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Article

The Interaction of a Gemini Surfactant with a DNA Quadruplex

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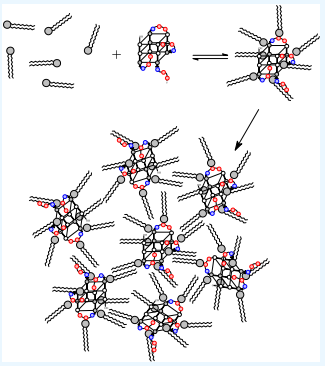
ABSTRACT: DNA secondary structures are stabilized by mono- and divalent cations. To examine the stability of the DNA quadruplex formed from $(TTAGGG)_4$, its interaction with a dicationic Gemini surfactant in standard phosphate buffer was investigated. The Gemini surfactant begins to form micelles in buffer at a *cmc* (critical micelle concentration) of 1.5 mM. In this study, solutions of DNA were prepared in buffer with surfactant concentrations ranging from 0.0 to 3.0 mM, i.e., above and below the *cmc* of the surfactant. In all samples of DNA and surfactant, a precipitate formed. The fraction of DNA precipitated depends upon both the initial DNA concentration and the initial concentration of the surfactant. In those samples where the DNA did not totally precipitate, the residual DNA assumed a quadruplex conformation. It was determined that two surfactant molecules per DNA phosphate are needed to completely precipitate all of the DNA in a particular sample. An estimated apparent K_{sp} for the DNA:surfactant complex was determined.



INTRODUCTION

Gemini surfactants are getting increased attention because of their unique physical properties, most notably their low *cmcs* (critical micelle concentrations), good wettabilities, and surface tension-lowering properties. Potential applications range from oil spill remediation agents,[1] anticorrosives,[2,3] and antimicrobial and antialgal agents[3−7] to gene therapy agents[8,9] for example.

We have been investigating the physical properties of the dicationic Gemini surfactant (**I**), shown in Figure 1, using conductivity, dynamic light scattering (DLS), and isothermal titration calorimetry (ITC) studies.[10] At low concentrations, this surfactant exists as a separate, unassociated entity. However, increasing the concentration of the surfactant results in self-assembly to micelles. We have determined that the critical micelle concentration (*cmc*—the concentration of surfactant when micelles begin to form) is 1.03 mM in water. In addition, DLS studies suggest that the micelles have diameters of 0.86 nm. Thus, this surfactant forms small micelles with high positive charge densities due to the close proximities of the quaternized ammonium groups to each other in the assembled state.

Our lab has also been interested in the biophysical properties of unusual DNA conformations such as DNA quadruplexes.[11−13] For these studies, DNA oligonucleotides related to the human telomere sequence $(TTAGGG)_n$ were synthesized and evaluated for conformation and stability. All sequence variations studied, $(XXXGGG)_4$ where X = A or T, form quadruplexes at 25 °C in standard phosphate buffer which unfold to single-stranded conformations with apparent melting temperatures (T_m) ranging from 44 to 75 °C, depending upon loop sequence.[12,13] The T_m is the temperature at the midpoint of the unfolding transition where the concentration of folded DNA equals the concentration of unfolded DNA and is a measure of DNA stability. For these quadruplexes, the sequence of the loops plays a significant role in the stability of the folded conformation. Thus, at 25 °C, these sequences form stable compact quadruplexes with high negative charge densities due to the close proximities of the backbone phosphates to each other in the folded conformation.

