Single-Molecule Visualization of Interaction between DNA and Oppositely Charged Mixed Liposomes

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The interaction between large single phage T4 DNA and mixed cationic dioctadecyldimethylammonium bromide/dioleoyl phosphatidylethanolamine (DODAB/DOPE) liposomes was studied with the use of a fluorescence microscopy technique. It was found that interaction started at very low liposome concentrations, far below the concentration of DNA. Also, the DNA size distribution was bimodal in a wide range of liposome concentrations. The critical concentration of the cationic lipid needed for the complete collapse of single DNA molecules strongly depended on the composition of the charged mixed DODAB/DOPE liposomes. The effect is supposed to be due to the concurrence between DNA−DODAB complex formation and DODAB incorporation into mixed liposomes with DOPE. In addition, the liposome surface charge density, which is dependent on the liposome composition and increases with the increase of the molar fraction of DOPE in the mixed liposome, is assumed to be a possible important factor. The report emphasizes the importance of the use of single-molecule detection methods in the DNA−liposome interaction studies.

The introduction of genetic material into cells has gained increased interest as a new therapy against various diseases.[1] One of the prerequisites for such therapy is an efficient and safe delivery system for the transfer of, e.g., DNA. Several types of delivery systems have been tested and have been found more or less efficient.[2a] Among these, liposomes of cationic lipids complexed with the DNA have been shown to be one of the candidate delivery systems.[2b] Very often neutral lipids are incorporated into the mixtures of liposomes and DNA. This modification has been found to be helpful for increasing liposome stability,[3] reducing the cytotoxicity of the cationic lipids,[4] and increasing membrane fluidity during transfection.[5−7] A large variety of experimental studies of the transfection of living cells via complex formation with oppositely charged liposomes led to the development of several commercially available liposome products,[8] which now are used less or more routinely in therapeutic studies. However, there have been almost no studies of the dynamic behavior of single DNA molecules in aqueous solution in the presence of liposomes. In this work we have examined the conformational behavior of single double-stranded T4 DNA (166 kbp) in the presence of mixed cationic DODAB/DOPE (dioctadecyldimethylammonium bromide/dioleoyl phosphatidylethanolamine) liposomes. The fluorescence microscopy (FM) technique, which has been applied for the investigation of DNA−surfactant interaction at the single-molecule level,[9] was also successfully used in the present study.

We found that the interaction between DNA chains and oppositely charged mixed liposomes started at very low lipid concentrations, far below the concentration of DNA utilized in the experiments. As soon as the mixed liposomes were introduced to the aqueous DNA solution, the single DNA molecules exhibited two limiting conformational states. They were characterized by substantially different linear dimensions and fluorescence intensities and corresponded to an extended coil and a compact globule conformation (Figure 1A). The coexistence of the two different conformational states of the stiff DNA chains in thermodynamic equilibrium in the presence of oppositely charged liposomes implied that under these experimental conditions the difference between the free energies of single coiled and globular DNAs did not exceed the thermal energy kT.[9b] Our results were in good agreement with those of Lasic and Frederik, who observed fully condensed particles, partially condensed structures, and unreacted DNAs in the same sample of plasmid DNA and cationic liposomes.[10] When the liposome concentration reached a critical value, the conformational distribution of DNA chains became unimodal and all DNAs were found to be in a collapsed globular conformation (Figure 1B).

Figure 2 exemplifies the phase diagram of single DNA molecules in the presence of DODAB/DOPE liposomes with various molar ratios between the cationic and neutral lipids. At all liposome compositions studied, compact DNA globules were formed, even at the extremely low lipid concentrations. This implied a strong cooperativity of the process.[9b,c] The width of the coexistence region was quite substantial, compared to that reported from studies of DNA−cationic surfactant interactions.[9b] The results correlated well both with the recent data from studies of DNA conformational behavior in the presence of DODAB[9c] and with the modern theory of polyelectrolyte collapse.[13]

Another important feature can be noticed in Figure 2. The concentration of mixed liposomes (expressed as the molar concentration of cationic lipid, DODAB), which corresponded to complete DNA collapse in the solution, depended strongly on the liposome composition, i.e., on the molar ratio between cationic and neutral lipids. It is commonly accepted that the interaction between cationic liposomes and DNA in aqueous

