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Effect of Cationic Strength and Species on 2-D Condensation of DNA

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The structure of DNA molecules condensed on cationic lipid bilayers changes in the presence of high-valence cations. For a plasmid DNA, it is found that the interhelical distance of condensed DNA has a minimum as the concentration of Mg^{2+} ions increases. However, divalent Mn^{2+} ions do not induce similar changes. More dramatically, the triamine spermidine causes most condensed DNA molecules to leave the bilayers at a concentration of only 0.05 mM. These findings may be useful for understanding how 2-D condensation of DNA occurs.

Introduction

Various in vitro studies have shown that ionic strength and ionic species have profound effects on the condensation of DNA into compact 3-D structures, such as toroids and rods.[1−9] Manning's theory explains the basic physical characteristics of these phenomena, suggesting that the screening of the electrostatic interaction among DNA helices by nonlocalized cations is the major factor leading to the condensation.[10−12] More elaborate studies of the DNA−DNA interacting forces under the influence of different cation species indicate that the DNA condensation is a more complicated phenomenon and may involve hydration layers and some cooperative phases.[13−15] For 2-D systems, the phenomenon of DNA condensation has recently been studied, both experimentally using atomic force microscopy (AFM)[16,17] and theoretically.[18] Intuitively, one would imagine that the reduced dimensionality might simplify some complexities to allow the construction of a comprehensive theoretical model that may explain experimental results. An encouraging example is the recent theoretical work by Dan, predicting that the ordering of DNA molecules occurs only on fluid phase cationic lipid bilayers.[18] The theory is consistent with recent experimental results that 2-D condensation of DNA is greatly facilitated by driving lipids to pass the main phase transition to the fluid phase.[17] Here, we report our experimental studies on how different cations induce structural changes of those DNA molecules that condense on supported bilayers of a cationic lipid, dipalmitoyldimethylammonium propane (DPDAP). For a plasmid DNA, it is found that the interhelical distance of condensed DNA has a minimum as the concentration of Mg^{2+} increases. Another divalent ion, Mn^{2+}, does not induce similar changes of condensed plasmid DNA molecules as its concentration increases. Large parts of the bilayer are free from any adsorbed DNA at a concentration of only 0.05 mM trivalent spermidine. These findings may stimulate further theoretical investigations to refine currently available models.

Experimental Section

Materials. Cationic DPDAP lipids, dissolved in chloroform, were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. All DNA molecules were obtained from Sigma (St. Louis, MO) and used after appropriate dilution. The DNA molecules include pZT plasmid DNA of 2880 bp, Col E1 plasmid DNA of about 6600 bp, and a DNA marker pUC 18 Hae III digest. All divalent and multivalent salts ($MgCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, and spermidine) were obtained from Sigma.

Supported DPDAP Bilayers. Supported unilamellar bilayers of cationic lipids were prepared with the vesicle-fusion method.[19]

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Briefly, 0.25 mL of 1 mg/mL lipids dissolved in chloroform was applied to a glass culture tube, and the sample was dried under a stream of nitrogen gas. The dried film coats the bottom of the glass tube. Then, 1 mL of 20 mM NaCl solution was added to the tube. The tube was then sonicated to a light cyanic color under nitrogen gas, using a Fisher FS3 ultrasonic cleaner. A droplet of about 0.2 mL of the lipid suspension was applied to cover a piece of freshly cleaved mica. The substrate was then store at 4 °C for about 24 h. Afterward, the substrate was heated to 50 °C for about 1 h. The existence and the quality of the bilayer were examined by AFM.

Initial DNA Incubation. DNA was allowed to incubate with those bilayers that have large surface coverage without excessive aggregates at 4 °C overnight. The typical concentration of DNA in the incubation is 0.001 or 0.002 mg/mL in a droplet of about 0.2 mL.

