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Efficient DNA Condensation Induced by Chiral β-Amino Acid-Based Cationic Surfactants

Bernat Pi-Boleda, Sravani Ramisetty, Ona Illa, Vicenç Branchadell, Rita S. Dias,* and Rosa M. Ortuño*

Cite This: ACS Appl. Bio Mater. 2021, 4, 7034−7043

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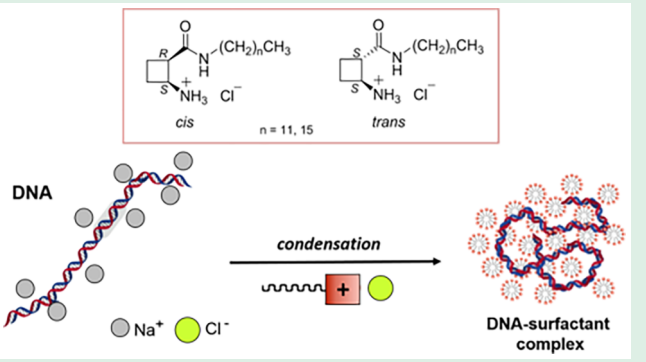
ABSTRACT: Four cationic chiral amino acid-based surfactants, *cis*- and *trans*-**1** and *cis*- and *trans*-**2**, have been studied as DNA-condensing agents with enhanced properties and the absence of cell toxicity. The polar head of the surfactant is made of a cyclobutane β-amino acid in which the amino group is a hydrochloride salt and the carboxyl group is involved in an amide bond, allowing the link with hydrophobic C_{12} (surfactant **1**) or C_{16} (surfactant **2**) chains. The ability of these surfactants to condense DNA was investigated using a dye exclusion assay, gel electrophoresis, and circular dichroism and compared with the well-studied dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB). The surfactant with the longest chain length and the *trans* stereochemistry (*trans*-**2**) was found to be the most efficient in condensing the DNA, including CTAB. Surfactant *cis*-**2** was found to be less efficient, probably due to its poorer solubility. The β-amino acid surfactants with the shorter chain length behaved similarly, such that the *cis*/*trans* stereochemistry does not seem to play a role in this case. Interestingly, these were also found to induce DNA condensation for the same concentration as *trans*-**2** and CTAB but showed a lower binding cooperativity. Therefore, a longer alkyl chain only slightly improved the effectiveness of these surfactants. Further, atomic force microscopy revealed that they compact DNA into small complexes of about 55−110 nm in diameter.



KEYWORDS: *DNA−surfactant complexes, surfactant headgroup, critical association concentration, dye exclusion, circular dichroism*

INTRODUCTION

Gene therapy has gained significant attention over the past two decades as a potential method for treating genetic disorders such as Severe Combined Immunodeficiency,[1] Hemophilia B,[2] cystic fibrosis,[3] Leber's Congenital Amaurosis,[4] and Parkinson's disease[5] as well as an alternative method to traditional chemotherapy for treating cancer.[6] It consists of introducing a target gene, which is encoded in a DNA or RNA chain, into a specific cell. Research efforts are currently focused on designing effective carrier vectors for gene therapy that efficiently compact and protect DNA,[7] as DNA is rapidly degraded by serum nucleases in the blood when injected intravenously.[8] Moreover, they must transfect into cells and release the target DNA within. Not surprisingly, viral carriers such as retroviruses and adenoviruses are found to exhibit a high efficiency in delivering both DNA and RNA to numerous cell lines. However, fundamental problems associated with viral vector systems, including toxicity, immunogenicity, and limitations with respect to scaled-up procedures, encouraged the investigation of other potential vectors for introducing the DNA into the targeted tissues.[9,10] The role of chemists in the field of gene therapy is to design and prepare new nonviral vectors, based on cationic lipids,[11−13] cationic surfactants,[12,14] cationic polymers,[15,16] metal cations,[17] dendrimers,[18,19] polypeptides,[20,21] and nanoparticles.[22,23]

Special attention has been dedicated to cationic amphiphilic molecules due to their properties.[24] The cationic polar heads interact with negatively charged phosphate groups of DNA, while the hydrophobic chains stabilize the formed aggregates. The degree of DNA condensation, the size of the complexes, and the neutralization of negative charges have been identified as crucial factors to transfect DNA into cells.[25] Complexes with a radius below 200 nm are likely to be transfected into cells through endocytosis and end up in endosomes that, in time, will develop into lysosomes where the complexes are degraded due to the presence of enzymes in these compartments.[26] Thus, it is most desirable that the vector facilitates the DNA release from the endosomes.[27,28]

