Morphology Characterization and Single-Molecule Study of DNA—Dodecyltrimethylammonium Bromide Complex

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ABSTRACT: DNA compaction induced by dodecyltrimethylammonium bromide (DTAB) is studied using atomic force microscopy (AFM) and magnetic tweezers. The morphology of DNA—DTAB complex is dependent on the DTAB concentration and incubation time. With magnesium ions, the complexes show rod- and network-like structures after approximately 5 min of incubation at low DTAB concentrations. With increasing incubation time, more toroids and globules appeared, resulting in the formation of scattered condensed particles. At high DTAB concentrations, the complexes show swollen globular structures independent of the incubation time. The compaction and unraveling of the DNA—DTAB complex are also analyzed at the single-molecule level using magnetic tweezers. The extension—time curves show a staircase structure with typical sizes of ~40, 60, 80, and 112 nm, suggesting that the complexes are well organized and more compacted than those induced by multivalent ions. Finally, the high DTAB concentration stabilized the complex and increased the unraveling energy barrier.

INTRODUCTION

DNA condensation is a key process in gene delivery. Many agents, including multivalent cations of valence three or greater, cationic polypeptides, basic proteins, alcohols, and neutral crowding polymers, can cause DNA molecules to collapse into more compact structures.5,6 Cationic surfactants can also induce DNA condensation and are reported to have potential uses in gene delivery.7,8

Due to the potential applications of a DNA—surfactant system, several studies have focused on elucidating the mechanism of surfactant-induced DNA condensation.5—15 Imaging techniques, such as atomic force (AFM) and electronic and fluorescence microscopies, along with single-molecule methods, such as optical and magnetic tweezers, are powerful tools that can be used to study DNA conformation under various condensation agents. Using three methods including electronic microscopy, Ghirlando et al. have studied the DNA packing process in the presence of positively charged micellar aggregates and flexible anionic polymers.15 On the basis of their results, they discovered the formation of a complex in which DNA molecules are partially embedded within a micellar scaffold and condensed into highly packed chiral structures. Using fluorescence microscopy, Mel’nikov et al. have shown that the compaction of DNA induced by cationic surfactant is a first-order transition.16 Depending on the surfactant concentration, the DNA molecules exhibit large discrete transitions between the elongated coil and compacted globular states. Husale et al. have recently employed optical tweezers to study the interaction between a single DNA molecule and cationic surfactants.6 Depending on the length of hydrophobic chain of the surfactant, they discovered different elastic behaviors of the complex, suggesting two different binding modes.

Apart from these studies that elucidated the interaction between DNA and cationic surfactants, there remains inadequate knowledge on the mechanisms involved and the relationships among factors that influence the conformation of a DNA—surfactant complex, such as time, surfactant concentration, and ionic condition. The structure of the DNA—surfactant aggregate, which is the foremost issue, is still ambiguous. Ghirlando et al. have proposed a close hexagonal packing model for the DNA—cetyltrimethylammonium bromide (CTAB) and DNA—tetradecyltrimethylammonium bromide complexes, which is supported by observed X-ray scattering peaks. However, such model is not applicable for the DNA—dodecyltrimethylammonium bromide (DTAB) complex because no similar peak has been observed.15

The interaction between DNA and DTAB is investigated systematically in the present study using AFM and magnetic tweezers. Imaging of the DNA—DTAB complex under varying conditions, namely, incubation time, surfactant concentration, and magnesium ions, is performed to characterize its morphology. Using magnetic tweezers, the process of DNA—DTAB complex formation unraveling at the single-molecule level is also studied to provide some clues on the dynamics of the complex formation.
MATERIALS AND METHODS

AFM Sample Preparation. DTAB (Sigma, USA) was dissolved to 100 mM in 1× TE buffer (10 mM Tris-Cl +1 mM EDTA, pH 8.0). Bacterial λ-phage DNA (0.5 μg/μL, 48 500 bp) were purchased from New England Biolabs and used without further purification. The DNA solution was diluted to 1 ng/μL with 1× TE buffer containing 5 mM MgCl₂. Several microliters of 100 mM DTAB solution were added to 500 μL of diluted DNA solution for incubation. The incubation time and DTAB concentration were varied in the sample preparation. Approximately 10 μL of the incubated solution was dropped onto a freshly cleaved mica surface and incubated for 3 min at room temperature. The surface was then washed with ∼100 μL of distilled water (purified by a Millipore system) and dried with nitrogen gas flow. After drying, the samples were kept in a desiccator ready for scanning.

