Structure of the Complex Monolayer of Gemini Surfactant and DNA at the Air/Water Interface

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Supporting Information

ABSTRACT: The properties of the complex monolayers composed of cationic Gemini surfactants, [C18H37(CH3)2N+-(CH2)2N(CH3)2C18H37]2Br− (18-ω-18 with s = 3, 4, 6, 8, 10 and 12), and ds-DNA or ss-DNA at the air/water interface were studied by the surface pressure–area per molecule (π-A) isotherm measurement and the infrared reflection absorption spectroscopy (IRRAS). The corresponding Langmuir-Blodgett (LB) films were also investigated by the atomic force microscopy (AFM), the Fourier transform infrared spectroscopy (FT-IR), and the circular dichroism spectroscopy (CD). The π-A isotherms and AFM images reveal that the spacer of Gemini surfactant has a significant effect on the surface properties of the complex monolayers. As s ≤ 6, the Gemini/ds-DNA complex monolayers can both laterally and normally aggregate to form fibril structures with heights of 2.0–7.0 nm and widths of from several tens to ~300 nm. As s > 6, they can laterally condense to form the platform structure with about 1.4 nm height. Nevertheless, FT-IR, IRRAS, and CD spectra, as well as AFM images, suggest that DNA retains its double-stranded character when complexed. This is very important and meaningful for gene therapy because it is crucial to maintain the extracellular genes undamaged to obtain a high transfection efficiency. In addition, when s ≤ 6, the Gemini/ds-DNA complex monolayers can experience a transition of DNA molecule from the double-stranded helical structure to a typical ψ-phase with a supramolecular chiral order.

1. INTRODUCTION

Over the past decades, studies on the interaction of DNA with lipids are motivated predominantly by gene therapy, which relies on the DNA carriers (viral and nonviral vectors).1−4 Up to now, viral vectors are the most efficient, but they have several potential disadvantages, such as unpredictable immune response which concerns the safety.2,3 As a result, there has been tremendous activity in developing synthetic nonviral vectors. It is well-known that cationic compounds, especially some cationic lipids or surfactants, can support gene transfection, since the strong binding of these compounds with DNA can compact DNA, protect them against degradation and deliver them to the cell membrane with efficiency and specificity, and finally facilitate the DNA transport through the cell membrane.4 Both in vitro and in vivo it is found that positively charged lipid–DNA complexes (lipoplexes) have a higher efficiency for transfection compared with neutral ones.

As a new class of amphiphilic molecules containing two head groups and two aliphatic chains connected by a rigid or flexible spacer, Gemini surfactants possess superior properties in the field of gene therapy, which has become a major focus of research, in part because of the extraordinary solution properties imparted by the molecular structure of the compounds, such as much lower critical micelle concentrations and levels of toxicity compared to monomeric surfactants.5,6 Although Gemini surfactants, either alone or in a mixture with helper lipids, have shown transfection efficiency comparable with or only slightly lower than that of commercially available transfection reagents, the interaction mechanism of cationic Gemini surfactants with DNA remains undefined, which results in hampering the further exploitations of these promising molecules. Progress in this direction can be expected by studying a Langmuir monolayer coupled with the Langmuir–Blodgett (LB) technique for the insoluble cationic Gemini surfactants with DNA dissolved in the subphase. On one hand, the Langmuir monolayer is widely used as a model interface for the study of interactions between amphiphilic compounds and film structure through film compression or change in subphase composition, pH, temperature, and so forth. On the other hand, the LB technique enables the monolayer to be transferred on to a substrate to carry out further studies such as atomic force microscopy (AFM).7

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So far, many studies have focused on the interaction of DNA with surfactants at the air/water interface.\(^8\)\(^–\)\(^16\) Such a system generally involves the electrostatic interaction of nucleic acid molecules with a closely packed monolayer of cationic surfactant. Several modern techniques such as fluorescence microscopy,\(^14\) Brewster angle microscopy,\(^17\)\(^,\)\(^18\) AFM,\(^9\) in situ grazing incidence X-ray diffraction,\(^19\)\(^,\)\(^20\) FT-IR,\(^10\) quartz-crystal microgravimetry,\(^10\) polarization modulation infrared reflection absorption spectroscopy,\(^20\)\(^,\)\(^21\) cryoelectron microscopy,\(^22\)\(^,\)\(^23\) neutron reflectometry,\(^24\)\(^,\)\(^25\) and vibrational sum frequency spectroscopy\(^26\) have been applied to obtain the detailed information on the organization of these monolayer systems. However, these results differ from each other notably. For instance, Sommerdijk et al. suggested that the formation of a self-assembled surfactant monolayer in the presence of DNA leads to a disordered complex rather than an organized monolayer, in which individual DNA strands do not bind completely to the monolayer surface in an orderly fashion but are partially suspended from the surface extending into the subphase.\(^27\) Symietz et al.\(^27\) put forward that DNA could align during compressing the complex monolayer, while Okahata et al.’s experiment\(^28\) shows that the ordering of DNA under a surfactant monolayer is formed in the presence of an intercalating dye. On the other hand, Shimomura et al.\(^14\) illustrated that DNA molecules bound to an amphiphile monolayer are in random coil state at the air/water interface before transferring onto the substrate, while DNA molecules are stretched during the lifting process. Moreover, Kago et al.\(^29\) demonstrated that the complex presented at the air/water interface changes its structure upon deposition to a solid substrate. Additionally, Ganesh et al. have studied the interfacial behavior of octadecylamine (ODA) and complementary single-stranded oligonucleotides (ss-DNA, \(<20\) bases) and discovered that the ss-DNA molecules could transform and form the double-stranded oligonucleotides (ds-DNA).\(^10\)\(^,\)\(^16\)\(^,\)\(^30\)\(^,\)\(^31\) On the contrary, other important results in the literature\(^32\)\(^–\)\(^34\) are connected to the denaturation of ds-DNA (\(<50\) base pairs) in ODA/ds-DNA complex monolayers. These indicate that different types of nucleic acids and/or different surfactants utilized would lead to different results.

