Compaction and Decompaction of DNA in the Presence of Catanionic Amphiphile Mixtures

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The dissociation of DNA−cationic surfactant complexes with the addition of a negative amphiphile was studied by fluorescence microscopy (FM). The unfolding of DNA molecules previously compacted with cationic surfactant was shown to be dependent on the anionic surfactant chain length; lower amounts of a longer-chain surfactant were needed to release DNA into solution. However, we observed no dependence on the hydrophobicity of the compacting agent. The structures of the aggregates formed by the two surfactants, after the interaction with DNA, were imaged by cryogenic transmission electron microscopy (cryo-TEM). We found that it is possible to predict the structure of the aggregates the surfactants will form, such as vesicle phases, from the behavior of mixed surfactant systems, which brings new insight to this subject and several interesting opportunities for applications.

Introduction

The interaction between DNA and cationic surfactants has long received great interest from the biomedical sciences. Recently, physical chemists have devoted particular attention to these systems in an attempt to better understand the driving forces behind the molecular interactions; this is also expected to increase the efficiency and number of uses for these systems.

The strong associative behavior displayed by DNA and cationic surfactant systems is well-known, and it is related to most interesting applications. In 1967, a procedure that used quaternary ammonium surfactants to precipitate DNA for its extraction and purification was first described.[1] The CTAB−DNA precipitation method is still in use, with more or less important modifications.[2−6]

Also, gene delivery and transfection constitutes a potential use of these systems. Though synthetic surfactants such as CTAB (cetyltrimethylammonium bromide) cannot be used alone for this purpose, because the complexes between DNA and cationic micelles do not result in effective transfection, these amphiphiles can be used for positive charging of neutral liposomes, improving their efficiency.[7]

In a previous study,[8] we found that an anionic surfactant could be used to unfold and release previously compacted DNA molecules by cationic surfactants. This can bring, in our opinion, some interesting new perspectives to our knowledge of DNA−cationic surfactant interactions and to the applications of these systems.

In this paper, we study the dependence of the anionic surfactant chain length as well as the dependence of the cationic surfactant hydrophobicity on the dissolution of the DNA−cationic surfactant complexes. The structure of the aggregates that the two surfactants formed after their interaction with DNA was also investigated by cryogenic transmission electron microscopy (cryo-TEM).

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Experimental Section

Materials. Synthetic surfactants cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), and dodecyltrimethylammonium bromide were obtained from Sigma, sodium octyl sulfate (SOS) was obtained from Merck, and sodium dodecyl sulfate (SDS) was obtained from BDH Chemical Lda. All were used as received. Coliphage T4 DNA (molar mass = 1.1×10^8 D, ca. 167 kbp) was purchased from Sigma. The DNA concentration was determined spectrophotometrically, considering the molar extinction coefficient of DNA bases to be equal to 6600 $M^{-1}cm^{-1}$.[9] The ratio of the absorbance of the DNA stock solution at 260 nm to that at 280 nm was found to be 1.9. The fluorescence dye 4′,6-diamidino-2-phenylindole (DAPI), the antioxidant 2-mercaptoethanol (ME), and the buffer Tris-Cl were supplied by Sigma.

Sample Preparation. DNA stock solutions were prepared by diluting DNA molecules with 10 mM Tris-Cl buffer (pH 7.6) containing an antioxidant (ME), the fluorescent dye (DAPI), and bidistilled water. The final concentrations were DNA in nucleotide units 0.5 μM, DAPI 0.5 μM, and ME 4% (v/v). Under these conditions, the binding number of DAPI per 1 bp DNA is, in an aqueous buffer solution, 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 dye.[10,11] A cationic surfactant stock solution (10 μL) was added to all samples. For the chosen surfactant concentration, 3.16×10^{-4} M, we could observe by fluorescence microscopy (FM) that all DNA molecules were in the condensed state.[12] The samples were left to equilibrate for at least 30 min before the addition of 15 μL of a solution of the desired concentration of anionic surfactant. The solution, after being gently mixed, was left for another 30 min before observation. The total volume of the samples was 0.5 mL. The vesicle solution for cryo-TEM control experiments was prepared by simply adding the surfactant stock solution of the desired concentrations and was left for a couple of hours prior to observation.