It is been long known that simple diammonium compounds, such as **II** in Figure 1, stabilize DNA through electrostatic interactions. In pioneering work from the mid-1960s, Gabbay used quaternized diammonium compounds to probe the structure of polymeric DNA and RNA. For example, Gabbay evaluated the effect of compounds of general structure $(CH_3)_3$-N^+-$(CH_2)_n$-N^+-$(CH_3)_3$ (n = 2,3,4) on the thermal stability of DNA. Interestingly, Gabbay found that the most stabilizing diammonium compound was the compound with n = 3. The distance between the two nitrogens, when n = 3, is 6.9 Å; the distance between adjacent phosphates in the DNA backbone was thought to be 7.0 Å. Hence, Gabbay was able to verify the

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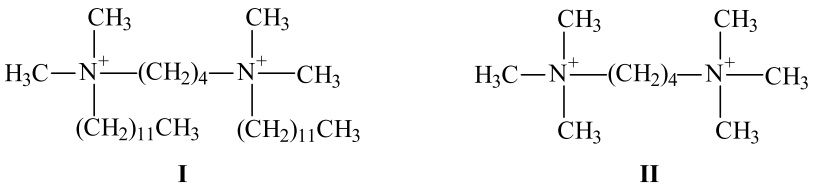

Figure 1. Gemini surfactant (**I**) and the simple diammonium compound (**II**). The surfactant is often designated as 12−4−12.

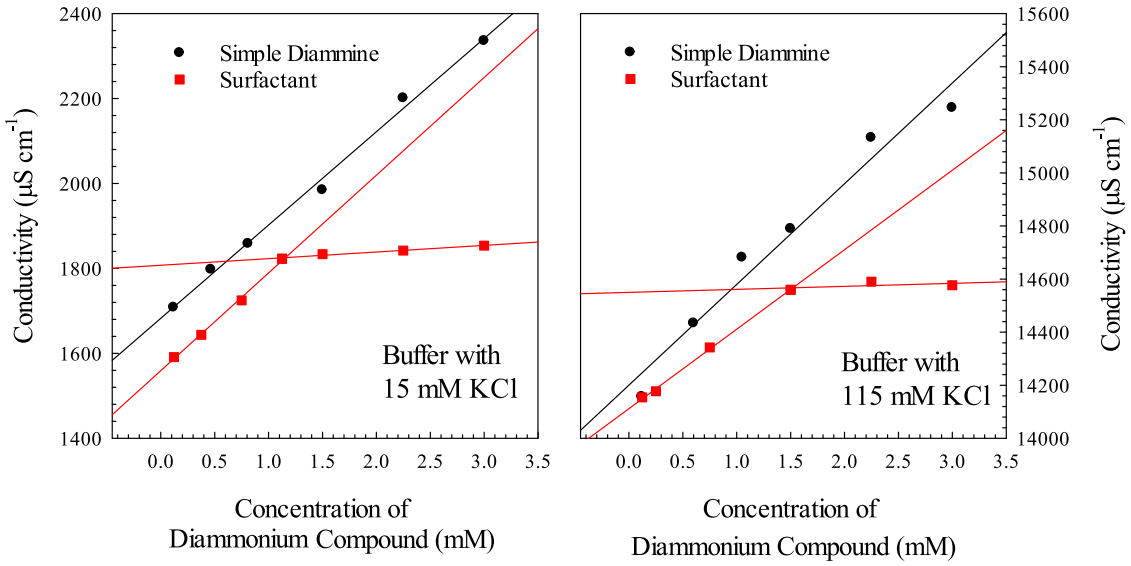

Figure 2. Plots of conductivity vs concentration of diammonium compound at 25 °C for the 12−4−12 surfactant **I** (red squares) and the simple diammonium compound **II** (black circles) in standard 10 mM phosphate buffer, 15 mM K^+ or 115 mM K^+, pH 7.0 with linear regression lines.

phosphate-to-phosphate distance in double-stranded DNA and RNA using these designed probes.[14]

