Control of the Compaction/Unfolding Transition of Genomic DNA by the Addition/Disruption of Lipid Assemblies

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We studied the interaction between individual long genomic DNA molecules and cationic lipid assemblies. The assembly of cationic lipid molecules into small unilamellar vesicles (SUVs) of about 50 nm diameter led to the compaction of DNA whereas the addition of a neutral surfactant resulted in the disruption of SUVs and the unfolding of DNA. This reversible process does not require any chemical reaction or change in the ionic strength of the solution. It was applied to switch DNA repeatedly between a compact and an unfolded conformation in a dynamic manner.

Introduction

Genomic DNAs are very long molecules (up to centimeters in contour length) that assume in pure water an elongated coil conformation as a result of the electrostatic repulsion between phosphate groups. In nature, DNA is compacted into a very dense state to fit within narrow spaces such as viral capsids (∼100 nm) or cell nuclei in eukaryotes (∼1–10 μm). The compaction process of DNA is crucial in regulating its biological activity, in particular, within chromatin.[1] A compact state is usually associated with gene silencing whereas the unfolded or open form of DNA or chromatin usually allow for transcription and gene expression. Therefore, active studies have been devoted to the control of compaction/unfolding of genomic DNA. Typical strategies to compact DNA in vitro are (i) to neutralize its charge by adding compounds such as polyamines,[2] multivalent metal cations,[3] cationic surfactants,[4] cationic polymers,[5] or cationic nanoparticles;[6] (ii) to decrease the dielectric constant of the solvent;[7] and (iii) to add crowding agents such as hydrophilic polymers.[8] However, in many cases, once it has been compacted DNA cannot be unfolded, and one the following specific methodologies has to be employed: (i) the addition of low-molecular-weight and low-valency salt (e.g., NaCl) to help unfolding by ionic exchange;[9] (ii) in situ modification of compaction agent valency by redox reaction;[10] (iii) the use of polyanions to release DNA from interpolyelectrolyte complexes;[11]
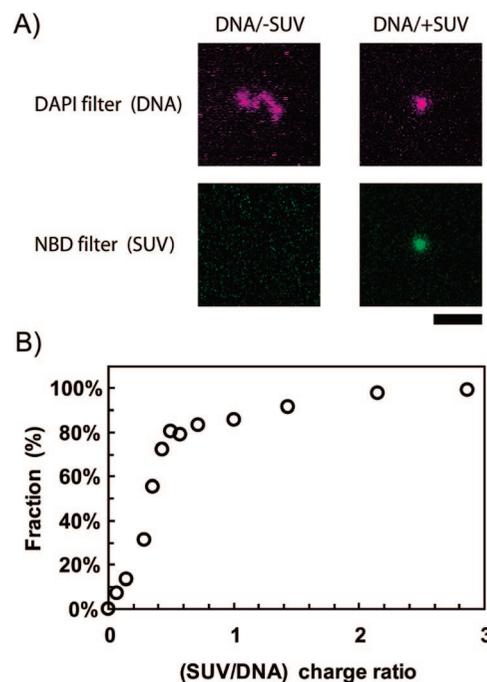
Figure 1. Compaction of T4 DNA (0.1 μM in 10 mM Tris-HCl) labeled by DAPI using small cationic unilamellar vesicles (SUVs) labeled by NBD. (A) Fluorescence microscopy (FM) images in false colors of an individual DNA molecule through a DAPI filter (fluorescence from DNA, top) and through an NBD filter (fluorescence from SUV, bottom) in the absence (left) and presence (right) of SUVs at a concentration corresponding to an SUV/DNA charge ratio of 2.1. The scale bar is 5 μm. (B) Fraction of DNA molecules in a compact state as a function of the SUV/DNA charge ratio.

(iv) the competitive interaction of surfactants;[12] or (v) DNA unfolding by light using photosensitive surfactants.[13] In addition, the compaction of DNA by cationic vesicles has been demonstrated to be a very efficient procedure for transfection applications.[14] Various studies have thus been devoted to understanding