Fluorescence Microscopy. The conformation map was drawn as follows. The samples were placed on microscope glasses (No. 0, Chance Propper, England) that had been previously cleaned to prevent DNA degradation as well as precipitation onto the

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glass surface[13] and 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 were digitized on a personal computer through a high-sensitivity 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 fluorescent image of the single DNA. The observations were carried out at 25 °C.

Light Microscopy. This technique was used to observe vesicles in solution. Because some samples, like surfactant aggregates, are not easily distinguished from the background if normal light is used in a normal apparatus, we used differential interface contrast (DIC)[14] lenses as well as a camera connected to an image processor, so-called video-enhanced microscopy (VEM).[15] The use of oil-immersion lenses also improves the resolution. A Zeiss Axion microscope was used, which was equipped with a 100× oil-immersed objective lens and DIC optics. The microscope images were treated the same way as for the FM studies.

Cryogenic Transmission Electron Microscopy (cryo-TEM). The samples for cryo-TEM were prepared on a controlled-environment vitrification system[16] at room temperature with controlled humidity (to minimize water loss) and temperature (to prevent temperature changes), allowing the specimen to relax for an indefinite time before being plugged into the cryogen. An 8-μL drop of the solution was deposited on a lacy carbon film supported by a copper grid, and then the drop was gently blotted with filter paper to create a thin film suspended over the holes of the grid. The sample was then vitrified by rapidly plunging it into the cryogen containing liquid ethane at its melting temperature. To avoid the formation of ice, the vitrified samples were stored and transferred onto the microscope under liquid nitrogen by means of a cold-stage transfer module (model 626, Gatan Inc., Warrendale, PA). A Philips CM 120 bio TWIN cryo electron microscope equipped with a postcolumn energy filter and an Oxford CT3500 cryoholder and its working station was used. The acceleration voltage was 120 kV, and the defocus was ca. 1 μm. Magnification of 55 000 allowed a pixel width of 5 Å. Images were collected under low-dose conditions, with the dose being less that 0.1 electron per nm^2. The images were recorded digitally with a CCD camera (Gatan MSC791).

Results and Discussion

Dependence of DNA Decompaction on the Anionic Surfactant Chain Length. The effect of SOS on the stability of DNA−CTAB globules was studied previously.[8] In the present investigation, we were interested in the influence of the hydrophobicity of the negatively and positively charged surfactants on the dissociation of the DNA−cationic surfactant complexes. For this, we started by compacting DNA with DTAB. For the chosen concentration of surfactant, 3.16×10^{-4} M, we could observe, by fluorescence microscopy, that all DNA molecules were in the compacted state.[12] These collapsed molecules presented a high fluorescence intensity and a long-axis length, *L*, of less than 1.0 μm and are usually dominated by globules. We then started by adding an anionic surfactant (SOS, for example). No visible effect was noted until a certain concentration, 3.80×10^{-4} M, where we observed a coexistence between globules and coils, DNA molecules with an extended conformation translating in solution with a wormlike motion and *L* around 3.7 μm. Above an SOS concentration of 6.31×10^{-3} M, only coils were observed in solution (Figure 1).