The Effect of Multivalent Cations. Once a sample was examined by the AFM after the initial DNA incubation, the sample was allowed to incubate with a solution containing multivalent cations. Through this process, the mica substrate containing membrane-adsorbed DNA was kept immersed in a droplet of about 0.25 mL. To change the droplet to a new salt solution, we withdrew 0.15 mL and added the same amount of the new solution and repeated the exchange procedure over 20 times. Thus, the specimen remains fully hydrated throughout the whole process. Then, the specimen was incubated with the new salt solution for about 2 h or overnight at room temperature. Afterward, we washed away the salt solution with 20 mM NaCl by repeating the above process. The specimen was then imaged by AFM in 20 mM NaCl to study the effect due to the incubation with the salt. The washing is necessary, because the image contrast with *in situ* AFM diminishes at high salt concentrations, consistent with our previous finding.[17] Trials of imaging in low concentrations of high-valence ions have set the following limits beyond which the image contrast diminishes: 0.1 mM Mg^{2+}, 0.01 mM Mn^{2+}, and 10^{-6} M spermidine. Longer incubation times have no additional effect on the structure of condensed DNA. Further, the structure of condensed DNA after the washing remained stable for periods over half a day to 2 days, during which AFM images were obtained.

Atomic-Force Microscopy. A NanoScope E AFM and oxide-sharpened Si_3N_4 tips with a nominal spring constant of 0.06 N/m, from Digital Instruments, were used in this work. AFM images were obtained in contact mode under 0.1−0.2 nN probe forces, at a pixel number of 512 × 512, and with a scanning line speed of 5 Hz. During the AFM scan, the drift in the imaging force was constantly adjusted to keep the imaging force from increasing.

Results

For a plasmid DNA, the effect of Mg^{2+} ions on condensed DNA molecules on a 2-D DPDAP bilayer is shown in Figure 1. The image in part a is an example of condensed DNA in 20 mM NaCl. In the absence of Mg^{2+} ions, the interhelical distance, determined by the radius of the Fourier transform ring, is 4.3 ± 0.2 nm (20). The value in parentheses indicates the number of AFM images used in the average, and the same convention is used throughout this paper. The image in part b shows an example of condensed DNA after incubating the same sample in 10 mM Mg^{2+} and washing. A decrease in the interhelical distance is observed, with an average value of 3.8 ± 0.2 nm (26). After the same sample is incubated at higher Mg^{2+} concentration and washed, the interhelical distance increases. An AFM image obtained after incubating the sample in 50 mM Mg^{2+} is shown in part c. In all the above cases, stable imaging is possible and repeated scans do not cause any degradation of the image quality. A summary plot in part d displays how the interhelical distance of condensed plasmid DNA changes as Mg^{2+} concentration increases. All data points in part d were obtained after averaging over 20 AFM images. We also tried to initially incubate DNA in 100 mM Mg^{2+} solution and found that DNA condensed onto the bilayer with similar structure. Thus, the ionic effect is independent of how Mg^{2+} ions are introduced into the system.