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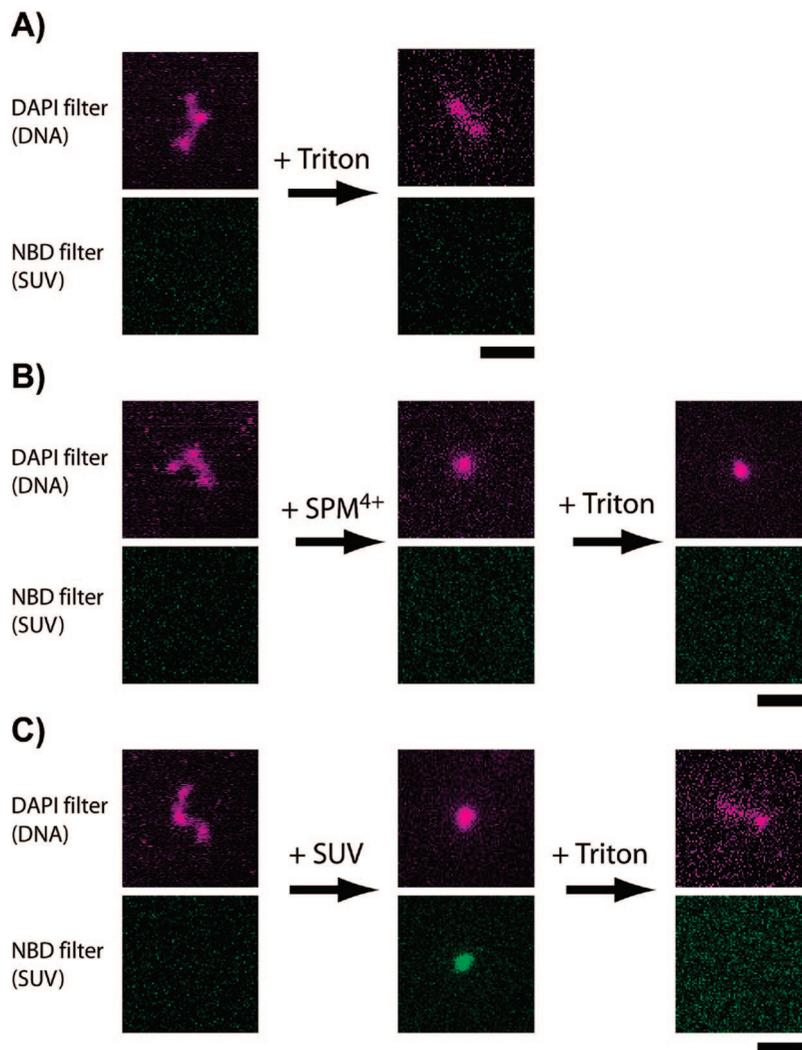
Figure 2. Fluorescence microscopy images (false colors) of an individual T4 DNA (0.1 μM in 10 mM Tris-HCl) labeled by DAPI through a DAPI filter (fluorescence from DNA, top) and through an NBD filter (fluorescence from SUV, bottom) under various conditions. (A) Left: DNA only; right: addition of Triton X-100 (6.3×10^{-4} M). (B) Left: DNA only; middle: addition of spermine SPM^{4+} (10 μM); right: addition of Triton X-100 (6.3×10^{-4} M). (C) Left: DNA only; middle: addition of SUV (SUV/DNA charge ratio is 2.1); right: addition of Triton X-100 (6.3×10^{-4} M). All indicated values correspond to the final concentrations in the observed sample. The scale bars are 5 μm.

the physicochemical principles underlying DNA−vesicle interaction.[15] However, very few of them have analyzed the phenomenon at the single-molecule level,[16] and the process of DNA unfolding after compaction was investigated only in the case of oligonucleotides.[17]

In this letter, we propose a strategy to switch genomic DNA reversibly between an unfolded and a compact state without any chemical reaction or change in the ionic strength of the medium. It is based on the controlled construction/disruption of nanoscale lipid assemblies. DNA was first compacted by cationic small unilamellar vesicles (SUVs) of 50 nm diameter. We found that the disruption of the lipid assemblies by the addition of a neutral surfactant was accompanied by the unfolding of DNA. We analyzed the DNA compaction/unfolding process by fluorescence microscopy observations of both individual DNA molecules and SUVs and explored the possibility of repeatedly switching DNA between a compact and an unfolded state.

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Materials and Methods

Materials. Bacteriophage T4 DNA (genomic duplex DNA carrying 166 000 base pairs) was from Wako Chemicals. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP), and 1-oleoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC) were from Avanti Polar Lipids. All other chemicals were purchased from Sigma. Deionized water (Millipore, 18 MΩ/cm) was used for all experiments.