The interactions between DNA and Gemini surfactant-based micelles have also been extensively investigated.[8,15−25] For these studies, a variety of surfactants as well as different DNAs were used. These interactions have been monitored by a broad range of biophysical techniques such as ITC,[15,16] DLS,[21,22] conductivity, atomic force microscopy (AFM),[20,22,25] low-angle X-ray diffraction,[23] and neutron reflectometry.[18] For example, Jiang et al. monitored the interaction of low-molecular-weight salmon sperm DNA with symmetric and dissymmetric Gemini surfactants via ITC and found that the interaction of the DNA with the micelles is entropically driven.[15,16] He et al. investigated the interactions of 12−3−12 and 12−4−12 Gemini surfactants with salmon sperm DNA using ITC, conductivity, and AFM.[19,20] They found, among other things, that increasing the salt concentration decreased DNA/micelle interactions but increased the formation of micelles.[20] Studies by Garcia et al. using 12−2−12 and 12−10−12 and calf thymus DNA indicated the interaction between the DNA and surfactant induced two conformational changes in the DNA: first, a compacted state that transitions to a more extended conformation at higher mole ratios of surfactant to DNA.[21] Recently, investigators have studied the interactions between plasmid DNA and Gemini surfactants as models for gene therapy.[8,23−25]

Since the surfactant molecule **I** is similar in structure to one of Gabbay's compounds (**II**) with the exception that one methyl is replaced by the much more hydrophobic $-(CH_2)_{11}CH_3$ chain, we decided to investigate the effect of the surfactant molecule on the thermal stability of the quadruplex forming sequence $(TTAGGG)_4$. Samples of the DNA of different concentrations were prepared using our usual protocols[11−13] but with added 12−4−12 surfactant (**I**) at concentrations ranging from 0.025 to 3.0 mM, i.e., straddling the *cmc* (1.5 mM) in standard phosphate buffer. In all samples of DNA and surfactant, precipitates formed. After centrifugation of these samples, the supernatant was separated from the pellet and the samples were analyzed for DNA by UV/vis. In some samples, 100% of the DNA had precipitated, while in others, only some of the DNA had precipitated. The percent of DNA precipitated depends on the surfactant/DNA ratio ([12−4−12]/[DNA]). Circular dichroism (CD) studies of the DNA from those samples indicated that the DNA assumed the normal quadruplex conformation for $(TTAGGG)_4$. In this work, we report the details of all of these findings.

RESULTS AND DISCUSSION

Conductivity Studies. As noted above, the *cmc* of the 12−4−12 surfactant is around 1.0 mM in water.[10] Since the DNA is normally prepared in standard 10 mM phosphate buffer with 115 mM K^+, the *cmc* of the 12−4−12 surfactant in this buffer needed to be determined. Earlier studies in our lab indicated that the *cmc* of the 12−4−12 surfactant increased with increasing ionic strength using KCl (Figure S1), contrary to that observed by He et al.[20] using NaCl and the 12−3−12 surfactant. The conductivities of solutions prepared from either the 12−4−12 surfactant (**I**) or the simple diammonium compound (**II**) with an increasing concentration of **I** or **II** were determined under two different buffer conditions. In both buffers, as shown in Figure 2, as the concentration of the surfactant increases, the conductivities of the solutions sharply

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increase linearly from 0.0 to 1.12 mM surfactant in buffer with 15 mM K^+ or to 1.50 mM surfactant in buffer with 115 mM K^+ followed by increasing linear conductivities but at a lower rate of change. The breakpoint is the critical micelle concentration (*cmc*) and represents the initiation of micelle formation. The observed change in conductivity upon micelle formation is typical for micelle formation[26−30] and is due to the lower mobility of the micelles. In contrast to the surfactant, the conductivity of the simple diammonium compound (**II**) continues to increase linearly with increasing concentration but with no breakpoint, consistent with the lack of micelle formation. As noted above, the *cmc* of the 12−4−12 surfactant in water is around 1.0 mM which increases with the increasing ionic strength of the solution. Hence the observed *cmc* of 1.5 mM for the surfactant in 10 mM phosphate buffer and 115 mM K^+ is not surprising. Assuming the micelles behave as polyelectrolytes, we rationalize that the observed increase in *cmc* with increasing counterion concentration (i.e., Cl^-) is due to the release of counterions (i.e., Br^-) from the surfactant upon micelle formation. However, for most of the studies reported here, surfactant concentrations are well below the *cmc* and, therefore, salt effects should only influence surfactant:DNA electrostatic interactions binding, not micelle formation.