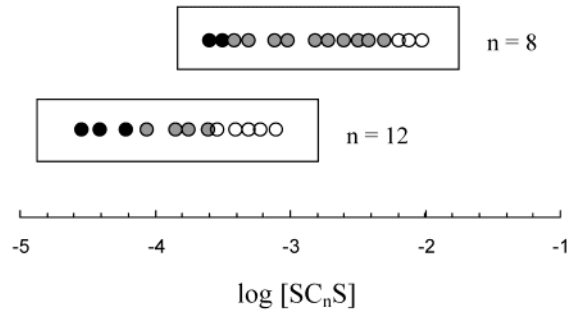

Figure 1. Dependence of the conformational behavior of single T4 DNA molecules, 0.5 μM in aqueous buffer solution and a constant DTAB (dodecyltrimethylammonium bromide) concentration of 3.16×10^{-4} M, on the stepwise addition of SDS (sodium dodecyl sulfate) and SOS (sodium octyl sulfate). Filled circles correspond to the globular DNA conformation, and shaded circles, to the coexistence between elongated coils and compacted DNA molecules, whereas open circles correspond to the extended conformation of DNA. $T = 25$ °C.

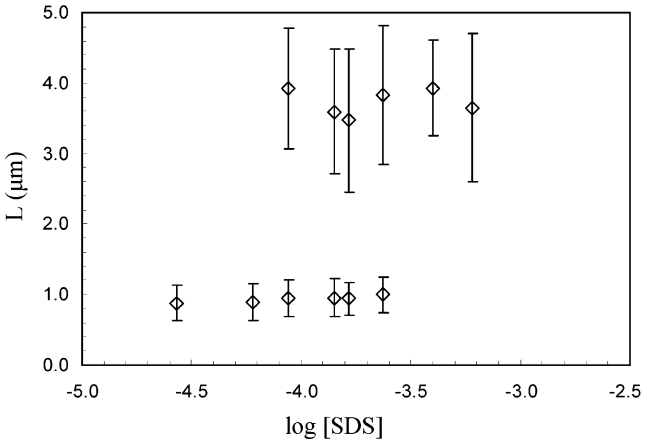

Figure 2. Long-axis lengths, *L*, of T4DNA molecules, 0.5 μM, vs the concentration of anionic surfactant, SDS, at a constant DTAB concentration of 3.16×10^{-4} M. Error bars indicate the statistical errors in the distribution and are given by the standard deviation. $T = 25$ °C.

For SDS, a smaller amount of surfactant was required to induce decompaction. As observed in Figure 1, a concentration of 8.71×10^{-5} M was enough to start DNA unfolding, and even for 2.88×10^{-4} M, below the charge neutralization of the surfactants, all DNA molecules were in the coil conformation. These conformation changes of the DNA molecules with the addition of positive and negative surfactants is well represented in Figure 2, where the dependence of the long-axis of DNA molecules, *L*, on the SDS concentration at a constant DTAB concentration of 3.16×10^{-4} M is presented.

Thus, we observed that SDS was much more efficient in unfolding DNA than the shorter-chain surfactant, SOS. This is easy to understand from the chain-length dependence of surfactant self-assembly. When adding the anionic surfactant to the solution of DNA and cationic surfactant, above a certain concentration, the anionic surfactant will associate and form mixed self-assemblies with the oppositely charged amphiphile and release DNA back into the solution as a coil. The onset of this association can be defined in terms of a critical micelle concentration for the mixture of the two surfactants (CMC_{mixt}). Because SDS is more hydrophobic than SOS, the CMC_{mixt} for that surfactant will be smaller than that for the short-chain one.

The above-mentioned coil and globule coexistence region is a known phenomenon for DNA molecules with the addition of condensing agents such as organic solvents,[13] flexible polymers,[17] multivalent ions,[18−20] and cationic surfactants.[21,22] The coil−globule transition of T4DNA is then discrete, a first-order