Received: June 13, 2021
Accepted: August 11, 2021
Published: August 25, 2021

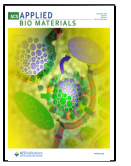
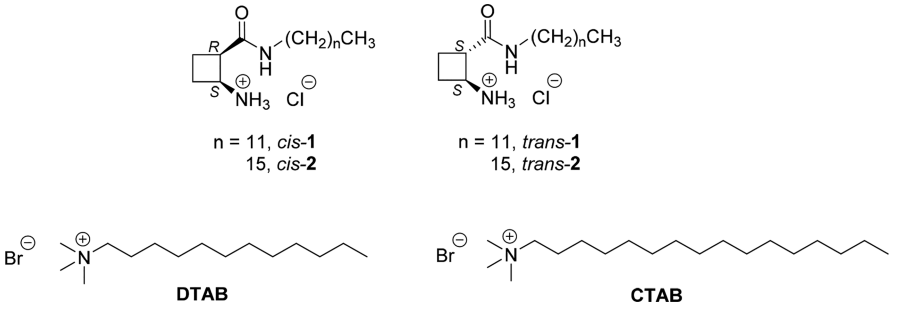


Chart 1. Structure of Chiral Cyclobutane β-Amino Acid-Based Cationic Amphiphiles[45] Studied in This Work and of Commercial DTAB and CTAB



The structure of the surfactant plays a very important role in the DNA condensation and complex formation, which can be controlled by modifying the structure of the polar head and the length of the hydrophobic chain.[29−31] However, the cytotoxicity of the most commonly studied cationic surfactants represents a shortcoming in their application. Amino acid-based surfactants are gaining relevance due to their good levels of biodegradability and biocompatibility.[32−36] The combination of amino acids or peptides with hydrocarbon chains of variable length has given rise to a variety of compounds with good amphiphilic properties and the possibility of tuning the amino acid composition to include, for example, pH-sensitive moieties, believed to facilitate the endosomal escape.[37] Moreover, intermolecular hydrogen bonding promoted by the amino groups can enhance the self-assembly of surfactants in these systems.[38] Furthermore, interactions of biodegradable amino acid-based surfactants with anionic polyelectrolytes have also been studied.[39] Indeed, the role of polyelectrolytes and their interactions with surfactants have been highlighted.[40,41]

Among amino acids, chiral cyclobutane-containing β-amino acid-based surfactants have shown interesting abilities. They are tuned by the relative *cis*/*trans* stereochemistry and by the stereochemical constraints imposed by the cyclobutane ring as well as the positive[42] or negative[43] charge or their nonionic character.[44] All these features confer great versatility to these derivatives as they strongly influence their aggregation properties. Recently, a new family of cationic surfactants, *cis*- and *trans*-**1** and -**2** (Chart 1), has been reported.[45] Their physicochemical properties are strongly dependent on the pH of the medium, such as the critical micellar concentration (CMC) and the surface tension. Further, the mode of aggregation is determined by the *cis*/*trans* stereochemistry with the *cis*-isomers forming micelles or vesicles while the *trans*-isomers predominantly form fibers. In addition, they were shown to be nontoxic for HeLa cells.

With these qualities in mind, we have, in this work, studied the condensation of DNA by chiral cyclobutane β-amino acid-based cationic surfactants using a range of complementary techniques and paying particular attention to the role of *cis*/*trans* stereochemistry and the alkyl chain length. For comparison purposes, some of the experiments were conducted using the commercial surfactants cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB), which have been extensively investigated. The obtained data should shed light on the potential of chiral cyclobutane β-amino acid-based surfactants to be used as nonviral vectors in gene therapy.

EXPERIMENTAL SECTION

Materials. Oligonucleotide primers were received from Sigma-Aldrich. For circular dichroism (CD) studies, which require high concentrations and volumes, DNA from salmon sperm (10 mg mL^{-1} solution, average size of ≤2 kbp) was obtained from Invitrogen (Fisher Scientific). Surfactants *cis*- and *trans*-**1** and -**2** were synthesized and purified following the procedures described in ref 45, and CTAB and DTAB were obtained from Sigma-Aldrich. Tris(hydroxymethyl)aminomethane and hydrogen chloride for Tris-HCl buffer preparation were purchased from Sigma-Aldrich. All stock, intermediate, and final solutions were made, unless stated otherwise, with 10 mM Tris-HCl, pH 7.4, prepared using Milli Q deionized water (18.2 Ω/cm resistivity at 25 °C).

DNA Preparation and Purification. For experiments other than CD, a linear double stranded DNA (4145 bp) harboring the gene-encoding green fluorescent protein (GFP mut3) under the control of a T7 promoter was generated by polymerase chain reaction (PCR) using the plasmid pSB-E1 g[46] as the DNA template and forward 5′-GCTGGCCGATAAGCTCTAAG-3′ and reverse 5′-GGTGC-ATTGCAAACGCTAGG-3′ primers. After PCR, the product was purified using DNA clean and a concentrator kit from Zymogen.