Formation of Precipitate in Samples of DNA and 12−4−12 Surfactant. In our initial studies, samples of $(TTAGGG)_4$ at a fixed concentration (15 μM) and 12−4−12 surfactant with various concentrations in buffer were prepared as described in the Methods section. In all samples of DNA with surfactant, a flocculent precipitate formed. The aqueous supernatant was separated from the precipitate by centrifugation and probed for the presence of DNA by UV/vis and CD. The initial results are listed in Table 1. The values of [I]/[DNA] in Table 1 are the ratios of the surfactant concentration divided by the input DNA concentration (i.e., 15 μM).

Table 1. $(TTAGGG)_4$ (DNA) with the 12-4-12 Surfactant (I) in Standard Buffer with 115 mM K^+

[I] mM	[DNA] μM^a	[I]/[DNA]	PPT[b]	DNA in supernatant	DNA conformation
0.00	15.0		ND	Y	quadruplex
0.094	12.5	6.26	Y	Y	quadruplex
0.188	6.3	12.5	Y	Y	quadruplex
0.375	ND	25.0	Y	ND	unknown
0.562	ND	37.5	Y	ND	unknown
0.750	ND	50	Y	ND	unknown
1.50	ND	100	Y	ND	unknown
3.00	ND	200	Y	ND	unknown

[a]In bases. [b]PPT = precipitate observed: Y = yes, ND = none detected.

Scanning the supernatants using UV/vis revealed no DNA present in samples prepared with surfactant concentrations greater than 0.375 mM, well below the *cmc* of 1.5 mM in the buffer. Thus, 100% of the DNA had precipitated. However, some DNA was detected in those supernatants from samples prepared with either 0.094 or 0.188 mM surfactant. Since the same amount of DNA was used for each sample in the original preparation, 17% of the DNA precipitated in the sample treated with 0.094 mM 12−4−12 while 58% of the DNA precipitated in the sample treated with 0.188 mM 12−4−12

$$\%\ \text{precipitation} = ([\text{DNA}]_i - [\text{DNA}]_s)/[\text{DNA}]_i \times 100\% \quad (1)$$

where $[DNA]_i$ is the concentration of DNA in the absence of **I** and $[DNA]_s$ is the concentration of DNA in the supernatant.

Circular Dichroism Studies. To verify the presence or absence of DNA in all supernatants, CD spectra of samples were determined. The resultant CD spectra at 25 °C are shown in Figure 3. As per the UV/vis results, no DNA is observed in

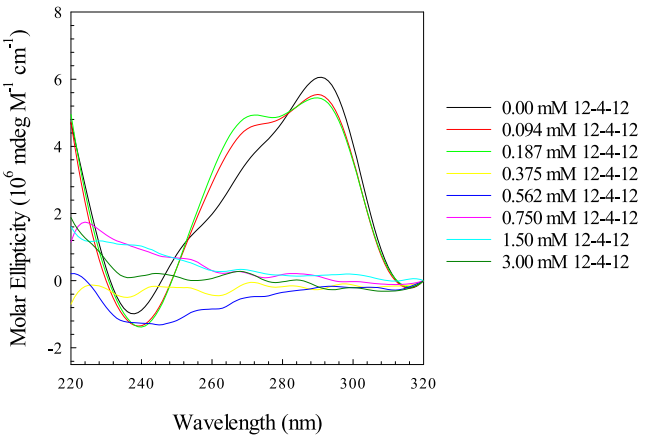

Figure 3. CD spectra at 25 °C for $(TTAGGG)_4$ after treatment with the 12−4−12 surfactant **I**. For these spectra, DNA samples were prepared in standard phosphate buffer, pH 7.0, and 115 mM K^+ with increasing concentrations of the 12−4−12 surfactant from 0.00 to 3.00 mM. In all samples with the surfactant, a precipitate was formed, which was separated from the supernatant by centrifugation. The CD spectra for these supernatants are shown with the corresponding concentration of the surfactant.