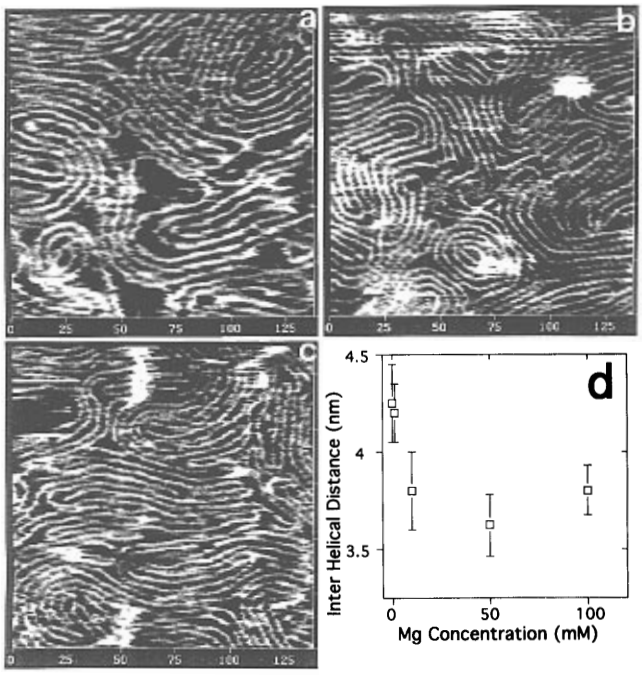

Figure 1. Three typical AFM images of Col E1 plasmid DNA molecules condensed on DPDAP bilayers and a summary plot. The Mg^{2+} concentrations used in the incubations are 0, 10, and 50 mM in parts a, b, and c, respectively. The summary of how the interhelical distance changes with the increase of Mg^{2+} concentration is shown in part d.

For the DNA fragments, the effect of Mg^{2+} ions on the structure of condensed DNA is less regular. An image in part a of Figure 2 represents condensed DNA fragments in 20 mM NaCl. The interhelical distance is 4.3 ± 0.2 nm (20). The image in part b shows an example obtained after incubating the same sample in 5 mM Mg^{2+} and washing, and the interhelical distance is 4.1 ± 0.2 nm (22). However, after the same specimen is incubated with 25 mM Mg^{2+} and washed, the interhelical distance increases to 5.4 ± 0.2 nm (20). An example is shown in part c.

For pZT plasmid DNA molecules on DPDAP, the presence of Mn^{2+} ions does not give rise to any significant change of the interhelical distance, although condensed DNA molecules remain closely packed after incubating with up to 5 mM Mn^{2+} ions. Figure 3 shows an example of the condensed DNA after an incubation with 1 mM Mn^{2+}. We found that condensed DNA remains on the bilayer after incubating the sample in 100 mM Mn^{2+} at room temperature. The contrast was much weaker in this case, and it was very difficult to obtain a stable image over one complete frame. However, after the same sample is incubated in 100 mM Mn^{2+} at 50 °C for 25 min, most DNA molecules leave the bilayer. The image in Figure 4, part a shows an example in which the dark areas are bilayer defects. The arrow in part a points to a DNA-containing domain, on which the adsorbed DNA is condensed, as shown in part b. A sectional plot, corresponding to the line in part a, is displayed in part c. The sectional plot shows that the bilayer defect has a thickness

Figure 2. Three typical AFM images of DNA fragments condensed on DPDAP bilayers after the incubations at different Mg^{2+} concentrations. The image in part a is original without any Mg^{2+} incubation. Images in parts b and c are obtained after incubations with 5 and 25 mM Mg^{2+} ions, respectively. Image size in the three images: 300 nm.

of about 5 nm and the DNA-containing domain has a thickness of about 7 nm. Note that the height of the DNA-containing domain corresponds to the sum of a bilayer and the diameter of the DNA.

The presence of trivalent cations shows a dramatic effect without the need for any heating. After specimens of condensed DNA are incubated in 0.05 mM spermidine and washed, most DNA molecules leave the bilayer. The image in part d of Figure 4 shows an example with the DNA fragments, in which only some domains of about 3 nm higher are seen, as shown in a sectional plot (part e) corresponding to the line in part d. However, stable imaging on the raised domains was not possible, although their heights suggest that these domains contain membrane-bound DNA molecules. We observed that the spermidine induced similar structural changes for the two kinds of plasmid DNA molecules.