At present, there are only a few of studies dealing with the interactions of DNA and novel surfactants such as gemini surfactant, double-tailed surfactant and bola surfactant, etc.\(^35\)\(^–\)\(^40\) Liu et al.\(^40\) observed that the width of the fibrils formed by DNA condensation increases with the disymmetric extent of surfactants in the tail chains owing to the increasing hydrophobic interaction. For gemini surfactants, it is shown that the spacer length plays an important role in determining the properties of monolayers at the air/water interface formed by DNA/gemini surfactant complexes,\(^35\) and the counterion has a marked influence on both micellization and aggregation of gemini surfactant in the presence of DNA.\(^37\) Furthermore, it was found that efficient compaction of DNA occurred with gemini surfactants with either short or long spacers, while surfactants having intermediate length spacers (\(s = 5–10\)) are less efficient.\(^38\) At the same time, the transfection efficiencies are shown to be the largest for spacer groups with \(s \leq 4\) or \(s > 12\).\(^41\) However, many details on the organization of gemini surfactant with DNA, together with the structure of the DNA itself, at the air/water interface are still elusive. As a consequence, it is necessary to elucidate the interactions and structure–function relationships of DNA with amphiphile monolayers, in particular formed by cationic gemini surfactants.

In this paper, we focused on the structure of the DNA in the gemini/DNA complex monolayers. For the sake of comparison, the effect of ds-DNA and ss-DNA on the properties of the complex monolayers was also investigated. The properties of cationic gemini surfactant/DNA complex monolayer at the air/water interface were in situ investigated by analyzing the \(\pi-A\) isotherm measurements and IRRAS. The corresponding LB films were characterized by FT-IR, AFM, and CD. To our knowledge, there were only a few of investigations devoted in the conformation change of DNA in the complex monolayer by means of CD.\(^42\) In order to clearly examine the structure of DNA, the effect of the humidity on the LB film was also studied. In addition, we investigated the influence of the spacer of geminis on the interfacial behavior within the complex monolayers.

2. EXPERIMENTAL SECTION

2.1. Materials. The cationic gemini surfactants \(((\text{C}_{18}\text{H}_{37})(\text{CH}_{2})_{3}\text{N}^+-(\text{CH}_{2})_{4}\text{N}^+-(\text{CH}_{2})_{3}\text{C}_{18}\text{H}_{37})2\text{Br}^-, \) abbreviated as 18-s, \(s = 3, 4, 6, 8, 10\) and 12) were synthesized in our laboratory by using the method described by Zana et al.\(^43\) and characterized by elemental analysis and nuclear magnetic resonance. Sodium salt of salmon sperm DNA was purchased from Sigma and used as supplied. DNA is double-stranded with \(~4500\) base pairs (bp) in length as declared by supplier and determined by gel electrophoresis method (EPS 100). DNA in salt solution (0.1 mM NaBr) under the experiment conditions is double-stranded with the conventional B-form conformation, here abbreviated as ds-DNA, while in pure water it is a single-stranded conformation, here abbreviated as ss-DNA, which can be viewed as a denatured DNA. This was confirmed by the UV–vis spectra and AFM images of the spin-coating films of the corresponding solutions on mica (more details are shown in the Supporting Information). Water was purified by a Milli-Q plus water purification system with the resistivity of 18.2 MΩ cm. Chloroform, analytical grade chemicals, was used as the solvent of the spreading solution.

2.2. Preparation of the LB Film. The surfactant concentration in the chloroform was \(5.0 \times 10^{-4}\) mol/L. The complex monolayers were obtained by dropping a certain amount of chloroform solution (40 or \(50 \mu\text{L}\)) on 0.1 mM NaBr solutions or the Milli-Q water containing 4 mg/L DNA with a microsyringe (Hamilton). In order to reach the adsorption equilibrium state of DNA, it was required to wait for 40 min, and then the compressing process was performed. The monolayer was compressed at a rate of 10 cm\(^2\)/min. The \(\pi-A\) isotherms were recorded during the compressing process. The LB monolayer was transferred on mica, CaF\(_2\), and quartz slide for AFM, FT-IR, and CD characterization, respectively, by a vertical method at a surface pressure of 20 mN/m. The more details of the controlling Langmuir trough are given in the Supporting Information. The deposition rate was 5 mm/min. The LB films for the AFM investigation were dried in a desiccator at room temperature.

2.3. Characterization. Ultraviolet–Visible Spectroscopy. UV–vis spectra of the 4 mg/L DNA aqueous solution (ss-DNA) or 0.1 mM NaBr solution (ds-DNA) were recorded on a Shimadzu UV-2450 spectrometer. All measurements were made with a quartz cuvette in the range of wavelengths 220–320 nm.

Atomic Force Microscopy. The AFM topographic images of the LB films of surfactant/(ds or ss)-DNA complex monolayers were obtained in constant repulsive force mode by AFM (AJ-III, Aijian Nanotechnology Inc., China) with a triangular microfabricated cantilever (Mikro Masch Co., Russia) with a length of 100 \(\mu\text{m}\), a Si pyramidal tip, and a spring constant of \(\sim 18\) N/m. A resonance frequency in the range of 100–190 kHz was used and resonance peaks in the frequency response of the cantilever typically at 330 kHz were chosen for the tapping mode oscillation. The measurements were carried out under ambient laboratory conditions.
Fourier Transform Infrared Spectroscopy. FT-IR spectra were recorded using an AVATAR360 infrared spectrometer. The 30-layer LB films of 18-s-18/(ds or ss)-DNA were used for the FT-IR measurement. A total of 500 scans were sufficient to achieve a high signal-to-noise ratio. The spectra with a resolution of 4 cm$^{-1}$ were obtained at room temperature. Prior to FT-IR measurement, calcium fluoride substrates with the 30-layer deposited LB films were kept in a desiccator with silica gel desiccant.