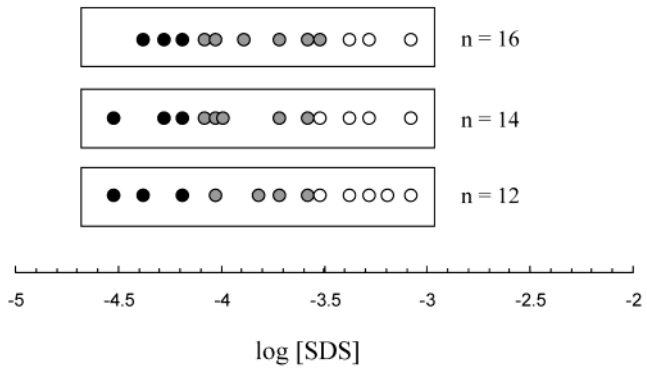

Figure 3. Dependence of the conformational behavior of single DNA molecules, 0.5 μM in aqueous buffer solution at constant concentrations of the cationic surfactants CTAB (cetyltrimethylammonium bromide), TTAB (tetradecyltrimethylammonium bromide), and DTAB, 3.16×10^{-4} M, on the total SDS concentration. Symbols are the same as in Figure 1. $T = 25$ °C.

transition, for individual chains but continuous for their ensemble average.[18,23] Compaction of DNA is driven by attractive interactions between different parts of the molecule by ion-correlation effects arising from the presence of multivalent ions, for example,[24] leading to the formation of a nucleation center in the DNA chain that grows along the molecule chain.[25] Because of hydrophobic interactions between the cationic surfactant molecules, these will self-assemble and act as a multivalent ion, inducing DNA compaction.

Dependence of DNA Decompaction on the Cationic Surfactant Chain Length. We also performed experiments to determine the dependence of DNA decompaction on the chain length of the compacting amphiphiles—CTAB, TTAB, and DTAB. Following the same procedure as before, we started by compacting DNA with 3.16×10^{-4} M of a cationic lipid (CTAB, for example) and added the anionic amphiphile stepwise. Contrary to the experiments varying the anionic surfactant, we found no dependence on changing the cationic surfactant chain length (Figure 3); that is, the amount of negatively charged surfactant necessary to unfold DNA is independent of the hydrophobicity of the compacting amphiphile.

This is explained as follows. Above a certain CMC_{mixt}, which is dependent on the anionic surfactant chain length, two types of structures can be formed in solution, DNA−cationic surfactant ($DNA-S^+$) globules and cationic−anionic surfactant aggregates (S^+-S^-). In the case of CTAB and SOS, for example, when we observe the unfolding of DNA molecules, there is a dissociation of DNA−CTAB globules, with CTAB molecules leaving DNA−CTAB complexes and transferring to CTAB−SOS aggregates. Because this "transfer" of molecules is occurring between two different surfactant aggregates, it will not depend on the surfactant chain length, or alternatively expressed, there is a cancellation in the alkyl chain-length effects in forming the two types of aggregates.

Surfactant Aggregate Structures. Mixtures of cationic and anionic surfactants are known as catanionic mixtures[26] and have been, because of their rich phase behavior and interfacial properties, the subject of many studies.[26,27] By varying the mixture composition (i.e., total surfactant concentration and