supernatants from samples prepared with [**I**] greater than 0.375 mM. The CD spectrum of $(TTAGGG)_4$ in just buffer is typical of the quadruplex conformation with one propeller loop and two transverse loops.[11−13] The CD spectra of the supernatants from samples prepared with [**I**] of 0.094 and 0.187 mM appear strikingly similar to each other and are not much different from the spectrum obtained in the absence of surfactant. However, both samples display an enhanced shoulder at 270 nm (Note: The spectra in Figure 3 are plots of molar ellipticity vs wavelength; thus, the spectra do not reflect differences in DNA concentrations but only similarities in conformation). Thus, the residual DNA in those samples also most likely assumes a quadruplex conformation with a propeller loop and two transverse loops.

A variety of solvents were used to try to dissolve the pellet to recover the DNA including water, buffer, methanol, and acetonitrile (with and without heating), but none worked. However, the pellet was soluble in 2 M KCl. Dialysis of the KCL solution vs water, followed by lyophilization and reconstitution in buffer, resulted in a nearly 70% recovery of the DNA. We are currently developing methods for higher recovery.

Dependence of Precipitation on [DNA]/[12−4−12]. The observation of residual DNA in samples prepared with low concentrations of surfactant led us to investigate the role that the ratio of the surfactant to DNA plays in the precipitation. Six series of samples were prepared, each series with [12−4−12] at 0.00, 0.025 0.050, 0.075, 0.10, or 0.15 mM, and each with the same concentration of DNA, ranging from 1.4 to 11.0 μM. All samples were centrifuged for 30 min at 13.3 K rpm.

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The supernatant was then probed for DNA by UV/vis. Figure 4 shows the representative UV spectra of three different concentrations of DNA.

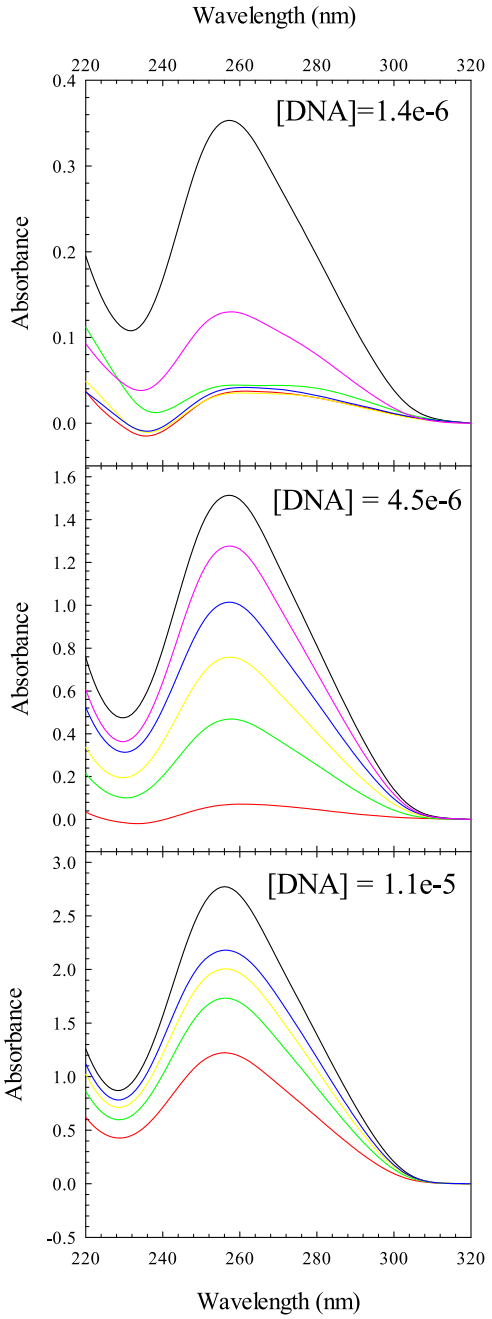

Figure 4. UV spectra at 25 °C of supernatants from samples prepared with concentrations of 12−4−12 of 0.00 mM (black), 0.025 mM (pink), 0.050 mM (blue), and 0.075 mM (yellow). 0.10 mM (green) and 0.15 mM (red) and charged with $(TTAGGG)_4$ at 1.4 μm, 4.5 μM, and 11.0 μM. All samples were prepared in standard phosphate buffer with 115 mM K^+.