Infrared Reflection Absorption Spectroscopy. IRRAS spectra of the monolayers at the air/water interface were recorded on an Equinox 55 FTIR spectrometer (Bruker, Germany) connected to an XA-511 external reflection attachment with a shuttle trough system suitable for the air/water interface experiments. Sample (film-covered surface) and reference (film-free surface) troughs were fixed on a shuttle device driven by a computer-controlled stepper motor allowing for collection from the two troughs in an alternating fashion. A KRS-5 polarizer was used to generate a perpendicularly polarized IR beam. The incident IR beam was conducted out of the spectrometer through the KRS-5 polarizer and focused onto the air/water interface. The reflected IR beam went to a liquid-nitrogen-cooled mercury–cadmium telluride (MCT) detector. These experiments were carried out at 20.0 ± 0.1 °C. The film-forming molecules were spread from the chloroform solutions of desired volumes, and 20 min was allowed for solvent evaporation. The measurement system was then enclosed for humidity equilibrium and monolayer relaxation for 4 h prior to compression. The monolayers were compressed from the initial surface pressures of ~0 mN/m to the surface pressures of 20 mN/m, and then the spectra were recorded with a resolution of 8 cm$^{-1}$ by coaddition of 1024 scans. The angle of incidence was 35° with respect to the surface normal, and the IR beam was polarized in the plane of incidence (p) and perpendicular to this plane ($s$).

Circular Dichroism Spectroscopy. CD experiments were carried out with a Jasco spectropolarimeter J-810 (Jasco). The 40-layer LB films deposited by 18-s-18/ds-DNA complex monolayers for CD spectrum were transferred onto the quartz slide at a surface pressure of 20 mN/m by the vertical method. In order to investigate the effect of the humidity on the ds-DNA structures in the films, the measurements were carried out with two different relative humidity (rh) values of atmosphere (12% and 85% rh). For this purpose, the samples were placed into the sealed chamber, where the defined relative humidity value was preset by means of saturated aqueous solution of appropriate salt. At the same time, a quartz cuvette of path length 1.0 cm with a PTFE plug was put in this chamber. After 6 h, the quartz slide was set into the cuvette then rapidly sealed by the PTFE plug for measurements. The quartz slide was fixed by two slits at the bottom of the cuvette and at the inner side of the plug. CD spectra were recorded as the average of three scans from 210 to 380 nm at a scan rate of 50 nm/min. In addition, the 4 mg/L DNA in 0.1 mM NaBr solution was in a 5 cm path length quartz cuvette, and its CD experiment was also performed under the identical conditions.

3. RESULTS

3.1. π–A Isotherm of Gemini/DNA Complex Monolayer. The π–A isotherms of 18-s-18 ($s = 3, 4, 6, 8, 10,$ and 12) on 4 mg/L DNA aqueous (ss-DNA) and 0.1 mM NaBr (ds-DNA) solution at 20 °C are shown in Figure 1. The adsorption kinetics of DNA from the bulk subphase to the surfactant monolayer was also studied. These curves were obtained by first spreading surfactant on the subphase and waiting for the different periods, i.e., 10, 20, 40, and 60 min, respectively, and then compressing the monolayer. The variation of π–A isotherms with the adsorption time are not given here. The results show that when the induction period is beyond 40 min, the isotherms have no noticeable change. Thus, 40 min was chosen as the adsorption equilibration period in this work. These curves were measured more than three times to confirm their reproducibility.

As shown in Figure 1, surface pressure increases smoothly with monolayer compression, suggesting no well-defined phase change. The characteristic properties such as the lift-off area ($A_{\text{L}}$), the limiting area ($A_{\text{∞}}$), the collapse pressure ($\pi_{\text{c}}$), and the molecular area at the collapse of the monolayer ($A_{\pi}$) could be obtained from these isotherms. Here, the limiting areas of geminis on ss-DNA and ds-DNA solutions including geminis themselves are compiled in Table 1. As for geminis/ss-DNA or ds-DNA systems, the shape of the isotherm is similar to that of 18-s-18 on pure water surface in our previous study, while the collapse pressure and the molecular area are different. First, there is a kink, identifying the collapsed monolayers, mostly at the surface pressure 30.5 ± 2.5 mN/m for isotherms of the gemini/ss-DNA or ds-DNA complex monolayers except for 18-3-18/ss-DNA, of which is higher. Second, when $s < 6$, $A_{\pi}$ of gemini/DNA complex monolayers are higher than those of geminis on pure water surface, while as
s \geq 6$, the values of $A_{\infty}$ are lower than them. This fact indicates that for the different geminis the isotherms could be expanded or contracted on the subphase containing DNA depending on the length of the spacer compared with the situation in the absence of DNA similar to that mentioned in the literature for other insoluble cationic amphiphiles\textsuperscript{22} (data given in Supporting Information). What is more crucial is that although DNA displays surface activity, it is not shown to be adsorbed at a clean air/water interface alone.\textsuperscript{46} The variations of the collapse pressures and the molecular areas compared with geminis on the pure water surface in this work provide a clue that the gemini/ss-DNA or ds-DNA complex monolayers might be formed, which would be verified by the AFM, FT-IR, and CD results as follows.

### 3.2. AFM Observation

The AFM morphologies of surfactant/DNA complex monolayers could provide further information about the structure variations of these monolayers at the air/water interface. The LB films of gemini/ds-DNA or ss-DNA monolayers could be transferred onto the mica substrates by the vertical dipping method easily and conveniently. Figure 2 shows the surface morphologies of gemini/ds-DNA complex monolayers transferred onto mica substrates at the surface pressure of 20 mN/m imaged by using tapping mode.

