Gemini Surfactant-Induced DNA Condensation into a Beadlike Structure

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Using a magnetic tweezers (MT) apparatus and an atomic force microscope (AFM), we studied the condensation of DNA induced by the cationic gemini surfactant hexyl-ω,ω-bisdecyldimethylammonium bromide (C12C6C12Br2). Stepwise condensation events were found for forces from 0.5 to 4 pN, with a decrease in DNA extension by ∼100 nm in each condensation event. Applied stretching forces larger than 6 pN were found to be able to decondense the condensates in a similar intermittent stepwise manner, but with ∼60 nm of DNA released in each decondensation event. These observations are consistent with AFM images that show beadlike structures on DNA. The results lead to a model in which, during condensation, independent beadlike structures are initially formed on DNA, and as the local density of the beads increases, they compact into higher-order structures while maintaining their independence. The condensation process is different from any previously reported condensation process, but it is somewhat similar to that of chromatin assembly.

1. Introduction

DNA condensation is a fundamental phenomenon in living matter and has long been the subject of many investigations.1–6 It poses a fascinating challenge to our understanding of semiflexible polymers. The condensation of DNA is also of biological and therapeutic importance. DNA compaction induced by gene vectors is a crucial step in gene therapy. The transfection activity of DNA is highly dependent on the structure of gene vectors turns out to be very important. Gemini surfactants are a novel class of DNA-condensing agents containing two aliphatic chains and two headgroups, linked by a spacer.10 They typically show greatly enhanced activity relative to conventional vectors.7–9 As the properties of the condensates, including morphology and size, are strongly influenced by the nature of the condensing agents, the choice of suitable gene vectors turns out to be very important. Gemini surfactants are a novel class of DNA-condensing agents containing two aliphatic chains and two headgroups, linked by a spacer.10 They typically show greatly enhanced activity relative to conventional surfactants and can thus bind and compact DNA efficiently at a very low concentration.11,12 This superior structure−activity correlation prompted us to study the kinetics of DNA condensation induced by the cationic gemini surfactant hexyl-ω,ω-bisdecyldimethylammonium bromide (C12C6C12Br2).

Previous studies have shown that DNA condensation in vitro usually exhibits first-order phase transitions.13,14 Three types of DNA condensates, i.e., toroids,15,16 globules,17 and rodlike structures,18 are usually found. Toroids bear an ordered crystallinelike structure formed by DNA wrapping upon itself circumferentially. Globules have a disordered structure comprising randomly packed DNA segments. Rodlike condensates come from parallel aligning of DNA segments. In this work, by gaining insight into the condensation process at the molecular level using an atomic force microscope (AFM) and a single-molecule magnetic tweezers (MT) setup, we found a new mode of DNA condensation that is different from any previously reported condensation process, but is somewhat similar to that of chromatin assembly.

2. Experimental Section

The measurements were performed in phosphate-buffered saline (10 mM PBS, 5 mM NaCl, pH 7.4). Gemini surfactants C12C6C12Br2 (n = 2, 4, 6) were synthesized and purified according to the method of Menger and Littau.19 λ-DNA (48.5 kbp, 16.4 μm, from New England Biolabs) ligated to biotin-and digoxigenin-modified DNA fragments (i.e., 3′-biotin-cccgccgctgga and 3′-digoxigenin-tcccagcggcggg) was used in the measurements.20 The biotin-modified 3′-DNA end was tethered to a 2.8-μm magnetic bead coated with streptavidin, and the other end modified with digoxigenin was tethered to the sidewall of a fluidic chamber coated with antidualogoxigenin (Figure 1a).21 The fluidic chamber was placed on top of an inverted microscope (IX71, Olympus, Tokyo, Japan), and a CCD camera was used to record the position of the bead in real time at 25 frames per second. The position of the bead was tracked using fast-Fourier-transform-based correlation technique.21 A magnetic force was exerted on the bead to create a tension on the DNA, which lies parallel to the focal plane of the microscope. The exerted force was calculated according to the position fluctuations of the microsphere in the direction perpendicular to the DNA extension.22,23 After the force exerted on the DNA had been adjusted, C12C6C12Br2 solution (1–15 μM) was loaded into the flow chamber to induce DNA condensation.