AFM Imaging. For the current study, an SPM-9600 system (Shimadzu, Kyoto, Japan) was equipped with a J-scanner for imaging. All samples were scanned in the tapping mode at an ambient condition. All images presented in the present report that were derived from the original data were flattened to improve the contrast grade. The imaging was performed at a scan size of either 2.5 × 2.5 μm or 5 × 5 μm. All images shown are phase images.

DNA Construction for Single-Molecule Study. For the single-molecule measurements, the bacterial λ-phage DNA (New England Biolabs, USA) molecules were functionalized by covalently attaching 12 bp chemically labeled single-stranded oligonucleotides (3’biotin-ccgcgctgga and 3’digoxygenin-tccagcggg) to their ends according to the procedure of Smith et al.16 Approximately 1 μL of ligated DNA solution was then mixed with ∼1 μL of 2.8 μm paramagnetic beads coated with streptavidin (M-280, Dynal Biotech) in 0.2 mM PBS, forming bead–DNA constructs.

Magnetic Tweezers Setup. The setup for the single-molecule measurement was similar to that described by Sun et al.17 A 0.17 mm thick coverslip with one side polished was sandwiched in between two glass slides; this served as a flow chamber by sealing the open side of the structure with polydimethylsiloxane. Next, two holes with a diameter of 1 mm each were created on the top glass slide and linked with a glass capillary to facilitate buffer out or in (Figure 1A). The flow chamber was placed on the 40× objective of an inverted microscope. The force exerted on the DNA in the focal plane was controlled by a micrometer-positioned permanent magnet placed lateral to the chamber. The polished sidewall was functionalized with antidigoxygenin to link with the dig-end of the λ-DNA. The DNA–bead constructs were dispensed into the cell to form a side wall–DNA–paramagnetic bead structure (Figure 1B). The distance between the bead and the surface of the sidewall can be deemed as the extension of the DNA. The structural image in the focal plane was monitored using a video camera (Figure 1C). After a single-suspended λ-DNA was checked, the DTAB solution was loaded to the chamber. The elastic response of DNA as a function of time was recorded and analyzed at different applied forces. The fluctuations transverse to the force direction are related to the applied force using $F = K_B T (L)/(δx^2)$, where (L) is the average extension of DNA and $δx$ is the fluctuation of the paramagnetic bead.18

Dynamic Light Scattering (DLS) Measurements. A DLS apparatus (Zetasizer μV, Malvern) was used to measure the size of the DNA–DTAB complexes. The sample was placed in a thermostated bath maintained at 25 °C. The scattered light was detected at an angle of 90°.

RESULTS

AFM Observation in the Presence of Magnesium Ions. The DTAB concentration and incubation time were varied to investigate their respective effects on the morphology of the DNA–DTAB complex. Figure 2 shows the morphologies of the complexes at different concentrations and incubation times. The top-down images of Figure 2 in each column exhibit the morphologies of the DNA–DTAB complex adsorbed on mica at four different DTAB concentrations of 0.1, 0.4, 2.0, and 4.0 mM, respectively, for a given incubation time. The left to right images in each row show the complexes at different incubation times of 5 and 30 min and 24 h, respectively, for a given concentration. The solvents were all 1× TE buffer (10 mM Tris-HCl + 1 mM EDTA, pH 8.0) containing 5 mM MgCl₂.