As can be seen in Figure 4, the amount of recovered DNA increases at each surfactant concentration with increasing concentration of DNA: at 1.4 μM DNA, nearly all of the DNA was precipitated, except at the lowest concentration of surfactant (0.025 mM); however, at 11.0 μM DNA, DNA was recovered from all samples. Using the extinction coefficient for $(TTAGGG)_4$ of 244,600 M^{-1} cm^{-1} in bases, the percent DNA precipitated can be determined using eq 1. Plots of percent DNA precipitated versus concentration of surfactant yielded linear correlations below 85% precipitation (Figure 5).

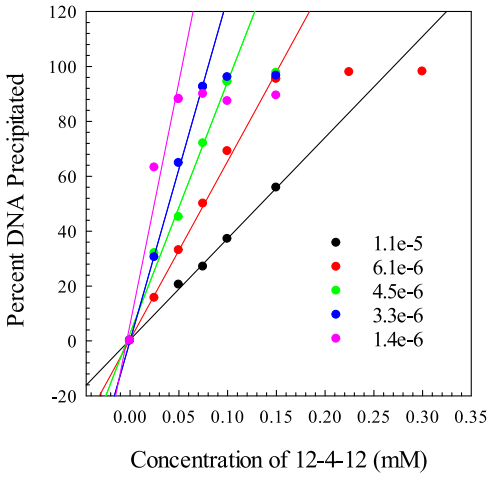

Figure 5. Plots of percent precipitated DNA as a function of the concentration of surfactant. The concentration of DNA was 11.0 μM (black), 6.1 μM (red), 4.5 μM (green), 3.3 μM (blue), and 1.4 μM (pink). The least-squares fits are for data from 0 to 85% precipitation.

Using the line parameters for the least-squares fits above, one can calculate the minimum concentration of 12−4−12 required to precipitate 100% of the DNA (Figure 6) in any

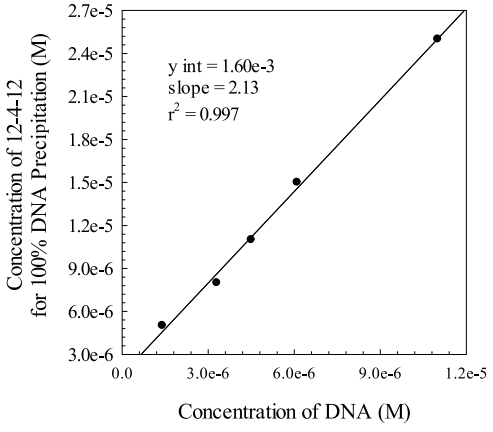

Figure 6. Plot of the minimum concentration of surfactant needed to precipitate all of the DNA in a sample of known DNA concentration.

sample. The slope of the least-squares fit indicates that it takes nearly 2.13 molecules of 12−4−12 to completely precipitate 1 DNA base. On a charge-to-charge basis, 2.04 molecules of 12−4−12 are required for every DNA phosphate for full precipitation. Thus, simple neutralization of the DNA by the surfactant, which would require only one surfactant for every two phosphates, cannot alone account for the precipitation.