A multimode Nanoscope IIIa AFM (Digital Instruments, Santa Barbara, CA) was used for AFM imaging. A 10–20 μl sample solution (20 pM DNA solution or a mixed solution of 20 pM λ-DNA and 10 μM C12C6C12Br2) was deposited onto a piece of freshly cleaved mica and left to adhere for 1–10 min. The sample was then dried with a gentle stream of nitrogen. Tapping-mode images were obtained.

3. Results and Discussion

We first studied the condensation kinetics using MT. Figure 1c presents a typical time course of the condensation process...
induced by C_{12}C_{6}C_{12}Br_{2}. It reveals that the condensation follows a slow overall shortening (referred to as “overall slow decay” in the figure) interposed by several fast events (referred to as “local fast decay”), implying a two-stage condensation process. The time course is different from that observed for DNA condensation induced by multication,

where linear time courses were observed because the condensation was controlled by a nucleation-and-growth process, leading to the formation of toroids. By further analyzing the time course in detail, a striking feature can be found: DNA shortens stepwise. Figure 2a presents typical steps recorded during a condensation process. We wrote a program to automatically detect the steps following the method introduced by Kerssemakers et al.\textsuperscript{24} The algorithm starts by fitting a single large step in the data and then adding the most prominent step to the fitting curve in each fitting circle until the data are well fitted. The detailed algorithm is described in the Supporting Information. The most probable size of the steps is about 100 nm (Figure 2b), which corresponds to the average length of a DNA segment imbedded in a condensed structure. Such discontinuous condensations were also observed for other forces. The force range for which we could see the discontinuous stepwise condensation is from 0.5 to 4 pN. At very small forces, the noise due to Brownian motion is too large for meaningful data to be obtained. The condensation rate decreases as the force is increased.

By applying a larger force (over 6 pN) to the condensed DNA, the DNA chain can be stretched back to its original length. Interestingly, the extension of DNA is also stepwise (Figure 3a). The distribution of step sizes shows two peaks (Figure 3b), one at about 62 nm and the other at about 125 nm. Here, the 62-nm steps should be caused by the disruption of a single structure on DNA, whereas the 125-nm step can be ascribed to the simultaneous disruption of two structures on DNA. Strikingly, the extending step size of \sim 62 nm is much smaller than the condensing step size (\sim 100 nm). This suggests that \sim 40 nm of DNA has been stripped off before the structure is fully disrupted (see discussion below).

Note that, basically, a DNA molecule condenses more rapidly at higher C_{12}C_{6}C_{12}Br_{2} concentrations and under smaller applied forces. However, we failed to obtain very good relationships between the shortening rate of DNA and the C_{12}C_{6}C_{12}Br_{2} concentration or the applying force. A possible explanation for this is the uncontrollable diffusion of surfactant molecules to the DNA chain in each experiment. However, we did find that, no matter what the surfactant concentration and applied force were, the condensation process produced approximately the same step-size values; that is, the peak distribution was the same for different applied forces and different surfactant concentrations. This indicates that the condensates formed at different surfactant concentrations and applied forces should be the same.

The regular step sizes must correspond to the formation of a certain regular structure on DNA. To verify this conclusion,
we measured the static structure of the condensates by AFM. Figure 4 presents morphologies of \( \lambda \)-DNA after interaction with 10 \( \mu \)M \( \text{C}_{12}\text{C}_{12}\text{Br}_2 \) for (a) 1 and (b) 8 min. The inset images show the three-dimensional morphologies of the chosen areas (white squares). Scale bars represent 200 nm in all of the images.