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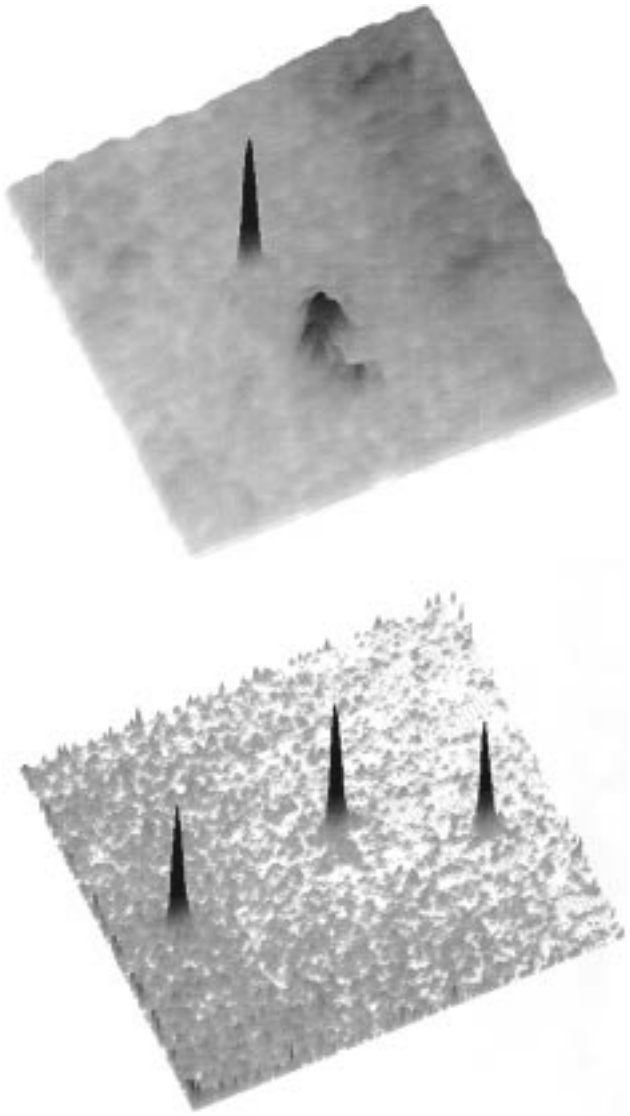

Figure 1. 3D fluorescence microscopy images of T4 DNA molecules in the presence of DODAB/DOPE liposomes. The higher fluorescence intensities correspond to the higher segment spatial densities along a single DNA chain. (A, top) Coexistence of coiled and compacted DNAs in the presence of 1:1 mol/mol DODAB/DOPE liposomes, [DODAB] $= 1.0 \times 10^{-6}$ M. The area corresponding to the photograph is 10.0×10.0 μm^2. (B) Globular T4 DNA in the presence of 1:5 mol/mol DODAB/DOPE liposomes, [DODAB] $= 1.0 \times 10^{-4}$ M. The area corresponding to the photograph is 10.0×10.0 μm^2.

solution, which leads to the formation of condensed structures, occurs at nearly equimolar ratio of components in the solution.[3] However, our results showed that with the increase of the neutral lipid content in a liposome, the amount of cationic lipid for complete DNA collapse also increased significantly. This should therefore be helpful for the rational development of a delivery system for therapeutic purposes. It should be noticed also that this result can hardly be perceived with the use of other experimental methods, since only FM allows the observation of single-molecule DNA dynamics in the solution.

At the moment it is difficult to make an indisputable description of the observed phenomenon. Tentatively, two different processes might have been of importance. First, the positively charged lipids could have been incorporated into mixed liposomes with the neutral lipids and concurrently could also have been forming complexes with DNA. This hypothesis agrees well with our previous observation of the formation of complexes between DNA and DODAB in the presence of

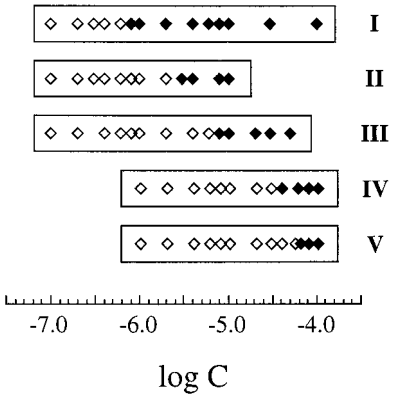

Figure 2. Phase diagram of T4 DNA−DODAB/DOPE liposome system at 25 °C in 10 mM TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH = 7.6). DNA molecules were stained with a fluorescence dye, 4′,6-diamidino-2-phenylindole (DAPI); final concentrations of both DNA (in nucleotide units) and DAPI were equal to 0.6 mM. 4% (v/v) of free radical scavenger, 2-mercaptoethanol, was added to prevent DNA photobleaching. Under these conditions, the binding number of DAPI per DNA base pair in an aqueous buffer solution was estimated to be ca. 0.05 and the persistence length of the DNA chain was expected to remain nearly the same as in the absence of DAPI.[11,12] Sample preparation and treatment of microscope glasses were carried out as previously described.[9a] Liposomes were prepared by sonication of a mixed lipid film, processed from lipid stock solutions in a chloroform: methanol (1:1) mixture, at 60 °C, and extrusion of a lipid aqueous solution through a polycarbonate membrane with a pore diameter of 200 nm (Poretics, Livermore, CA). Open diamonds correspond to the coexistence of coiled and globular DNAs in the solution, and filled diamonds correspond to the complete compaction of single DNA molecules in the sample. Measurements were performed using a Zeiss Axioplan microscope, equipped with a 100× oil-immersed objective lens, at various DODAB/DOPE molar ratios: (I) $Y = 1.00$; (II) $Y = 0.80$; (III) $Y = 0.50$; (IV) $Y = 0.17$; (V) $Y = 0.09$, where $Y =$ [DODAB]/([DODAB] + [DOPE]). C is the molar concentration of DODAB in the mixed liposomes.