Methods. *Dye Exclusion Assay (DEA).* The accessibility of the DNA molecules to the fluorescent dye GelStar was determined by exciting the DNA−GelStar samples, possessing varying concentrations of surfactant, with light at a wavelength of 493 nm (corresponding to the excitation maximum of GelStar) and measuring the respective emitted fluorescence intensity at 500−550 nm. The emitted fluorescence is linearly dependent on the "free" DNA concentration (in the absence of compacting agent).[47] A reference sample with DNA and GelStar (without surfactant) was prepared simultaneously and used to normalize the data. Furthermore, to check that the surfactant aggregates did not interfere with the fluorescence intensity of the dye, control experiments were performed using the protocol described below but replacing DNA with the buffer solution, that is, by measuring samples with buffer, GelStar, and the highest surfactant concentration studied, which was above the CMC. No significant differences were found in the fluorescence intensity of the dye in the presence or absence of surfactant micelles.

Samples were prepared in triplicate using the following procedure: First, 5 μL of 20 μg mL^{-1} DNA was mixed with 5 μL of 100× GelStar (prepared from 10 000× GelStar stock solution obtained from Lonza) and left to equilibrate for 15 min. Afterward, 5 μL of surfactant solutions with varying concentrations was added to the DNA−GelStar mixtures. Finally, 35 μL of 10 mM Tris-HCl was added to the samples, which were left to incubate for 1 h. After incubation, the fluorescence emission of the samples (500−700 nm) was measured using an Infinite M200 Pro Tecan spectrophotometer. The experimental parameters are shown in Table S1. As an example, the emission spectra of the DNA−GelStar at different *cis*-**1** concentrations, as well as the results of all studied systems, are also provided in Figures S1 and S2, respectively. Concentration ratios were expressed as a function of the surfactant μM concentration divided by ppm of DNA. In addition, results are also presented as a function of the charge ratio (Z_{hp}), defined as the ratio between the molar

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ACS Appl. Bio Mater. 2021, 4, 7034−7043

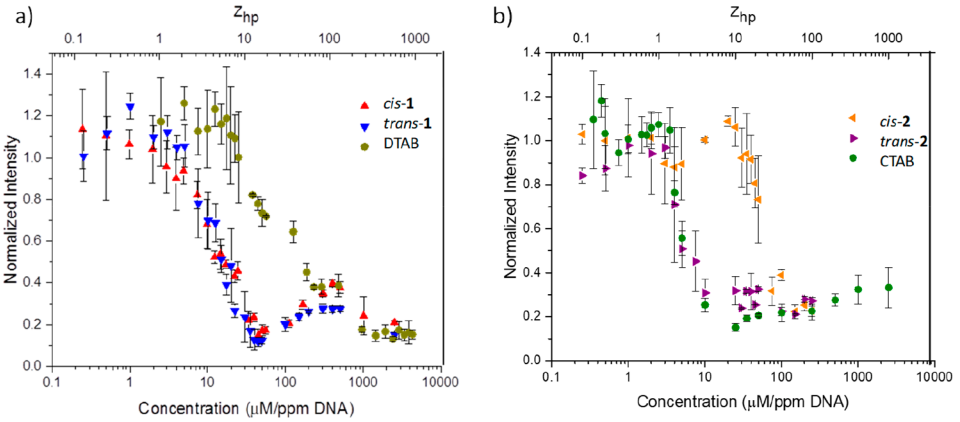

Figure 1. Fluorescence intensity of DNA−surfactant complexes normalized to DNA solutions in the absence of surfactant, as a function of surfactant concentration, for surfactants possessing (a) 12 carbons in the hydrocarbon chain: *cis*- and *trans*-**1** and DTAB; (b) 16 carbons in the hydrocarbon chain: *cis*- and *trans*-**2** and CTAB. The concentration of surfactant is given as both μM/ppm and Z_{hp}. The DNA concentration was 2.0 μg/mL, and the excitation and emission wavelengths were 493 and 540 nm, respectively.

concentration of charges from the surfactants, that is, NH_3^+ for *cis*- and *trans*-**1** and -**2**, assuming that all the surfactants are protonated, and $N(CH_3)_3^+$ for CTAB and DTAB, and the molar concentration of DNA nucleotides (PO_4^-, *p*).

Electrophoretic Mobility Shift Assay (EMSA). 20 μL samples were prepared by adding 5 μL of a 20 μg mL^{-1} DNA solution, 5 μL of a surfactant solution at different concentrations, and 10 μL of Tris-HCl buffer and incubating for 1 h. To compare the results with those obtained from the DEA, the concentration of surfactant will be expressed as a function of the charge ratio (Z_{hp}). The samples were loaded in a 0.8% agarose gel (SeaKemRLE agarose from Lonza, Rockland, ME USA) and run for 20 min at 100 V. Afterward, the gels were visualized under UV light using a ChemiDoc instrument.