Figure 4. DNA morphologies obtained by AFM after interaction with 10 \( \mu \)M \( \text{C}_{12}\text{C}_{12}\text{Br}_2 \) for (a) 1 and (b) 8 min. The inset images show the three-dimensional morphologies of the chosen areas (white squares). Scale bars represent 200 nm in all of the images.

Yoshikawa and co-workers\(^{15}\) previously found a “rings-on-a-string” structure in surfactant-induced DNA condensation by single-molecule fluorescence. The rings in their study were toroids. One might think that the beads observed here are toroids as well. However, the two types of structures are different. First, \( \lambda \)-DNA with a contour length of about 57 \( \mu \)m was used in Yoshikawa et al.’s study, and one DNA chain gave at most five rings. This means that one \( \lambda \)-DNA with a contour length of about 16 \( \mu \)m should have given only one or two beads on average if the beads in Figure 4 were toroids. However, the number of beads in Figure 4 is large, and the beads do not fuse into larger structures. Second, the sizes of the beads in the present study are much smaller than that of the toroids. Theoretical and experimental studies have revealed that DNA toroids favor diameters of 70–140 nm.\(^{12}\) Our previous study\(^{26}\) also revealed that one ring of the toroids contained DNA strands of around 300 nm in length, which means that the toroid rings are \( \sim \)86 nm in diameter. However, in the present study, because we ascribe each stepwise shortening event to the formation of a bead, thus the step size of \( \sim \)100 nm should correspond to the length of DNA strands in one such bead. In this case, even though we consider the bead as a single ring formed by a DNA chain, its diameter is only about 30 nm, which is much smaller than the typical diameters of the toroids. Third, whereas the decondensation process is just the reverse of the condensation process in the case where toroids are formed on DNA,\(^{26}\) the kinetics of decondensation is different from that of condensation in the present work, for instance, the step sizes in the two processes are different. In conclusion, our results indicate that the beadlike structure is a new type of condensate that has not been observed before in surfactant-mediated DNA condensation.

The appearance of the beadlike structures in the AFM images is consistent with the kinetics observed with the MT. It is reasonable to assume that each stepwise shortening event in the MT measurements corresponds to the formation of a single beadlike structure. The images also help explain the unique condensation kinetics in Figure 1c. The slow overall shortening is caused by the independent and random formation of the beads on DNA, in which case the growth rate of the number of the beads is proportional to the length of the remaining naked DNA. The fast shortening events can be ascribed to further aggregation of the beads, which can interact with each other when they are close enough. Each fast shortening process corresponds to the aggregation of some of these beads. At the end of each such process, the shortening rate decreases because of the lower amount of free space available or the steric hindrance caused by aggregate overlapping.

The driving force for the formation of the beadlike structure might arise from the hydrophobic interaction between the hydrocarbon tails of the surfactant molecules bound to DNA. However, the molecular structure of the surfactant itself might play an even more important role in determining the morphology of the condensates. First, conventional surfactants usually lead to the formation of toroids and globules.\(^{15–17}\) We have also measured the condensation of \( \lambda \)-DNA induced by multicationics and single-chained surfactants. Only toroids and random globules were observed, respectively (data not shown), which are completely different from the beadlike structures reported here. Second, we studied the effect of the spacer length of the gemini surfactants on the condensation. The results (Figure 5) indicate that gemini surfactants with a spacer length of \( n = 2 \) or 4 do not lead to the formation of beadlike structures. In agreement with this, no steps were observed in the MT
measurements, in either the condensation process or the decondensation process (data not shown).