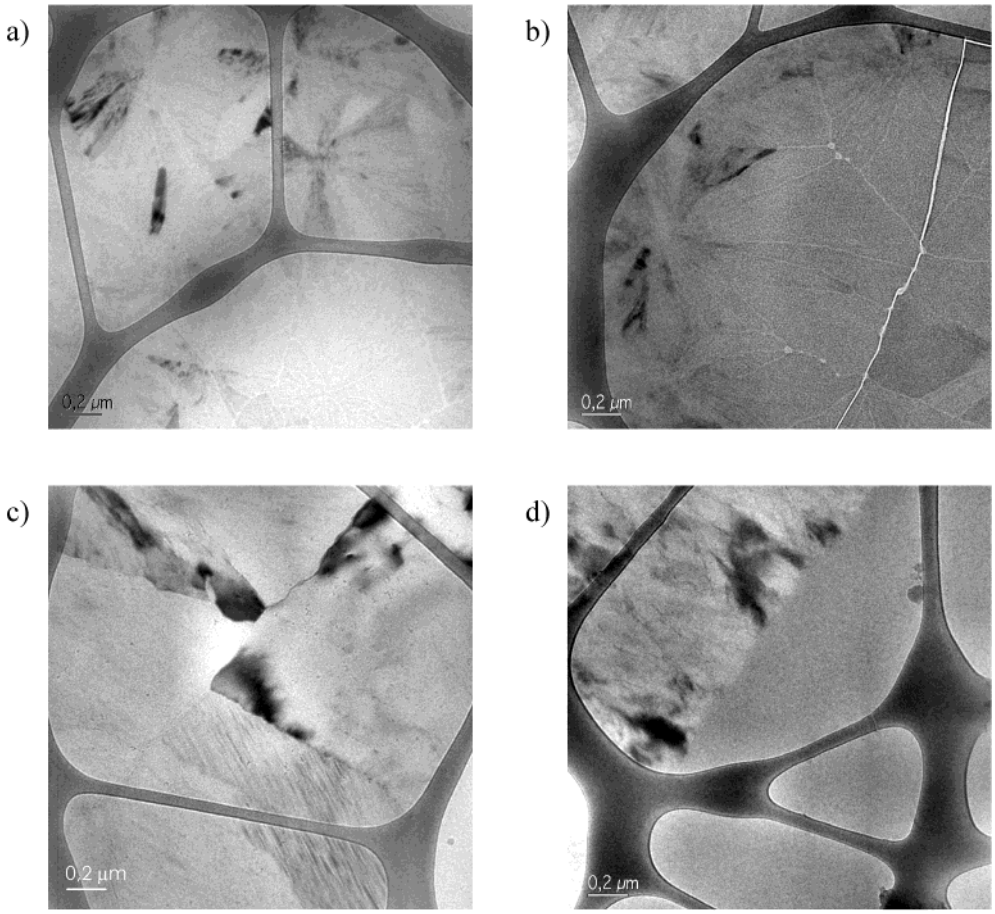

Figure 4. Cryo-TEM images of the surfactant structures formed after DNA compaction with DTAB and addition of the anionic amphiphile SDS (0.5 μM of T4DNA in buffer solution; 3.16×10^{-4} M of DTAB). In (a), (b), and (c), $[SDS] = 8.13 \times 10^{-4}$ M, and in (d), $[SDS] = 1.48 \times 10^{-4}$ M. In all samples, crystals can be observed.

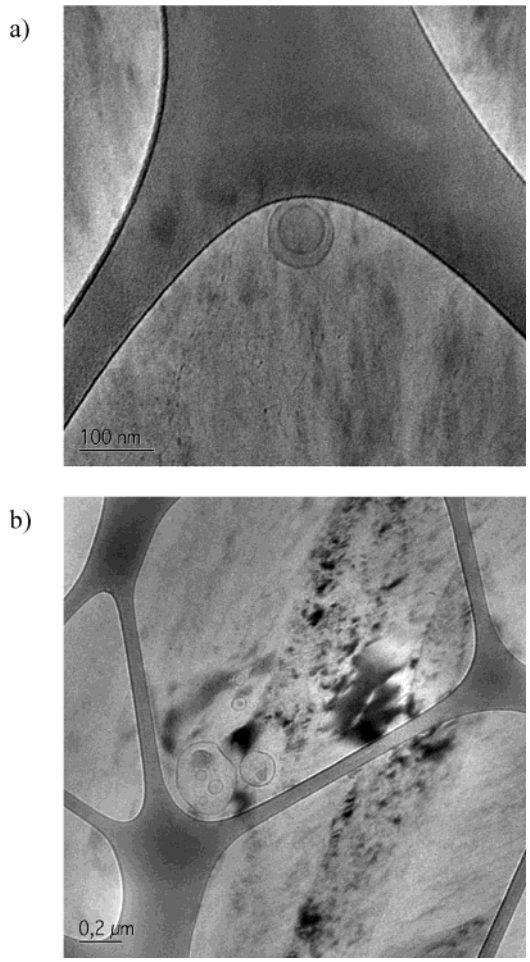

Figure 5. Cryo-TEM images of the surfactant structures observed after DNA, 0.5 μM in buffer solution, interactions with DTAB, 3.16×10^{-4} M, and SOS, 7.94×10^{-3} M. The coexistence of crystals and vesicles can be observed.

mixing ratios), one can obtain aggregates with different geometries ranging from spherical to cylindrical to planar.