![Figure 2](image)

**Figure 2.** AFM images (10 × 10 μm²) of LB films of gemini/ds-DNA complex monolayers at surface pressure of 20 mN/m. (A) 18-3-18; (B) 18-4-18; (C) 18-6-18; (D) 18-8-18; (E) 18-10-18; (F) 18-12-18. The inset image is the amplified image of the corresponding complex film, with size of 2 × 2 μm².

Three kinds of structures, i.e., the apparently parallel fibril, the relatively irregular fibril, and the main platform with a few fibrils, can be distinguished. These morphologies are attributed to the formation of the complex monolayer because pure surfactants and pure DNA molecules under the same experimental conditions do not form the similar structures. Furthermore, the fibril structures are observed in all images, and the width and the distance of the fibrils are dependent on the length of the spacer group. These fibrils of the gemini/ds-DNA complex monolayers are aligned in the same direction to some extent parallel to the lifting direction of the film. In 18-3-ss-DNA and 18-4-ss-DNA systems, fibrils are obviously parallel to each other as shown in Figure 2A,B. In Figure 2A, a large number of fibrils are observed with a height of 2.0−4.0 nm and a width of about 50 nm. In Figure 2B, however, the number of the fibrils decreases markedly and the branched fibrils arise appreciably, and the height and the width of the fibrils increase up to about 4.0−6.0 and 150 nm, respectively. In the 18-6-18/ds-DNA system shown in Figure 2C, the number of the fibril with visible branched fibrils further decreases, while the corresponding width rises up to about 270 nm and the height remains about 4.0−6.5 nm. At the same time, although they are prone to be parallel with each other, these fibrils connected by branched fibrils have a tendency to form the network structures. As $s \geq 8$, it is a special interesting feature that the main morphologies are of the platform structures as shown by the white arrow in Figure 2F, together with a few of fibrils as shown by the black arrow in Figure 2F, where the heights of the platform and the fibril are approximately 1.4 and 5.0−7.0 nm, respectively.

Native DNA molecules are double-stranded helix, of which the diameter is ca. 2.0 nm, while the height of the fibril or thread obtained at low surface pressure (data given in Supporting Information), as well as the thickness of the platform, are only ~1.4 nm in this work. In our previous work, except for the surface cluster, the thickness of the main structure in LB films of gemini surfactants is very close to 0.5 nm, which is consistent with the diameter of the alkyl chain.\textsuperscript{45} The smaller height of 1.4 nm than 2.0 nm might originate from two conditions: (i) the denaturation of ds-DNA molecules and (ii) the variation of the conformation of ds-DNA molecules in the complex monolayers. Therefore, in order to compare with gemini/ds-DNA, the gemini/ss-DNA complex monolayers were also carried out in this work, as shown in Figure 3.

![Figure 3](image)

**Figure 3.** AFM images (5 × 5 μm²) of LB films of gemini/ss-DNA complex monolayers at surface pressure of 20 mN/m: (a) 18-3-18; (b) 18-4-18; (c) 18-6-18; (d) 18-8-18; (e) 18-10-18; (f) 18-12-18.

As seen from Figure 3, treelike structures with the height of 1.0−1.8 nm appear and the width of them increases with the
length of the spacer of gemini. The special interesting is that the morphologies of gemini/ss-DNA complex monolayers are markedly different from those of gemini/ds-DNA. This fact leads to the suggestion that the denaturizing of ds-DNA molecules in those complex monolayers might not happen. This would be further examined by FT-IR, IRRAS and CD.

3.3. FT-IR of LB Films. FT-IR allows one to consider DNA component bands and to examine the influence of surfactant on each of these bands and identify the conformational changes in the light of distinctive IR “fingerprints”. The most informative FT-IR vibrations are the guanine/thymine carbonyl (C=O) stretch (1715 cm$^{-1}$), thymine aromatic amine stretch (1328 cm$^{-1}$), symmetric and asymmetric phosphate (PO$_2^-$) stretches (1088 and 1222 cm$^{-1}$, respectively), and a strongly coupled sugar–phosphodiester signal (970 cm$^{-1}$). Each of the different forms of DNA helix has its own distinctive IR “fingerprints”, which allows the secondary conformational changes to be identified. Thus, the 1715, 1328, 1222, 1088, and 970 cm$^{-1}$ bands are traditionally taken as the markers of the B-form of DNA. A favorable feature of the systems under this study is that 18-s-18 has no remarkable absorption in the region of 1250–950 cm$^{-1}$, and the carbonyl groups absorption is around 1700 cm$^{-1}$. This fact enables one to make a conclusion about DNA conformational state in LB film with a surfactant. The FT-IR spectral features for 30-layer LB films of gemini/ds-DNA or ss-DNA complex monolayers are presented in Figure 4. In this spectrum, the main bands of DNA can be assigned: vibration of C=O of the base pairs in the 1650–1700 cm$^{-1}$ region, the asymmetric vibrations of PO$_2^-$ at about 1226 cm$^{-1}$ (ds-DNA) and 1236 cm$^{-1}$ (ss-DNA), the symmetric vibrations of PO$_2^-$ at about 1096 cm$^{-1}$ (here, a shoulder can be seen), vibration of C=O of deoxyribose around 1062 cm$^{-1}$, and ribose–phosphate main chain vibration around 962 cm$^{-1}$.

Here, strong gemini–PO$_2^-$ interaction is evident from shifting of the PO$_2^-$ asymmetric band from the native origin of 1222 cm$^{-1}$ to a higher frequencies around 1236 cm$^{-1}$ for gemini/ss-DNA and 1226 cm$^{-1}$ for gemini/ds-DNA, respectively, and symmetric band from the original of 1088 cm$^{-1}$ to about 1096 cm$^{-1}$, in the spectra of the LB films. In addition, the bands at 2922 and 2852 cm$^{-1}$ are attributed to asymmetric and symmetric vibrations of $-\text{CH}_2$, respectively. The other mode of $-\text{CH}_2$ such as at $\sim$1460 and 1380 cm$^{-1}$, are not stressed here partly because they are weaker than asymmetric and symmetric vibrations.

