the phase diagram in [14])

Sample	[SOS] (%)	[CTAB] (%)	[H_2O] (%)	[SOS] (mM)	[CTAB] (mM)	[CTAB]/[SOS]
I	1.1	0.2	98.7	45.9	6.0	0.13
II	1.9	0.7	97.4	80.0	19.0	0.24
III	2.4	1.0	96.6	103.2	29.0	0.28
IV	0.1	0.2	99.7	3.0	6.0	2.00
V	0.4	0.7	98.9	16.5	19.0	1.15

solution leads to the collapse of individual DNA chains, which were detected in a compact globular conformation with L values of 0.6–0.8 μm. The dimensions and fluorescence intensities of single DNA globules were quite similar to those reported previously for DNA compaction in the presence of various condensation agents [7]. However, the fluorescence microscopy experiments revealed that in some of our samples, DNA globules exhibited much slower a Brownian motion, compared to that in DNA-cationic surfactant systems [9].

During the observation of single DNA globules in the presence of positively charged catanionic vesicles, we noticed that in sample V, the diffusion rate of DNA globules was substantially lower than that for DNA globules formed in the presence of other condensation agents, as well as in sample IV. Since this observation might indirectly point to the aggregation between single DNAs for the experiment of sample V or to the precipitation of collapsed DNA chains onto the surface of catanionic vesicles, we performed additional experiments in order to determine translational diffusion coefficients and, consequently, hydrodynamic gyration radii of DNA globules. Recently, we demonstrated that the actual size of DNA globules may be evaluated from the translational diffusion coefficients for the individual DNA molecules [8]. In the present study, we also analyzed video tapes with the recorded Brownian motion of DNA-vesicle complexes in aqueous solution and calculated the hydrodynamic radii of DNA-catanionic vesicle complexes.

The translational diffusion coefficient of DNA-lipid complexes, D, was evaluated from the time, t, dependence of the mean square displacement (MSD) of the center of the mass of DNA macromolecules in a two-dimensional area of observation, as is exemplified in Fig. 2. During the observation, a minor spontaneous convective flow in the sample solution was recognized, probably due to the illumination effect. As the bulk flow rate was almost constant for the observation area, one can eliminate the effect of the convection from the two-dimensional diffusional movement, based on the following equation:

$$<(R_G(t)-R_G(0))^2> = 4Dt + At^2, \quad (1)$$

where $R_G(t) = (R_x, R_y)$ is a coordinate of a center of the DNA globule at a given time t and A is a numerical constant as a scale of a convective flow magnitude. From the least squares fitting to the second order polynomial of t for the various time intervals observed, we have evaluated the actual diffusion constant D. The hydrodynamic gyration radius ξ_H was calculated from D according to the Stokes-Einstein equation:

$$\xi_H = \frac{k_B \cdot T}{6\pi \cdot \eta_S \cdot D}, \quad (2)$$

where T is the temperature, k_B is the Boltzmann constant and η_S is the viscosity of the solvent. In Table 2, we present the hydrodynamic diameters of DNA globules in two of the examined samples, namely IV and V, which were calculated at $T = 298$ K.

The obtained values of ξ_H for the DNA complexes are substantially different. Whereas in sample IV, the size of DNA globules is consistent with the previously reported values [8], in sample V, the value of the hydrodynamic diameter of the DNA globule is much higher and is almost equal to the apparent long-axis length L of DNA globules. This effect may be explained by (i) DNA aggregation in our experimental conditions or (ii) adsorption of DNA globules onto the larger particles, moving freely in the solution and invisible with the fluorescence microscopy.

The second hypothesis on the adsorption of DNA globules on the surface of catanionic vesicles is directly supported by the results of optical microscopy observations. Fig. 3 demonstrates images of DNA globules adsorbed onto the surface of CTAB/SOS vesicles. In this particular experiment, we illuminated sample with both UV- and visible light simultaneously. Therefore, the observation of both fluorescently labelled DNA and large catanionic vesicles was possible. The vesicles in our sample were quite polydisperse and some of them were large enough to be detected with the use of optical microscopy.

Therefore, the final question remained about the observed difference in the diffusion rates of DNA globules in the samples IV and V. The high diffusion rate of DNA globules in sample IV implied that either there are no vesicles in the solution or the dimensions of the vesicles are comparable with the sizes of compact DNA globules. Using an optical microscopy technique, the objects with the dimensions less than the wavelength of light could not be visualized. At the same time, other microscopic methods, like, for instance, cryo-TEM, could be successfully applied for the visualization of the nm-sized objects. Hence, cryo-TEM experiments with samples IV and V were performed.