Network structures consisting of rods and spherical structures appeared at 0.1 mM DTAB concentration (Figure 2A). As the incubation time increased to 30 min, the compositions of the network structures became mainly toroids and spheres. These toroidal networks consisted of more than one DNA molecule, indicating that intermolecular contacts between the DNA molecules were involved in the complex formation. Unexpectedly, scattered spheres and rods were exhibited after 24 h incubation instead of fibrous networks, corresponding to reduced intermolecular contacts in the complexes. When the concentration increased to 0.4 mM, more condensed globular structures and branchlike structures appeared, indicating that increasing DTAB concentration facilitated the complex formation kinetically. Increasing incubation time also led to a similar result as that of 0.1 mM; that is, scattered structures began to replace the networks. At concentrations higher than 2 mM, the morphologies of the complex became dramatically different from those in the low-concentration regimes (Figure 2H–L). These structures are called swollen globules and consist of DNA and DTAB, as shown by the linking extended DNA molecules between them due to the washing flow (Figure 2I). For the 2 mM DTAB, the
image first exhibited fibrous networks similar to those observed at low concentrations. This indicates that it is still kinetically favored to form such structures even if the incubation time is insufficient. In comparison, prolonged incubation time results in typical swollen globules whose sizes are larger than those in the low-concentration regimes. Apparently, when the DTAB concentration is as high as 4 mM, the incubation time becomes insignificant in the determination of the morphologies of the complexes. All images at the three incubation times display similar swollen conformations.

**AFM Observation in the Absence of Magnesium Ions.** DNA molecules can be adsorbed onto mica with the aid of divalent magnesium ions under TE buffer conditions. However, magnesium ions enhance intermolecular contacts, which can lead to networks. In the current study, scanning was performed in the absence of magnesium ions in order to check its effects on the DNA–DTAB interaction (Figure 3).

Additional DTAB molecules are needed to compact DNA without the aid of magnesium ions. Few structures can be observed at DTAB concentrations of less than 1 mM, indicating that the complexes cannot bind on mica effectively without magnesium ions. When 2 mM DTAB was added to the DNA solution, flat structures appeared independent of the incubation time (Figure 3, A and B). These structures most frequently occurred at the beginning of the incubation time (Figure 3, A, C, and E). Therefore, they should be intermediate structures resulting from kinetics. Without the presence of sufficient surfactant molecules, the flat structure remained in its original conformation (Figure 3B). Increasing DTAB concentration by up to 4 mM initially yielded pancakes after the 5 min incubation period, after which globules appeared after ≥30 min incubation. Further increase in the DTAB concentration by up to 8 mM led to swollen globules similar to those observed in Figure 2H–L (Figure 3F). When DTAB concentration was ≥12 mM, the DTAB caused the DNA to condense as small globules (Figure 3G) and finally aggregate as swollen globules (Figure 3H). In contrast to the case with magnesium ions, network structures seldom appeared in this regime, suggesting that magnesium ions contributed significantly to the formation of the networks.

**Single-Molecule Measurements.** To elucidate the dynamic process of the interaction between DNA and DTAB, varying forces were exerted on a single DNA molecule to investigate its delicate interaction mechanism. As the DTAB solution was loaded into the flow chamber, the extension of the DNA molecule began to transform step by step due to the condensing effects of DTAB. Figure 4A presents a typical extension–time curve which reflects the stepwise condensation at 5 mM DTAB
for an exerted tension of ~12 pN. The abrupt jumps in the curve are essentially reflections of the step-by-step formation of orderly structures. The single-molecule measurements were performed at different DTAB concentrations. Results showed that, below 0.5 mM, DTAB hardly caused any DNA condensation even at forces as low as 1 pN. At 1 mM DTAB, typical DNA stepwise condensation was observed by applying a tension of <5 pN. In contrast, at DTAB concentration ≥5 mM, DNA condensation occurred at forces of 7–12 pN. The final extension of DNA was also related to the exerted forces. Specifically, the paramagnetic bead came close to the sidewall of the chamber at low forces (∼5 pN), whereas DNA retained an end-to-end length of ~6–8 μm at high forces (7–12 pN). After condensation, DNA was released again by exerting larger forces. The unraveling process was also discontinuous, stepwise, and concentration dependent. Figure 4B shows a typical unraveling curve at a force of ~28 pN for 5 mM DTAB. At a low DTAB concentration of 1 mM, the unraveling occurred by applying a tension of ~8 pN, whereas at a high DTAB concentration of ≥5 mM, the regime required higher forces (>20 pN) to unravel the complex.