3.4. IRRAS. To go further in the organization of ds-DNA and ss-DNA under gemini monolayers, IRRAS was performed, as given in Figure 5. The spectral region of 1800–700 cm$^{-1}$ in solution is rich with so-called DNA marker bands. These can be assigned as following: vibrational peak of C=O of the base pairs (ds-DNA) at 1724 cm$^{-1}$, that of bases (ss-DNA) at 1712 cm$^{-1}$, the asymmetric vibrations of PO$_2^-$ at 1227 cm$^{-1}$ for the gemini/ds-DNA and ss-DNA complex monolayers, the symmetric vibrations of PO$_2^-$ around 1087 cm$^{-1}$, vibration of C=O of deoxyribose in the range of 1054–1066 cm$^{-1}$ for ds-DNA or ss-DNA, and that ribose–phosphate main chain vibration around 970 cm$^{-1}$. In the literature, cationic ion–base interaction could also lead to the vibrational band of adenine shifting toward lower frequencies in the 1606–1609 cm$^{-1}$. Tajmir-Riahi et al. also pointed out that the bands at 1606 and 1590 cm$^{-1}$ are also due to the drug–DNA interaction via guanine and adenine N7 atoms and thymine O2 in the major and minor grooves of DNA duplex, which is not normally involved in Watson–Crick hydrogen-bonding network and does not bring about helix destabilization. Therefore, the adsorption peaks at 1606 and 1590 cm$^{-1}$ are tentatively ascribed to the interaction of the quaternary ammonium headgroups with the dipoles of bases (adenine N7 atoms or thymine O2). As a result, these spectral changes are indicative of the presence of ion–dipole interaction in the gemini/ds-DNA or ss-DNA complex monolayers. In addition, the bands at 2924 and 2854 cm$^{-1}$ are also attributed to the asymmetric and symmetric vibrations of $-\text{CH}_2$, respectively.

3.5. CD Measurements. Small amounts of molecules that bind strongly to DNA can cause a change from one conformation to another. CD spectroscopy is one of the most useful techniques for probing the conformation of DNA in aqueous solution as well as in gels, films, and fibers. The “native” B-form helix of DNA has a distinctive spectrum, which consists of a positive band at $\sim$275 nm, a negative band of equal intensity at $\sim$245 nm, and a crossover point near 258 nm. Changes in the shapes and intensities of these bands reflect rearrangements of the helical structure. In order to determine whether the binding of surfactant influences the helical structure, the CD spectrum of DNA between 210 and 380 nm was measured for the DNA solution and LB films of gemini/ds-DNA complex monolayers shown in Figure 6.
Taniguchi et al. suggested that water has a significant influence on the structure and properties of DNA; therefore, the effect of the relative humidity on the DNA structure was also investigated. For the sake of clear comparison, the CD spectrum of “native” B-form helix of DNA in solution was given in three cases. The corresponding bands are listed in Table 2.

On one hand, at high relative humidity, the peak positions shift up 10−20 and 45−60 nm for the ∼245 and ∼275 nm bands, respectively; i.e., the negative band moves from ∼245 nm to the region of 255−267 nm as well as the positive bands from ∼275 nm to the region of 320−332 nm. Simultaneously, for the spectra of 18-3-18/ds-DNA and 18-6-18/ds-DNA LB films, the magnitudes of the negative and positive bands are different: the negative CD band slightly increases (i.e., becomes more negative) while the magnitude of the positive band decreases. On the contrary, as for 18-8-18/ds-DNA LB films, the CD spectrum consists of a positive band around 332 nm, a negative signal at 258 nm, with approximately the same intensity. At low relative humidity, on the other hand, for the spectra of 18-3-18/ds-DNA and 18-6-18/ds-DNA LB films the intensity of the negative bands further increase, whereas the positive band almost disappears. On the contrary, irrespective of the high or low relative humidity, the shape, location, and magnitude of CD spectra of 18-8-18/ds-DNA remain almost the same. The CD spectra of condensed DNA are characterized by an enhanced negative ellipticity, an overall shift of the bands toward higher wavelengths, a flattening of the positive band, and the appearance of long “tails” above 300 nm. This is in good consistency with that observed by Bombelli’s group, indicating that DNA is in a ψ-phase, which is the supramolecular chiral order of a cholesteric-like phase. In such a ψ-phase, DNA molecules are thought to be tightly packed together to form highly condensed structures that exhibit negative CD signals.

### Table 2. CD Bands of 18-s-18/ds-DNA Complex LB Films at Relative Humidity 85% and 12%

<table>
<thead>
<tr>
<th>bands</th>
<th>18-3-18</th>
<th>18-6-18</th>
<th>18-8-18</th>
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<tr>
<td>+ (nm)</td>
<td>275</td>
<td>319</td>
<td>na</td>
</tr>
<tr>
<td>− (nm)</td>
<td>245</td>
<td>267</td>
<td>256</td>
</tr>
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</table>

“na means almost no absorption.