neutral L-α-phosphatidylcholine (PC) liposomes.[9c,14] In those studies it was concluded that the introduction of neutral PC liposomes to an aqueous solution of DNA−DODAB compact globules leads to the unfolding of DNA chains owing to the partial transfer of lipid molecules from the bulk to the liposome. In our experiments, DODAB molecules might have been forming complexes with DNA polyanions stabilized due to the strong electrostatic attraction and hydrophobic interactions between hydrocarbon chains of DODAB. At the same time DODAB molecules might have been incorporated into the mixed liposomes with DOPE owing to the hydrophobic interactions between hydrocarbon lipid moieties. In other words, the partitioning of DODAB between mixed DODAB/DOPE liposomes and DODAB−DNA complexes takes place in the system. With the increase of DOPE concentration in the sample, when the neutral lipid concentration is several orders of magnitude higher than that of DNA polyanions, the effect due to the presence of DOPE in the solution must be quite substantial. Obviously, the free fraction of DODAB in the solution, which has a determining influence on the coil−globule transition in large DNA, will decrease.[9c] Clear support for this speculation is found in Figure 3, which presents the dependence of C^* (the critical DODAB concentration needed for complete DNA collapse in the sample) on the ratio between DODAB and DOPE in the initial liposomes, Y. As is seen in Figure 3, the C^* value underwent an almost 2 orders of magnitude increase while Y was changed from 1.00 down to 0.09.

Second, the liposome surface charge density may also have been important for the understanding of the interaction mech-

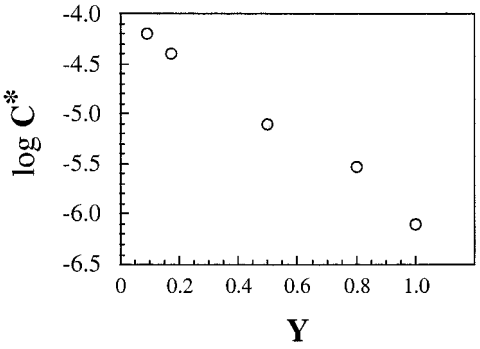

Figure 3. Dependence of the critical DODAB concentration (*C**) in the DODAB/DOPE liposomes, corresponding to complete DNA collapse in the sample solution, on the liposome composition *Y*.

anism. It has clearly been shown by Dubin et al.[15] that the interaction between polyelectrolytes and oppositely charged mixed micelles strongly depends on the molar fraction of ionic surfactant in the micelle. The trend in Figure 3 is quite clear: the decrease in the liposome surface charge density corresponds to the increase of the critical concentration of liposomes that induced a complete DNA collapse. This hypothesis about the surface charge density effect can be convincing if the liposome structures did not undergo significant changes during the formation of DNA−liposome complexes. However, taking into account the notion of Lasic that during the interaction between DNA and cationic liposomes DNA compaction is accompanied by the disintegration of liposomes,[3] we may conclude that our explanation based on the redistribution of charged lipid molecules between a DNA−DODAB complex and mixed DODAB/DOPE liposomes is quite plausible. In this case the surface charge density of liposomes is changed during the process owing to the strong electrostatic interactions between DNA and DODAB and preferential binding of cationic lipid onto the DNA chains.

The result presented in this study is important for the understanding of the molecular mechanisms of genosome formation. It may be quite helpful for the formulation of mixed liposome-based transfection agents with the optimal molar ratios between cationic and neutral lipid molecules. At the same time, the existence of a bimodal distribution of DNA in the presence of oppositely charged liposomes emphasizes the importance of single-molecule detection methods in the DNA studies, since the methods that provide the "ensemble average" characteristics of the system often fail to present its true description.[9b]

In conclusion, we have shown that the critical concentration of the cationic lipid needed for the complete collapse of single DNA molecules strongly depends on the composition of the charged mixed DODAB/DOPE liposomes. The increase of the molar fraction of the positively charged lipid in the mixed liposome resulted in the decrease of this critical concentration. A detailed study of the observed phenomenon with the use of cryo-TEM and light-scattering techniques is now in progress in our group. It is also of importance to perform similar experiments with other lipid combinations at various ionic strengths to better understand the general features of single DNA compaction in the presence of mixed liposomes.

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