Preparation of Small Unilamellar Vesicles (SUVs). In a glass tube, a lipid film was obtained by the evaporation of a mixture of lipid solution in chloroform (88 wt % DOPC, 10 wt % DOTAP, and 2 wt % NBD-PC) for 20 min under vacuum. A solution of Tris-HCl (10 mM) was then added to obtain a final lipid concentration of 1 mg·mL^{-1} and sonicated for 30 min at 35 °C. The solution was extruded 10 times through a polycarbonate Nucleopore membrane (Whatman) with a pore size of 50 nm using a miniextruder (Avanti Polar Lipids). Under these conditions, we obtained unimodal populations of SUVs with the most probable size ranging between 45 and 55 nm according to dynamic light scattering measurements. We assumed that the final composition of the SUV membrane was identical to that of the initial lipid film. Following the titration method proposed by Barenholz et al.,[18] we estimated that the fraction of lipid in the outer leaflet was 48% of the total lipid amount in the

Figure 3. (A) Fraction of DNA molecules (0.1 μM in 10 mM Tris-HCl) in the compact state in the presence of SUV (2.1 SUV/DNA charge ratio) as a function of Triton X-100 concentration as established by FM. (B) Fraction of DNA molecules in the compact state for the successive and alternative additions of SUV and Triton X-100 in one tube containing DNA (0.1 μM in 10 mM Tris-HCl). (1) DNA only, (2) addition of SUV (2.1 SUV/DNA charge ratio), (3) addition of Triton X-100 (6.3 $\times$ 10^{-4} M), (4) addition of SUV (45.1 SUV/DNA charge ratio), (5) addition of Triton X-100 (1.2 $\times$ 10^{-3} M), (6) addition of SUV (131.0 SUV/DNA charge ratio). All indicated values correspond to the final concentrations in the sample.

membrane, which shows that most SUVs are bilayered. The SUV suspension was used within 1 week of preparation.

Preparation of DNA Samples. Water, Tris-HCl buffer, fluorescent dye 4′6-diamidino-2-phenylindole (DAPI), and SUV (or spermine) were mixed in this order prior to careful T4 DNA introduction (under low shear conditions to avoid DNA breakage). For unfolding experiments, nonionic surfactant Triton X-100 was added to the DNA solution. For all experiments, we used T4 DNA at a final concentration of 0.1 μM (concentration in nucleotides) in Tris-HCl buffer (10 mM, pH 7.4) with DAPI (0.1 μM) as a DNA fluorescent dye (the total volume of each sample was 1 mL). When DNA was in the presence of SUVs, we calculated the SUV/DNA charge ratio by dividing the final concentration of the cationic lipid (DOTAP) by that of DNA. Samples were equilibrated 15 min prior to DNA characterization by fluorescence microscopy. All experiments were performed at room temperature.

Fluorescence Microscopy. Fluorescence microscopy (FM) was performed on an Axiovert 200 inverted microscrope (Carl Zeiss) equipped with a 100× oil-immersion objective lens. Images were acquired by using a highly sensitive EM-CCD camera (Photonmax 512B, Princeton Scientific) and Metaview image acquisition software. Observations were made in 20 μL microdroplets of sample solution deposited on a clean cover glass slide. A combination of two filters was used for the dual observation of DNA fluorescence (DAPI filter) and SUV fluorescence (NBD filter) on each characterized individual

DNA molecule. For each condition, a minimum of 200 individual DNA molecules were characterized to determine the fraction of molecules in the compact state.

Results and Discussion

First, we studied the compaction of DNA by SUVs. To a very dilute solution of T4 DNA (0.1 μM in 10 mM Tris-HCl) we introduced a solution of about 50-nm-diameter SUVs composed of 88 wt % DOPC (zwitterionic lipid), 10 wt % DOTAP (cationic lipid), and 2 wt % NBD-PC (a lipid carrying a fluorescent NBD group in one apolar tail). We characterized the conformation of individual DNA molecules and their interaction with SUV by fluorescence microscopy (FM). Typical FM images are shown in Figure 1A. In the absence of SUV, all DNA molecules are in the typical coil state, which is characterized by intrachain fluctuations and slow translational diffusion. The observation through the NBD filter indicates that there is no DNA signal emerging from the background. In contrast, when a significant amount of SUV has been introduced into the DNA solution, a large majority of observed DNA molecules were in the compact state, which is observed as a quickly diffusing bright spot through a DAPI filter. Through an NBD filter, at the exact location of the compact DNA, strong fluorescence was observed, which indicates that SUVs have been complexed to DNA to form the DNA compact state. By using FM, we systematically analyzed the conformation of a large number of DNA molecules and calculated the fraction of DNA molecules in the compact state as a function of the SUV/DNA charge ratio. Figure 1B shows that DNA molecules are progressively compacted with an increase in SUV concentration. For an SUV/DNA charge ratio larger than 2.1, more than 98% of all individual DNA molecules have been compacted by SUVs. Analogously to the mechanism of interpolyelectrolyte complexation[19] between DNA and polycations,[5] this charge ratio close to unity ($\sim$1.05 if we take into account only the cationic lipid molecules in the outer leaflet assuming a homogeneous distribution of lipid in the SUV bilayer) can be explained by a strong DNA–SUV electrostatic interaction accompanied by the entropically favorable release of counterions from DNA and SUV. This process relies on the presence of a large density of cationic charges within individual lipid assemblies (SUVs). Therefore, we studied the response of DNA when such assemblies were destroyed but the total amount of charge in solution remained constant. To this end, we used Triton X-100, a neutral surfactant that is known for its ability to break SUVs.