On one hand, at high relative humidity, the peak positions shift up 10−20 and 45−60 nm for the ∼245 and ∼275 nm bands, respectively; i.e., the negative band moves from ∼245 nm to the region of 255−267 nm as well as the positive bands from ∼275 nm to the region of 320−332 nm. Simultaneously, for the spectra of 18-3-18/ds-DNA and 18-6-18/ds-DNA LB films, the magnitudes of the negative and positive bands are different: the negative CD band slightly increases (i.e., becomes more negative) while the magnitude of the positive band decreases. On the contrary, as for 18-8-18/ds-DNA LB films, the CD spectrum consists of a positive band around 332 nm, a negative signal at 258 nm, with approximately the same intensity. At low relative humidity, on the other hand, for the spectra of 18-3-18/ds-DNA and 18-6-18/ds-DNA LB films the intensity of the negative bands further increase, whereas the positive band almost disappears. On the contrary, irrespective of the high or low relative humidity, the shape, location, and magnitude of CD spectra of 18-8-18/ds-DNA remain almost the same. The CD spectra of condensed DNA are characterized by an enhanced negative ellipticity, an overall shift of the bands toward higher wavelengths, a flattening of the positive band, and the appearance of long “tails” above 300 nm. This is in good consistency with that observed by Bombelli’s group, indicating that DNA is in a ψ-phase, which is the supramolecular chiral order of a cholesteric-like phase. In such a ψ-phase, DNA molecules are thought to be tightly packed together to form highly condensed structures that exhibit negative CD signals.

### 4. DISCUSSION

The π−A isotherms of the symmetric and asymmetric cationic gemini surfactant monolayer are known to change significantly when it is compressed on the subphase in the presence of DNA. Compared with the geminis on pure water surface alone, the variations of A∞ and collapsed surface pressures indicate that geminis and DNA can form complex monolayers at the air/water interface. Furthermore, the conformation of DNA (ds-DNA or ss-DNA) can also remarkably influence the interfacial behavior of the complex monolayers.

Wu et al. have reported that the thickness of a LB film of the lipid/DNA monolayer is about 0.9 nm, thicker than that of the pure lipid monolayer from neutron and X-ray reflectivity techniques. Kago et al. have confirmed that the structure and conformation of DNA molecules in the lipid/DNA LB film are largely different from its conformation at the air/water interface by means of a direct in situ X-ray reflectivity technique. They suggest that the thickness of the lipid/DNA complex monolayer at the air/water interface is ca. 2.5−2.8 nm, which is comparable to the diameter of a DNA molecule. However, when this monolayer is deposited on the solid substrate, this thickness decreases to about 1.1 nm, which is much smaller than the diameter of the DNA molecule. Erokhina et al. have
investigated the interaction of DNA with ODA monolayers at the air/water interfaces and suggested that the protonation of the ODA head groups leads to the local modification of the water properties in the very thin surface within the Debye length close to the ODA head groups and forms an elevated pH surface layer environment.\textsuperscript{52} The latter would result in DNA becoming partially denatured due to the basic pH and then ss-DNA attach electrostatically to positive headgroups of ODA. On the contrary, they have also studied the formation of a complex of DNA with dipalmitoylphosphatidylcholine (DPPC) monolayer at the air/water interface in the presence of Ca\textsuperscript{2+} ions and discovered that the presence of Ca\textsuperscript{2+} is essential for the formation of the complex and DNA attached to the DPPC monolayer maintains its native double-helix form when DNA is dispersed onto the water surface. The surfactant molecules are dissociated carrying a positive charge when they are dispersed onto the water surface. The concentration of the original counterion Br\textsuperscript{−} becomes negligible immediately after spreading. At the same time, this concentration at the air/water interface becomes the strongly hydrated OH\textsuperscript{−} ion. As a result of the headgroup charge in the monolayer, counterions are attracted to the interface; the interfacial ion concentration is then significantly higher than that in the bulk. This situation is similar to that of ODA. Therefore, from the above information, we thus could not simply rule out the possibility of the denaturation of ds-DNA in this study.

For the LB films of gemini/ds-DNA systems, the bands around 1690 cm\textsuperscript{−1} appear in the FT-IR spectrum and when \( s \geq 8 \), the thickness of the platform including DNA and gemini is only about 1.4 nm, which is smaller than the size of 2.0 nm. Both of them seem to further support the denaturation of ds-DNA. However, for the IRRAS spectra of ds-DNA, on one hand, the absorption band at \( \sim 1724 \) cm\textsuperscript{−1}, attributed to C=O stretching vibrations of stacked guanine/thymidine base pairs, indicates that DNA molecules are regular double helices. If the double helix is destroyed, a new band at 1692 cm\textsuperscript{−1} due to carboxylic group vibrations of unstacked bases usually appears.\textsuperscript{56} On the other hand, the morphological comparison between the gemini/ds-DNA and gemini/ss-DNA complex monolayers indicates that the double-helical structure of ds-DNA is not destroyed, which could be verified by the thorough assay of FT-IR, IRRAS, and CD results.

So far, several models have been developed to describe the complex properties of the cationic lipid/DNA complex.\textsuperscript{57–59} The electrostatic interaction between lipid headgroup and DNA backbone phosphate, and the base binding between lipid polar group and the bases donor atoms, as well as the cooperatively hydrophobic interaction between aliphatic tails are regarded as the major force. Vibrational spectroscopy can provide, as expected, the information on the electrostatic, ion-dipole, and hydrophobic interaction as well as a complementary technique with which to examine the form of the DNA helix. Although a new band around 1690 cm\textsuperscript{−1} appears in the FT-IR spectrum, in the IRRAS spectrum the peaks of C=O stretching vibrations appear near 1724 and 1712 cm\textsuperscript{−1} for ds-DNA and ss-DNA, respectively. Thus, it is rational to consider that the shift of the peaks of C=O in the range of 1712–1724 cm\textsuperscript{−1} to the bands in the vicinity of 1690 cm\textsuperscript{−1} could be ascribed to the change of the environment from solutions to LB films, which is similar to CD results during the desiccation of LB films. Furthermore, compared with the result of IRRAS, a new peak around 1650 cm\textsuperscript{−1} appears in the FT-IR spectrum. The band centered at 1690 cm\textsuperscript{−1} arises due to the C6=O6 stretch of base paired guanine plus C2=O2 stretch of uracil and thymine. The band centered at 1660 cm\textsuperscript{−1} arises mainly from the stretching vibrations of C6=O6 of free guanine, C2=O2 of free cytosine, and C4=O4 of free uracil or thymine. A double strand to single strand transition results in a decrease in the intensity of the band at \( \sim 1690 \) cm\textsuperscript{−1} with a concomitant increase of the band at \( \sim 1660 \) cm\textsuperscript{−1}.\textsuperscript{60–62} Here, the gemini/ds-DNA complex exhibits a stronger absorption at 1690 cm\textsuperscript{−1} relative to the band at 1650 cm\textsuperscript{−1}, while the gemini/ss-DNA complex experiences a stronger absorption at 1650 cm\textsuperscript{−1} relative to the band at 1690 cm\textsuperscript{−1} (for the sake of clarity, an enlarged image was given in the Supporting Information). As a consequence, these results imply that DNA molecules maintain the double-stranded state in the gemini/ds-DNA complex monolayers, whereas DNA is in single-stranded motif within the gemini/ss-DNA complex monolayers.