We are not able to declare the exact internal structure of these beadlike structures because of the finite resolution of the AFM. However, our measurements do provide some clues about it. Surfactant molecules can form some sort of micellar aggregates in the vicinity of oppositely charged macromolecules. A micellar aggregate can act as nucleation center around which DNA can wrap to form a DNA spool, i.e., the beadlike structure. This model is attractive because it is similar to that of a nucleosome in which DNA wraps around a core histone octamer. However, the hydrophobic interaction between the hydrocarbon tails is not strong enough to overcome the bending energy of DNA because the curvature of DNA in the beads must be extremely high to agree with the observed small step sizes (~62 nm; note that the persistence length of DNA is ~50 nm under solution conditions similar to ours). We propose that the double heads of the surfactants undertake the very task of bending DNA locally. The double heads connected by the spacers could lead to a remarkable alternation in the DNA structure including a rigid bending. However, this is not enough. The observation that only simple exponential time courses were observed in the MT measurements when gemini surfactants with a spacer length of \( n = 2 \) or \( 4 \) were used (data not shown) and the AFM images in Figure 5 imply that the length of the spacer is also a very important parameter in determining the structure of the DNA-C\(_{12}\)C\(_6\)C\(_{12}\)Br\(_2\) complex. The two-headed surfactant molecules with a spacer length of \( n = 6 \) may match well with the geometrical arrangement of the anionic phosphate groups on the DNA helix, thus causing the specific binding of the two cationic headgroups of C\(_{12}\)C\(_6\)C\(_{12}\)Br\(_2\) to DNA. The peculiar beadlike structure must then result from the coordination between the self-assembly of the surfactants and the specific bending of DNA induced by the double heads of the surfactants. Further detailed experimental and theoretical studies are awaited to clarify the exact nanostructure of these beadlike structures, as well as the whole story of C\(_{12}\)C\(_6\)C\(_{12}\)Br\(_2\)-mediated DNA condensation.

It was mentioned above that the step size in the decondensation process is only ~62 nm, which is 40 nm shorter than the step size in the condensation process. This means that the outermost 40-nm segment in a beadlike structure is stripped off before the structure is fully disrupted. Using the above model, we now try to interpret the difference in step size. When a beadlike structure forms on DNA, a stretch of DNA about 100 nm in length wraps on the micellar aggregate to form a spool. A theory has already been conceived to describe the unraveling of such DNA spools. It suggests that the unraveling of a full loop in the spool is a force-induced transition from one metastable state to another metastable state via a saddle point because the DNA spool is strongly kinetically protected from mechanical disruption. The energy barrier leads to the stepwise opening of the loop, similar to that observed for nucleosome unwrapping under tension. The circumference of the spool in our case is about 60 nm according to the decondensing steps. The 100-nm DNA segment can wrap only 1\( \frac{3}{4} \) cycles on the micellar aggregate such that the outermost two DNA segments (each ~20 nm in length) of the spool can be easily stripped off by the applied force because they are not kinetically protected, leaving behind a full loop. The loop is now kinetically protected to give a step in the decondensation process.

4. Summary

The present work shows that C\(_{12}\)C\(_6\)C\(_{12}\)Br\(_2\) can induce a new type of DNA condensate. The kinetic measurements and static images are consistent with a two-stage condensation process, during which beadlike structures are formed at the beginning and these structures further aggregate into higher-order structures but do not fuse with each other. Both the condensation and (tension-induced) decondensation processes are discontinuous, meaning that each beadlike structure assembles and disassembles independently on the DNA. This is somewhat similar to the assembly of chromatin, although the beadlike structures are less regularly distributed than in a chromatin. Finally, the finding that the length of the spacer of the gemini surfactants can greatly affect the condensation of DNA should be instructive in designing new agents for gene therapy, although why this effect occurs is still an open question. Obviously, techniques with higher resolution are needed to elucidate the structure.

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Supporting Information Available: More experimental results including extension and contraction curves and AFM

Figure 5. DNA morphologies obtained by AFM after interaction with 10 \( \mu \)M (a) C\(_{12}\)C\(_6\)C\(_{12}\)Br\(_2\) and (b) C\(_{12}\)C\(_6\)C\(_{12}\)Br\(_2\). No obvious beadlike structures are observed. Similar images were obtained at other concentrations. Scale bars represent 200 nm.
morphologies and the detailed step detection algorithm. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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