We also prepared samples of DNA with **II** in a buffer. No precipitation of DNA was observed. Thus, although charge−charge interactions between DNA and **I** and **II** are strong, they are not strong enough alone to account for the precipitation of the DNA by **I**. Further, even concentrations of surfactant well below the *cmc* led to complete precipitation of >99% of the DNA except at the lowest concentrations where only some of

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the DNA was precipitated. Hence, the hydrophobic interactions of surfactants play a significant role in the precipitation. A possible mechanism for the formation of the insoluble complex is shown in Scheme 1:

Scheme 1. Possible Mechanism for the Precipitation of the DNA in the Presence of the Surfactant. DNA = $(TTAGGG)_4$; SUR = 12-4-12 Surfactant

$$DNA + nSUR \rightleftharpoons DNA{\bullet}SUR_n + mSUR \longrightarrow DNA{\bullet}SUR_{n+m}$$

Initially, nanomolecules of surfactant interact reversibly with the DNA strictly by electrostatic interactions leading to a water-soluble complex ($DNA{\cdot}SUR_n$). As the concentration of surfactant increases, additional association of surfactant with the water-soluble complex ultimately leads to the irreversible formation of a precipitate ($DNA{\cdot}SUR_{n+m}$) after the complexation of m additional surfactant molecules. If 2 surfactants are indeed needed to precipitate 1 phosphate (from Figure 6), then $n + m = 46$ since the DNA possesses 23 phosphates. Complete neutralization of the DNA by the surfactant, which could lead to precipitation alone, should require only 11.5 surfactants. Hence, the additional surfactants required for complete precipitation are associated with the already formed DNA:surfactant complex strictly through hydrophobic interactions.

Based on the above stoichiometry, one can calculate an apparent solubility product, K*sp*, for the DNA-SUR complex

$$K_{sp} = [DNA]_s[12-4-12]_s^2 \quad (2)$$

where $[DNA]_s$ is the concentration of DNA in the supernatant, determined from the UV absorption at 260 nm using ε_{260} = 244,600 in bases, and $[12{-}4{-}12]_s$ is the estimated concentration of the surfactant in the supernatant

$$[12-4-12]_s = [12-4-12]_i - [12-4-12]_p \quad (3)$$

$[12{-}4{-}12]_i$ is the initial concentration of the surfactant and $[12{-}4{-}12]_p$ is its concentration in the precipitate

$$[12-4-12]_p = 2[DNA]_p \quad (4)$$

$$[DNA]_p = [DNA]_I - [DNA]_s \quad (5)$$

The concentration of DNA in the precipitate is calculated as the difference between the initial concentration and its concentration in the supernatant.

The concentration of the DNA in the supernatant can be determined using the UV data for each set of DNA concentrations ($[DNA]_s$) at each known initial concentration of surfactant ($[12{-}4{-}12]_i$) and proceeding using eqs 3 to 5 to determine the estimated concentration of surfactant in each supernatant ($[12{-}4{-}12]_s$) and then calculating the apparent K_{sp} using eq 2. Calculated values ranged from 3.45×10^{-14} to 9.06×10^{-15} with an average value of 8.46×10^{-15}. Although the supernatant concentration of DNA can be quantitatively determined, the concentration of the surfactant in the supernatant is estimated using our assumptions noted above. We are currently developing methods to accurately quantify the concentration of surfactant in the supernatant. Nonetheless, this estimated apparent K_{sp} indicates that DNA:surfactant complex is not very soluble under the buffer conditions used.

In early studies, the interactions between low-molecular-weight salmon sperm DNA and different salts of the 12−6−12 surfactant were investigated by DLS and ITC determinations. The titration of the surfactant into the DNA leads to the formation of DNA-surfactant aggregates that behaved more as a dispersed solid state rather than precipitate.[15,16] A later study investigated the interaction between the 12−3−12 surfactant and polymeric salmon sperm DNA using a variety of biophysical techniques. The findings of these studies indicated that the surfactant modulates the conformation of the DNA from a chain-like structure to structures of toroidal aggregates.[21]

The above and other studies[18−25] used high-molecular-weight DNA which would be highly negatively charged and surfactants similar to the 12−4−12 surfactant used in our experiments and reported formations of DNA-surfactant aggregates without the formation of precipitates. We have shown here the surprising result of the formation of a water-insoluble DNA-surfactant aggregate using a small, low-molecular-weight DNA molecule of high negative charge density with the dicatioinc surfactant. The formation of the aggregate is clearly dependent on the concentrations of both DNA and surfactant. Factors that could also contribute to precipitation include: (1) the length of the DNA, which would also affect its total charge; (2) the conformation of the DNA, which would also affect its charge density; (3) the ionic strength of the solution; and (4) the temperature of the solution. We are now investigating this phenomenon in a systematic fashion to address these questions.