A series of curves exhibiting well-defined jumping steps were used to obtain the statistics of the step sizes. The distribution of the step sizes is shown in Figure 4C. The peak in the frequency
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Assessment of the Single-Molecule Measurements. Previous reports have reported on the occurrence of jumps with step sizes resulting from the formation or disruption of substructures (e.g., toroidal spools and nucleosomal spools).\textsuperscript{19,21} Kulic et al. have concluded that the formation or unraveling of the spools is a transition between two states, corresponding to a jump in the extension—time profile.\textsuperscript{22} Thus, the jumps observed in extension—time curves may be assumed to correspond to the formation or disruption of similar spools, and the step size may represent the length of DNA stored in the spool. Jumps with large sizes can be considered as several spools that concurrently formed or unraveled.

As shown in the previous section, the obtained typical step size is usually smaller than those shown by other condensing agents. The toroid observed in the polylysine-induced system has a typical size with a contour length of \( \sim 339 \text{ nm} \).\textsuperscript{23} Single measurements show that the typical size in the compaction curve for hexammine cobalt chloride-induced toroids is \( \sim 270 \text{ nm} \), which is the length of a typical toroidal spool.\textsuperscript{21} In contrast, our measurements showed smaller step sizes including 40, 60, 80, and 112 nm, indicating that the DNA—DTAB complex adopted a highly condensed structure.

Mel'nikov et al. have shown that the collapse of a single DNA caused by surfactants is a first-order transition.\textsuperscript{4} However, in the present single-molecule experiment, the compaction process exhibited consecutive jumps instead of a sharp transition, which may be due to the exerted force. In the experiments by Mel'nikov, the DNA molecules are not subjected to directional forces. In contrast, an external force was applied on the DNA in the present measurement, thereby creating an energy barrier. The compaction process became very fast in the absence of applied tension. At a force of \( \sim 1 \text{ pN} \), DNA can be compacted within \( \sim 15 \text{ s} \) with \( \sim 7 \text{ μm} \) decrease of extension. These results indicate that, in the absence of an applied force, the compaction process is a first-order transition, whereas the energy barrier created by the applied force leads to a continuous transition.

The experimental conditions of single-molecule measurements, in which external forces are applied to DNA molecules and no intermolecule interaction is included, are indeed a little different from those of AFM characterization. However, both experiments are consistent in the feature of having complex structures. The step sizes observed in the former indicate a highly condensed structure. Correspondingly, in the AFM scanning images, the most frequently characterized structures of the DTAB—DNA complex are highly compacted globules.

**AFM Sufficient Characterization of the Complex.** In the AFM sample preparation, the deposition, washing, and drying processes on mica may influence the morphology characterization. As a complementary approach, DLS was used in this study to improve the data robustness. The hydrodynamic diameters of the complexes obtained from the DLS measurements are shown in Figure 5A, and the corresponding AFM images and analysis are presented in Figure 5B—D. The sizes of the complexes were consistent in the two measurements.

**Morphology of the DNA—DTAB Complex.** According to the scanning results, the DNA—DTAB interaction is concentration and time dependent at low surfactant concentrations. In the presence of magnesium ions (5 mM), the brief 5 min incubation at the low DTAB concentration of 0.1 mM caused the formation of networks, whereas the prolonged 24 h incubation resulted in the formation of scattered condensed particles. High DTAB concentration at \( \geq 2 \text{ mM} \) led to swollen globule conformation. Finally, globules and swollen globules can be created with DTAB alone.

The network structure consisting of rods and toroids is an interesting morphology characterized by AFM. The formation and disappearance of this network after prolonged incubation must be elucidated further. Considering the kinetics of complex formation and the roles of magnesium ions,\textsuperscript{24} a typical reaction route at low DTAB concentrations can be visualized. In the beginning, different DNA molecules interlaced with each other, and the magnesium ions played a role in maintaining these structures. From these metastable states, the DNA molecules started to interact with the DTAB molecules and began to compact to form similar networks consisting of compacted parts, including rods, toroids, and globules. However, the resultant compacted network was not thermodynamically stable because it exposed too much surface to the bulk solution. In order to reduce the surface energy of the structure, it became segregated into compacted particles (Figure 6A).