We can note from the above discussion that the interaction between the oppositely charged surfactants is stronger than the one between DNA and cationic amphiphiles. A question that remains is the type of aggregates the surfactants will form. To answer this, we performed some cryo-TEM experiments because this technique allows a good visualization of nanometer-size objects. We found what looked like crystals in most of the samples (Figure 4). This is not unexpected because both surfactants had the same chain length and approximately the same concentrations. It is known that most catanionic systems precipitate at equimolar concentrations even at very high water content.[27]

Interestingly, except for the precipitate, we observed under other conditions the formation of vesicles (Figure 5). Above, the ability of catanionic mixtures to display rich phase behavior with a profusion of surfactant aggregate structures was mentioned. The most notable of them is the formation of vesicles, which are not observed when only one of the surfactants is present in solution.[28,29] Catanionic vesicles are believed to be thermodynamically stable because they are formed spontaneously and reversibly and remain stable for a long period of time.[30] Their formation and stability are dependent on the surfactant chain type and length. Thus, vesicle regions are usually larger when one of the amphiphiles has a double chain or when two single-chain surfactants have asymmetric chains, which has been referred to as packing conditions.[31,32] The

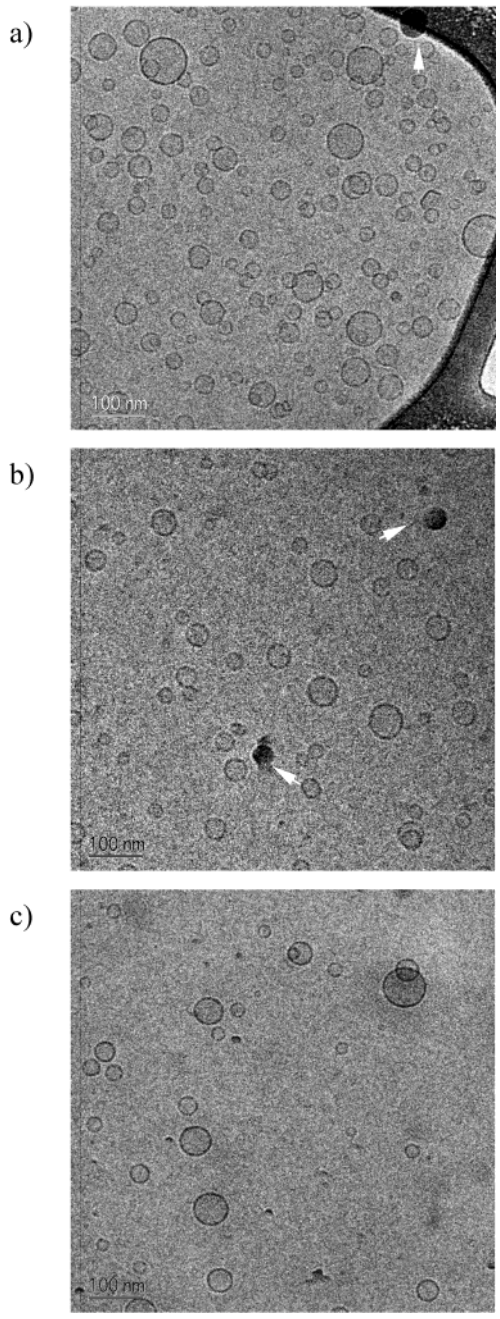

Figure 6. (a) Cryo-TEM images of an aqueous solution of SOS, 4.3×10^{-2} M, and CTAB, 9.88×10^{-3} M; (b) and (c) cryo-TEM images of samples with the same surfactant compositions as (a) but prepared under conditions needed for FM experiments in the presence (b) and absence (c) of DNA, 5.0×10^{-7} M; (a), (b), and (c) unilamellar vesicles. Arrows denote crystallized water.