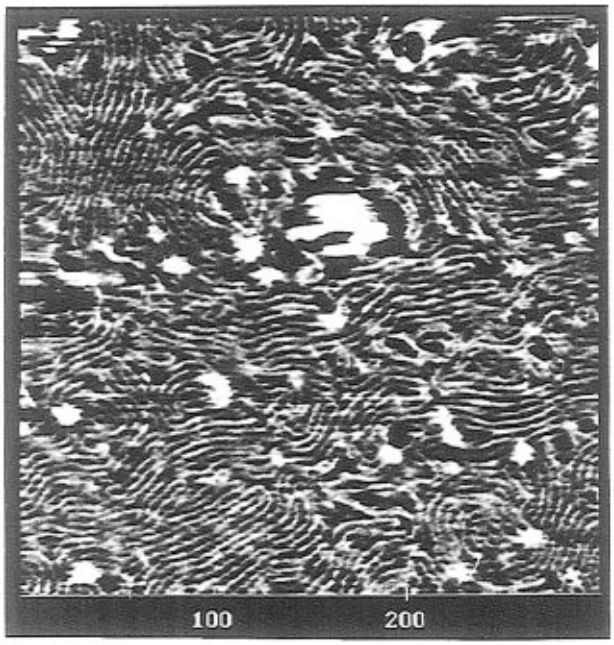

Figure 3. Image of condensed pZT plasmid DNA molecules on DPDAP bilayers after incubating the sample in 1 mM Mn^{2+}.

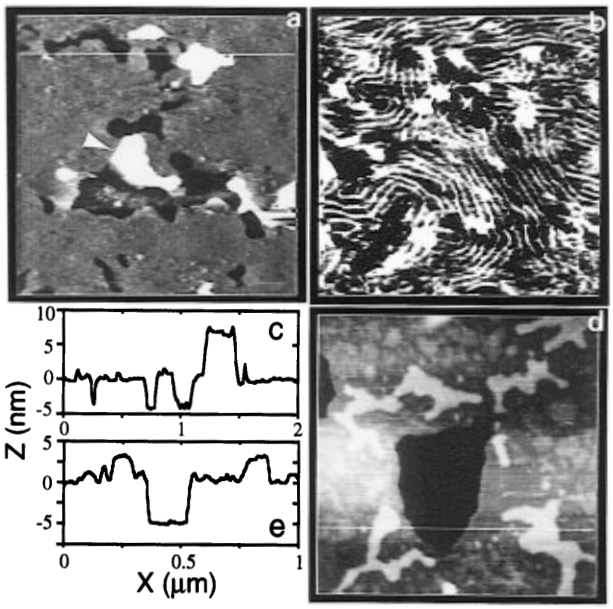

Figure 4. Images of DNA fragments and sectional plots. The image in part a was obtained after heating the condensed pZT DNA molecules to 50 °C for 25 min in 100 mM Mn^{2+}. The image in part b was obtained on top of the white island pointed to by the arrow in part a and shows the presence of condensed DNA molecules. Image size in part b: 200 nm. A sectional plot corresponding to the line in part a is shown in part c. An image obtained after incubating a specimen of condensed DNA fragments with 0.05 mM spermidine is shown in part d, and a sectional plot corresponding to the line in part d is given in part e.

Discussion

Our results have several interesting features that are consistent in some aspects with available models[10−12,18,20] but are not fully explained by these theories. According to the electrostatic interaction, one expects those cations to screen the electrostatic attraction between DNA and the cationic bilayer by binding to DNA. Thus, the presence of more cations screens more effectively and may cause the DNA to leave the bilayer plane. It has been known that divalent and trivalent cations bind to DNA more strongly than do monovalent cations.[7,8] It is possible that trivalent ions bind to DNA more strongly than the attraction

between the bilayer and DNA, causing DNA to leave the bilayer. However, the electrostatic interaction alone would not explain the more complicated phenomena in our experiments. The interhelical distance of condensed plasmid DNA exhibits a minimum as the concentration of divalent Mg^{2+} ions increases. This nonmonotonic behavior suggests the existence of some cooperative behavior of the DNA. At higher concentration of the cations, the electrostatic interaction is overwhelmed so that the cations function as simple screening agents. The different behavior between divalent Mg^{2+} and Mn^{2+} ions, also reported elsewhere on different systems,[21] may suggest that factors other than the electrostatic interaction play a role in the condensation of DNA on 2-D membranes. A more comprehensive theoretical model is needed to explain details of the molecular processes involved.

In conclusion, we have demonstrated that divalent and trivalent ions induce changes of the structure of DNA condensed on 2-D cationic bilayers. Our experimental results reveal how the degree of the changes depends on the ionic strength and ionic species. Thus, some experimental bases are established to provide clues for future theoretical studies.

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