The swollen globule, another remarkable structure, is larger than the regular globule. According to the present experimental results, at least two factors influenced the size of the complex, namely, the amount of DNA molecules included in the complex and the internal structure of the complex. The more DNA molecules are included, the larger the size became. The internal structure of the complex depends on the properties of surfactant. The surfactants are amphiphilic molecules that have at least two parts, namely, the hydrophobic and hydrophilic parts. At a concentration higher than the critical micelle concentration (cmc), the surfactants can aggregate and form micelles driven by the hydrophobic force. The micelle formation is the most probable factor causing the swollen globular conformation at high DTAB concentration. The cmc of DTAB is 15 mM.\textsuperscript{25} However, the DNA molecules can induce surfactant aggregation in the vicinity of the DNA. Consequently, micelle formation can occur far below the cmc of the surfactant.\textsuperscript{5} Ghirlando et al. have shown that the surfactants can bind onto the DNA in micellar form.\textsuperscript{15} Mel'nikov et al. have also found an increase in the size of the DNA globule corresponding to a swollen globule at high CTAB concentrations, indicating the penetration of CTAB molecules inside the DNA globule.\textsuperscript{9} In the present experiments, swollen globules appeared when the DTAB concentration was \( \geq 8 \text{ mM} \) (DTAB alone) or \( \geq 2 \text{ mM} \) (DTAB with 5 mM Mg\textsuperscript{2+}). Accordingly, it is reasonable to assume that micelle formation contributed to the formation of the swollen structure.

Thus, a model is proposed to illustrate the formation mechanism of the swollen globules. Micelle formation is difficult when the surfactant concentration is far below the cmc. In the present case, surfactant molecules were bound to the DNA backbone through electrostatic and hydrophobic interactions in a regular manner, forming globules or toroids (Figure 6B). At high surfactant concentrations close to cmc, the DNA molecules served as scaffolds to help the surfactants form micelles (Figure 6C). Each micelle had multivalent charges and served as a bridge to link different condensates, such as the magnesium ion. In addition, DTAB molecules that were bound to DNA...
Figure 5. (A) Hydrodynamic diameter of the complex measured by DLS vs the concentration of the surfactant. The DTAB concentrations are 0.1, 2, and 4 mM containing 5 mM Mg$^{2+}$, and the incubation time is 24 h. (B–D) Corresponding measurements through AFM offline analysis, which resulted in typical sizes of 80 nm for 0.1 mM DTAB, 210 nm for 2 mM DTAB, and 181 nm for 4 mM DTAB. Note that the images used for measurements are the heights images illustrated in Figure 2, C, I, and L.
molecules in a unit of micelle tend to occupy more space and exhibit a swollen conformation. As a result, DTAB molecules not only induce compact swollen DNA—DTAB complexes but also serve as effective bridges to link different complexes. Nevertheless, the proposed model is tentative and needs further experimental tests and theoretical investigation.

■ CONCLUSION

In summary, the compaction of DNA induced by DTAB has been studied using AFM scanning method and magnetic tweezers. At low DTAB concentrations aided by magnesium ions, the complexes showed rod- and network-like structures after undergoing incubation for around 5 min. With increasing incubation time, more toroids and globules appeared, eventually leading to the formation of scattered condensed particles. At high DTAB concentrations, the complexes exhibited swollen globular structures independent of incubation time. In comparison, without the aid of magnesium ions, more surfactants are needed to induce DNA condensation.

The formation and unraveling processes of DNA—DTAB complex have been analyzed using magnetic tweezers. Both processes displayed stepwise jumps, indicating that the complexes are well organized and more compacted than those induced by multivalent ions. The stability of the complex is also related with the surfactant concentrations. Micelle formation is suggested to produce swollen globules.

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■ ACKNOWLEDGMENT

The authors thank Xinyu Yang and Zhaolun Wang for their technical assistance. This work was supported by National Natural Science Foundation of China (Grant Nos. 20934004 and 10974146), and Zhejiang Provincial Natural Science Foundation of China (Grant No. Y4110357).

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