Further evidence regarding the double-stranded state in the gemini/ds-DNA complex monolayers also comes from asymmetric stretching vibration of the nonbridging PO\textsuperscript{2−} groups, which is a strong marker for the different backbone conformers of DNA. Usually, in B-DNA, a strong absorption is seen around 1225 cm\textsuperscript{−1}. In contrast, this band shifts to 1245–1240 cm\textsuperscript{−1} upon transition into the A-type of conformation. All the sequences studied here show a strong absorption in the vicinity of 1225 cm\textsuperscript{−1} irrespective of FT-IR or IRRAS results, reflecting the B-form character of the backbone in the gemini/ds-DNA complex monolayers.

The other evidence come from the parameters of narrow intensive 1088 cm\textsuperscript{−1} band and 1053 cm\textsuperscript{−1} band, which may also be the criteria of double-stranded DNA in 1250–900 cm\textsuperscript{−1} range. The former of the band is assigned to the symmetric vibrations of the PO\textsuperscript{2−} groups. The last one is associated with complex vibration motions of O5−C4−C5−O4 atoms of sugar–phosphate backbone. When DNA is denatured, both of these bands are widened, decreased in intensity, especially the 1088 cm\textsuperscript{−1} band, and their frequencies are shifted. The greatest change is observed for the 1053 cm\textsuperscript{−1} band which is shifted to 1069 cm\textsuperscript{−1}.\textsuperscript{56,63} In the IRRAS, the bands at 1088 cm\textsuperscript{−1} almost show no shift for all complex monolayers. Moreover, as for the gemini/ds-DNA systems, the bands at 1053 cm\textsuperscript{−1} show no apparent shift, though those of gemini/ss-DNA complex monolayers are shifted toward a higher frequencies near 1063 cm\textsuperscript{−1}.

The CD results indicate that: (i) The spacer of gemini has a great effect on the properties of the complex monolayer; namely, when \( s \leq 6 \), the tertiary structure of DNA is changed in the LB films, whereas when \( s > 6 \), the transition of the secondary conformation of ds-DNA in LB films occurs, but DNA molecules still retained the chiral or double helical structure, similar to the native DNA structure in aqueous solution. (ii) The water content can significantly influence the structure of ds-DNA molecules when \( s \leq 6 \). The former case is based on two observations: first, as \( s \leq 6 \) gemini binding causes the long wavelength peak decreases in height and the negative band slightly increases at the high rh, while the former disappears and the latter further becomes more negative at the low rh; second, as \( s > 6 \), irrespective of the high or low rh, the negative and positive bands remain their intensity. As \( s \leq 6 \), on one hand, CD studies suggest cationic geminis induce the change of the DNA structure from the secondary (the double-stranded helix) to tertiary (a \( \psi \)-phase, i.e., a supramolecular
chiral order of a cholesteric-like phase) conformation, in the complex monolayers. Such structure is usually thought to arise from long-range interactions primarily within DNA molecules but not necessarily in a condensed state. Combined with the fibrils with the height of ~2.0 to ~7.0 nm, CD spectra indicate that DNA within the gemini/ds-DNA complex monolayers is a \( \chi_2 \)-phase, which is made up of these fibrils. On the other hand, although the magnitudes of the negative and positive bands remain unchanged as \( s > 6 \), both of them shift toward the long wavelength, similar to the case of \( s \leq 6 \). This indicates the base stacking mode undergoes a significant change, which deserves further investigation. Anyhow, the helical structure still conserves within the gemini/ds-DNA complex monolayers, so that it is reasonable to conclude that the helical structure of ds-DNA is not appreciably disturbed by the binding of gemini.