In sample IV, we found that spheroidal and rod-like surfactant micelles co-exist in the bulk of the sample, whereas vesicular structures were not detected (Fig. 4A). This can be due to the addition of salt (Tris-Cl buffer) and organic solvent (ME) to the sample solution and a shift in the phase behavior of the catanionic mixture induced by the change in electro-

Table 2
Viscosities, η_S, translational diffusion coefficients, D, and hydrodynamic gyration diameters, $d = 2\xi_H$, of single T4 DNA globules in samples IV and V at $T = 25$°C

Sample	η_S, mPa s	D, μm^2/s	d, nm
IV	1.26	5.58	55
V	1.26	0.47	650

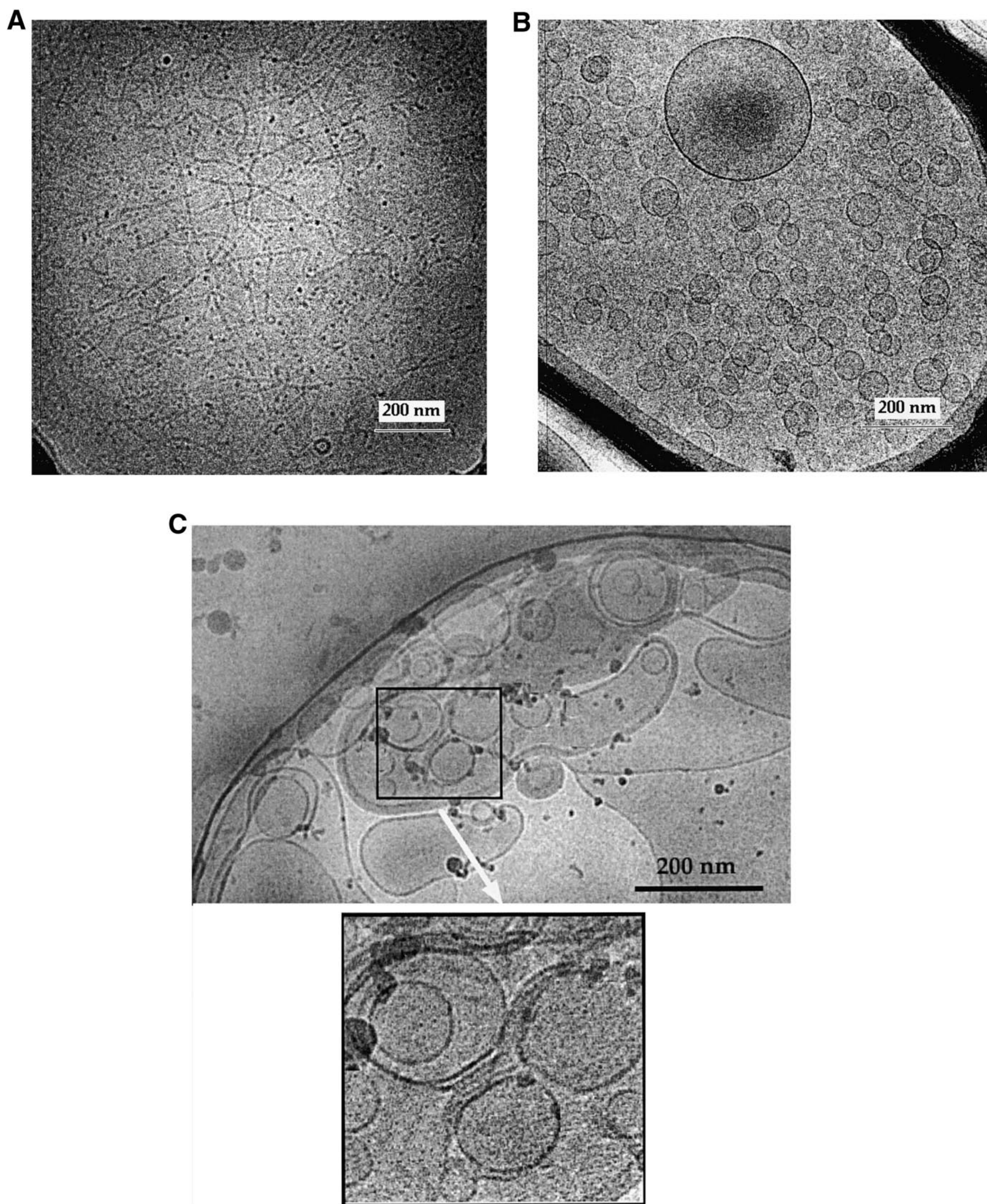

Fig. 4. Cryo-TEM images, (A) co-existence of spherical and rod-like surfactant micelles in sample IV, the sample composition is identical to that for the fluorescence microscopy measurements, [DNA] = 5.0×10^{-7} M. (B) Catanionic vesicles in sample V, the sample composition is identical to that for the fluorescence microscopy measurements, [DNA] = 5.0×10^{-7} M. (C) DNA complexes in sample V at [DNA] = 5.0×10^{-5} M.

static interactions in the sample. This process may lead to the variation of the coordinates of the vesicle region on the map and disappearance of vesicles in the aqueous solution. In this case, the observed co-existence of spheroidal and rod-like micelles can be attributed to the change of the ionic strength of the solution. Contrary to this observation, in sample V, polydisperse vesicles were successfully imaged (Fig. 4B). Moreover, if DNA is added to sample V at a higher concentration, compared to that in fluorescence microscopy experiments, DNA molecules adsorbed onto the catanionic vesicles were visualized, similarly to DNA chains enclosed between liposomes observed in [26], confirming data of optical microscopy experiments (Fig. 4C).

In conclusion, our results suggest that the positively charged catanionic vesicles may be successfully applied to induce the folding transition in a large single linear DNA and adsorption of DNA globules onto the surface of the vesicles. Recharging of the vesicles through change of the molar ratio between cationic and anionic lipids in the solution leads to the DNA unfolding and release to the bulk solution. Therefore, our observation might be of interest from the perspective of a controlled DNA delivery in vitro and in vivo. For this purpose, the conditions for the formation of catanionic vesicles from the mixtures of oppositely charged biocompatible lipids should be additionally studied.

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