CONCLUSIONS

Gemini surfactants have physical properties that make them ideal candidates for a variety of potential applications. After many years of focusing on the biophysical properties of DNA using designed DNA oligomers, we started looking at Gemini surfactants. The observation of the precipitate in samples prepared with both the surfactant and a DNA quadruplex was quite surprising. The results presented here suggest that the precipitate is a surfactant-quadruplex complex, stabilized by electrostatic interactions between the negatively charged quadruplex and positively charged surfactant and hydrophobic interactions between the hydrophobic tails with hydrophobic regions on the quadruplex and with each other. Many dicationic surfactant molecules bind to the quadruplexes, leading to net neutralization or near net neutralization, resulting in precipitation of the complex. Hence, the complex formed between the quadruplex and the simple diammonium compound (**II**) molecule is strictly electrostatic in nature, and the complex formed between the quadruplex and the surfactant molecule (**I**) is stabilized by electrostatic interactions as well as hydrophobic interactions leading to additional stabilization. To address the balance between electrostatic and hydrophobic interactions, we are currently investigating the interactions of this surfactant with DNA oligomers of different sequence contexts, lengths, base content, and conformations, preparing a surfactant with only one dodecyl chain as well as investigating environmental effects on the formation of the DNA:surfactant complex and the stabilization of the DNA by the surfactant.

METHODS

Materials. The DNA oligomer $(TTAGGG)_4$ was synthesized and purified using RP-HPLC by Biosynthesis, Inc. (Lewisville, TX) and used without additional purification. The Gemini surfactant **I** was synthesized and kindly provided by

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Prof. Steve Bachofer. The simple diammonium compound **II** was synthesized as previously described.[14]

Preparation of DNA-Surfactant Solutions. For all studies, samples of DNA with or without surfactant were prepared in standard potassium phosphate buffer: 10 mM phosphate, 0.1 mM EDTA, and 115 mM K^+, pH 7.00, prepared using KH_2PO_4, K_2HPO_4, KCl, and EDTA (Sigma Chemical Co) in ultrapure water. The samples were then incubated at 95 °C, allowed to slowly cool, and then stored at 5 °C. All samples were then centrifuged at 14K rpm for 30 min to separate the supernatant from the precipitate, if any.

Conductivity Studies. Conductivity measurements were determined with a Mettler Toledo SevenCompact Duo S213 Benchtop pH/Conductivity Meter with a standard micro conductivity probe at 25 °C. Measurements were repeated until 3 consecutive identical readings were obtained.

UV/vis Spectroscopy. UV/vis spectra were determined using a Varian Cary 100 Bio model (Varian Associates, Palo Alto, CA) or an OLIS 8453 UV/vis model (OLIS, Inc., Athens, GA). Spectra were recorded from 320 to 220 at 1 nm intervals at 25 °C. DNA concentrations in all samples were determined using ε_{260} = 244,600 in bases.

Circular Dichroism. An OLIS RMS 1000 CD spectrophotometer (OLIS, Inc., Athens, GA) was used to perform all circular dichroism studies. Spectra were determined at 25 °C in a 2 mm quartz cuvette with scanning from 320 to 220 nm with ellipticity recorded every nm. For optical melting studies, spectra were recorded from 320 to 220 nm, at 1 nm intervals, every 5 °C from 25 to 95 °C after a 5 min equilibration at each temperature.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03739.

Dependence of the cmc for the 12−4−12 surfactant on the concentration of KCl (Figure S1) (PDF)

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Notes

The authors declare no competing financial interest.

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