In addition, the infrared spectral features of the methylene stretching vibration are very informative, since their peak positions are strong indicators of the chain conformation. The symmetric and asymmetric stretching vibrations of the methylene groups usually present in the narrow ranges 2846–2850 and 2915–2918 cm\(^{-1}\), respectively, for all-trans conformations of the fully extended tail chains and in the distinctly different ranges 2854–2856 and 2924–2928 cm\(^{-1}\) for disordered chains characterized by a significant presence of gauche conformations. Porter et al.\(^{64}\) and Byrd et al.\(^{65}\) have reported that when the alkyl chains are highly ordered (trans-zigzag conformation), the \(-\text{CH}_2\)- bands appear near 2918 and 2850 cm\(^{-1}\), respectively, while if conformational disorder is included in the chain, they shift up to near 2927 and 2856 cm\(^{-1}\) depending on the content of gauche conformations. Tang et al.\(^{66}\) and Cea et al.\(^{67}\) reported that the hydrocarbon tails in the LB films located at 2918 and 2850 cm\(^{-1}\) indicate well-ordered alkyl chains with a trans-zigzag conformation. Thus, the peak frequencies 2924 and 2854 cm\(^{-1}\) in IRRAS indicate that alkyl chains of 18-s-18/ds-DNA or ss-DNA complex monolayers at the air/water interface are in disordered conformations distinguished by a significant presence of gauche chains. However, the peak frequencies 2920 or 2922 and 2850 or 2852 cm\(^{-1}\) in FT-IR spectra imply that alkyl chains in the LB films undergo a conformation change from the gauche to trans-zigzag conformational state. Especially, in the gemini/ds-DNA systems, alkyl chains of gemini inclined to exhibit a great deal of the trans-zigzag conformation. In addition, the FT-IR transmission spectrum of 30-layer gemini/ds-DNA or gemini/ss-DNA complex LB films could also give the information on the orientational changes in aliphatic chain of the gemini molecules. The ratio of absorbance of the CH\(_2\) asymmetric and symmetric stretching bands in the transmission of the 30-layer gemini/ss-DNA LB films is calculated to be about 2.2 (Figure 4), while that of gemini/ds-DNA LB films is only around 1.9. The value of the former is larger than that of the latter, which indicates that the alkyl chain in the gemini/ss-DNA complex films tends to become more perpendicular to the substrate surface than that in the gemini/ds-DNA films. This might be caused by the hydrophobic interaction of tail chains with the relative hydrophobic region of the major and minor grooves after the deposition for the gemini/ds-DNA complex monolayers. For the gemini/ss-DNA systems, on the contrary, the bases of ss-DNA have a tendency to escape out of water and stretch toward the air since they are comparatively hydrophobic, which can constrain the motion of tail chains and does not favor the parallel configuration with respect to the substrate surface.

Overall, DNA molecules take on a sparsely expanded random coil form in good solvents or at high temperatures because of the strong electrostatic repulsion between the negatively charged phosphate groups, whereas they can be in a spherical, toroidal, rodlike, and highly folded solid-like form in poor solvents or at low temperatures because of the suppressed electrostatic repulsion. When gemini is spread on the ds-DNA or ss-DNA solution subphase, gemini/ds-DNA or gemini/ss-DNA complexes are formed due to the electrostatic interaction. At the same time, the tail chains of gemini almost exhibit the gauche conformation. The attraction between quaternary ammonium ions and phosphate groups reduces the surface charge of ds-DNA, so that ds-DNA molecules can be adopted in a tight pack manner by van der Waals interaction after the deposition of the complex monolayer. As a consequence, gemini surfactants can condense ds-DNA in the complex monolayer, resulting in the decreasing size of ds-DNA, so that the height of the platform and the structures at the lower surface pressure is only ~1.4 nm. At this moment, although ds-DNA molecules undergo the structure transition, they retain the double-stranded hexical conformation. In particular, as \( s \leq 6 \) the complexes with 1.4 nm height can laterally and normally aggregate to form the fibril with 2.0–7.0 nm height and several tens to ~300 nm width with the increasing amount of DNA at higher surface pressures, which is a typical \( \chi_2 \)-phase, while when
s > 6, they can laterally aggregate and mainly form the platform. The mechanism can be described in Figure 7.

5. CONCLUSIONS

The formation of the gemini surfactant/ds-DNA or ss-DNA complex monolayers at the air/water interface has been investigated. AFM images indicate that the morphologies of the LB films vary with the increasing length of the gemini spacer, and the structures of gemini/ds-DNA complex monolayers remarkably differ from those of gemini/ss-DNA systems. It is reasonable to conclude that DNA molecules in gemini/ds-DNA complexes primarily maintain the double-stranded helical structures, which is further supported by the combination of FT-IR, IRRAS, and CD spectra. However, the shifting negative and positive peaks and the various intensities in the CD spectra reveal that the ds-DNA undergoes a conformational transition, which deserves further investigation. Anyhow, the double-stranded structure of DNA within the gemini/ds-DNA complexes is not destroyed.

In summary, we have observed that the nature of the spacer in gemini surfactants plays an important role in determining the surface properties of gemini/ds-DNA or ss-DNA complex monolayers in this study. As s ≤ 6, the gemini/ds-DNA complex monolayers can both laterally and normally aggregate to form the fibril structures with heights of 2.0–7.0 nm and widths of several tens to 300 nm. Here, ds-DNA molecules exhibit a tertiary conformation, which is the supramolecular chiral order of a cholesteric-like phase (a ψ-phase). On the contrary, when s > 6, they can only experience lateral condensation to produce the platform structure with only 1.4 nm heights, made up of ds-DNA and geminis themselves, but ds-DNA molecules still conserve the double-stranded helical structure. At the same time, after the deposition of the gemini/ds-DNA complex monolayers, a certain amount of tail chains experiences the changes from the gauche to trans-zigzag conformations. In addition, the alkyl chains in the gemini/ss-DNA complex films incline to be comparatively more perpendicular to the substrate surface, while they in the gemini/ds-DNA films are favor of the parallel configuration with respect to the substrate surface.

The present work indicates that an effective complexation between DNA and liposomes will lead to the compaction of the nucleic acid. This will protect the genetic exogenous material from nuclease degradation and will promote the penetration of the nucleic acid into the target cell because of the reduced size. This will help in the delivery of therapeutic genes undamaged. Therefore, the results of the present work support that cationic geminis could be useful as potential vectors for transfer of genetic material into mammalian cells by nonviral gene therapy.

**ASSOCIATED CONTENT**

* Supporting Information

Examining DNA conformation in solution with UV−vis spectra and AFM characterization of the spin-coating films on mica; figures showing π−A isotherms of 18-s/18-ds-DNA complex monolayers compared with those of geminis alone; some details of measuring π−A isotherms and preparing LB films; the effect of surface pressures of the morphologies of 18-s/18-ds-DNA complex monolayer; FT-IR spectrum of 18-s/18-ds-DNA and ss-DNA LB films. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

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