By FM, we systematically studied the effect of Triton X-100 on DNA conformation under various experimental conditions. We found that for a concentration smaller than 6.3 $\times$ 10^{-4} M, Triton X-100 had no detectable effect on a solution containing only DNA in a coil state (Figure 2A). This is in agreement with the investigations of Melnikov et al., who showed that significant effects on DNA conformation appear only at much larger Triton X-100 concentrations.[20] It is also interesting to characterize the effect of Triton X-100 on DNA in a compact state. For this purpose, DNA molecules were first compacted by spermine (SPM^{4+}), a natural tetravalent cation and a well-known DNA compaction agent. For [SPM^{4+}] = 10 μM, all DNA molecules were observed to be in the compact state (Figure 2B, middle). After the addition of Triton X-100 up to 6.3 $\times$ 10^{-4} M, no

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change was observed, with all DNA molecules remaining in the compact state (Figure 2B, right). All of these results indicate that under our experimental conditions Triton X-100 has no detectable effect on DNA regardless of its conformational state. In contrast, when Triton X-100 was added to a solution containing DNA compacted by SUVs, all DNA molecules initially in a compact state (Figure 2C, middle) unfolded to an elongated and fluctuating structure having all of the characteristics of a DNA coiled state in the absence of a compaction agent (Figure 2C, top right). Interestingly, the observations of the same objects using NBD filter showed that the unfolding of DNA was accompanied by the vanishing of SUV fluorescence together with an increase in the fluorescence of the background (Figure 2C, bottom right). This indicates that the disassembly of SUVs into lipid monomers, small lipid aggregates, or mixed micelles induced by Triton X-100 is most probably at the origin of DNA unfolding. We hypothesize that the disassembled lipid molecules can no longer act in a synergetic way and that their DNA binding activity strongly decreases because of the competitive binding of DNA counterions.

Then, we systematically investigated the effect of Triton X-100 concentration once DNA had been compacted by a fixed number of SUVs (SUV/DNA charge ratio is 2.1). Figure 3A shows that the fraction of DNA in a compact state strongly decreases with an increase in Triton X-100 concentration. For a concentration of Triton X-100 larger than 3.8×10^{-4} M, all DNA molecules have been unfolded into a coil or slightly shrunken coil state. It is interesting that this concentration is slightly higher than the critical micelle concentration (cmc) of Triton X-100 ($\sim 3 \times 10^{-4}$ M).[21] It is thus possible that dissembled lipid molecules form mixed micelles that contain lipids and Triton. Finally, we studied the possibility of repeating with the same solution a sequence of compaction/unfolding processes using the methodologies exposed above. Starting from a DNA solution, we alternatively introduced SUV and Triton X-100 into the same sample tube and measured by FM the fraction of DNA molecules in a compact state. Figure 3B shows that it is possible to switch the conformation of the majority of DNA molecules a few times between compact and unfolded states. To our knowledge, the only system where the DNA conformation could be repeatedly switched in a similar way was reported by Le Ny and Lee.[13a] In that case, the authors used photosensitive surfactant binding to DNA in a light-dependent manner. A few cycles of compaction/unfolding were also obtained, but the kinetics of the process was extremely slow because each cycle required about 2 days. In our system, both compaction and unfolding were obtained in a few minutes.

Conclusions

We demonstrated that genomic DNA can be compacted by the addition of cationic small unilamellar vesicles and unfolded afterward by the simple addition of a neutral surfactant. This process, which is based on the construction/destruction of the lipid nanoscale assemblies, was interpreted in terms of charge distribution in the DNA microenvironment. It was applied to switch DNA repeatedly from an unfolded to a compact state in a dynamic way. Potentially extensible to other nanoscale assemblies, this methodology paves the way for the dynamic control of DNA conformation and should find fundamental and practical applications in various domains such as gene therapy, self-assembly, and nanobiotechnology.

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