presence of vesicles in the sample is then due to a large excess of the anionic surfactant and an asymmetry in the surfactant chain lengths, SOS versus DTAB.[29,30]

These findings led us to inquire whether it was possible to predict the structures formed by the surfactant mixture, after DNA compaction and decompaction, if we had knowledge of the phase diagram of the mixture. In particular, we investigated this issue by additional cryo-TEM experiments. We chose a point in the CTAB/SOS/water phase diagram,[33,34] with 9.88 mM of CTAB and 43.00 mM of SOS, which corresponds to the region of negatively charged vesicles. This region was selected because vesicles are self-assembly structures that are easy to observe and recognize as well as one of the most interesting for application purposes. In the solution, as expected, we

observed only the presence of somewhat polydisperse unilamellar vesicles with sizes ranging from 20 to 100 nm (Figure 6a).

One sample was prepared using the normal FM procedure (Experimental Section). We observed on the microscope that in a DNA buffer solution with 9.88 mM of CTAB all DNA molecules presented a compact conformation. With the addition of the anionic surfactant, SOS at 43.00 mM, only coils were present in solution. These concentrations of amphiphiles were enough to induce compaction and decompaction of the DNA molecules. Then, observing the surfactant aggregates by cryo-TEM, we realized that there was no visible difference between this sample and the previous one, which was prepared by mixing surfactants alone (Figure 6b vs a). Again, only small unilamellar vesicles were present in solution.

One should, of course, realize that though necessary for FM experiments, some of the added compounds might shift the phase boundaries on a phase map. The addition of salt (TrisCl buffer) and organic solvent (ME) to the samples changes the ionic strength of the solution and alters the electrostatic interactions between the components. For example, the addition of ethanol to surfactant systems influences both the CMC and the surface tension at the CMC.[35] Also, the addition of a monovalent salt changes the phase diagram markedly; it was shown[34] that as little as 0.5 wt % is enough to induce major changes in the phase diagram of the CTAB/SOS/water system. However, in control experiments, where samples were prepared with and without these FM conditions, it was shown that there was no measurable effect (Figure 6c vs a).

The ability to control the structures formed by the surfactants is very interesting and promising for a number of applications. For example, when using cationic surfactants to compact DNA for purification purposes, the addition of a certain amount of anionic surfactant would both release DNA back into solution and form a precipitate with the oppositely charged surfactant. That would enable a simple and efficient separation of DNA from the surfactants in solution. From another point of view, for an eventual application of these types of systems to gene therapy, the presence of crystals would be catastrophic for the cells.

All of this discussion was conducted by focusing on the amphiphile aggregates and the DNA conformational behavior. We do not discuss in this paper a matter that is currently being studied in our laboratory—the effect of these compaction and decompaction procedures on the DNA molecule. There is no indication that the DNA molecule undergoes denaturation or degradation; however, studies on the reversibility are being performed and will be reported soon.

Conclusions

It was observed earlier[8] that anionic amphiphiles can be used to unfold and release DNA previously compacted by cationic surfactants. We found that by using surfactants with longer chains the decompaction of DNA is more efficient, whereas it does not depend on the hydrophobicity of the compacting amphiphile. Our results also suggest that one can predict and control the aggregates that the oppositely charged surfactants will form, after DNA folding and release, simply from the added amounts of the amphiphiles and knowledge of their phase map. Therefore, our observations might be of some interest for some relevant applications such as DNA purification or controlled DNA delivery. Specifically, for DNA delivery purposes and taking into account toxicity factors, the phase behavior for mixtures of oppositely charged biocompatible lipids, with a special interest in possible vesicle regions, should be additionally studied.

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