Circular Dichroism (CD) Spectroscopy. CD spectra were obtained in a Jasco-715 spectropolarimeter. 350 μL solutions of 0.333 μM (1 mg mL^{-1}) Salmon Sperm DNA in the absence and presence of known concentrations of surfactants were prepared, left to equilibrate for a couple of minutes, and placed into a 1 mm length-path cuvette. The CD spectra of the solutions were recorded.

Atomic Force Microscopy (AFM). Samples were prepared by mixing 80 μM solutions of the respective surfactants with an 8 μg mL^{-1} solution of DNA. After 1 h of incubation, 10 μL of the resultant solution was transferred to a freshly cleaved 5 mm diameter mica disk. Then, the sample surface was rinsed rapidly with pure water (Milli-Q) to obtain a clean surface and then dried under a stream of N_2 followed by vacuum-drying at a pressure of 1.3×10^{-4} Pa for 5 h.[48] The imaging was performed in a Multimode 8 from Bruker using ScanAsyst mode in air and employing ScanAsyst-air cantilevers with nominal spring constants of 0.4 N/m.

RESULTS AND DISCUSSION

Several techniques were used to study the surfactant features favoring the interactions between these and DNA and concomitant DNA condensation.

The binding of cationic surfactants to DNA and surfactant self-assembly in its vicinity is known to occur at concentrations much below the CMC of the DNA-free surfactant solutions.[49] The binding of the monovalent surfactant itself is not expected to affect the DNA conformation; however, the formation of surfactant aggregates in the vicinity of the DNA, driven by hydrophobic interactions between surfactant tails and counterion release, leads to attractions between DNA chains and/or within a single chain, leading to the condensation of DNA.[50,51] Accordingly, binding isotherms of cationic surfactants to DNA show a sigmoidal shape, indicating cooperativity.[52] The concentration at which the surfactant starts forming micelles in the vicinity of an oppositely charged polyelectrolyte is called the critical association concentration (CAC).

In this work, DEA experiments were performed to estimate the CAC values for the DNA−surfactant complexes. The results obtained for the novel *cis*- and *trans*-**1** and -**2** surfactants were compared with those of commercial DTAB and CTAB, which have been extensively studied as DNA-compacting agents. Further, the obtained results were complemented with EMSA experiments that also explore the degree of complexation of DNA by the cationic surfactants, while CD spectroscopy was used to monitor the variation of the DNA structure upon surfactant self-assembly. AFM was used to probe the geometry of the complexes formed at intermediate surfactant concentrations.

Accessibility of a Fluorescent Dye to DNA in the Presence of Surfactants. As described in the experimental part, the fluorescence emission spectra of DNA−GelStar complexes were measured for increasing concentrations of all the surfactants under study. All the studied systems were found to have a maximum of emission at 540 nm, and thus, to more easily analyze the results, the intensity of each sample was normalized with respect to the intensity of the DNA−GelStar complex (absence of surfactants) and represented as a function of the surfactant concentration (Figure 1). The general trend shows that an increase in cationic surfactant amount leads, at low concentrations, to a small increase in the emission intensity, followed by a significant decrease in fluorescence. Some of the systems show a further increase in fluorescence intensity with increasing surfactant concentrations. The reason for the initial increase in fluorescence is not clear but could be due to an extension of the DNA chain, which makes it more available to the fluorescent dye upon the addition of small amounts of surfactant. Such a chain extension at low concentrations of condensing agent (polycations) has been observed using coarse-grained systems and Monte Carlo simulations.[53] Upon further addition of surfactant, the fluorescence intensity decreases due to the exclusion of the dye from the DNA molecules. The increase in fluorescence intensity observed at larger surfactant concentrations for some of the systems is likely due to the formation of overcharged DNA−surfactant complexes, which leads to an expansion of the complex and consequently an increase in the accessibility of the DNA to the dye.[54]

https://doi.org/10.1021/acsabm.1c00683
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Panels a and b in Figure 1 show the results obtained for surfactants possessing 12 (**1** and DTAB) and 16 (**2** and CTAB) carbons in the hydrocarbon chain, respectively. Starting with an overall assessment, it can be observed (Figure 1a) that the behavior of *cis*- and *trans*-**1** is very similar, suggesting that *cis*/*trans* stereochemistry does not play a significant role in the interaction of the surfactants with the DNA in these systems. Both surfactants are found to be much more efficient than DTAB in inducing DNA condensation, that is, the decrease of fluorescence intensity occurs at a lower concentration of surfactant. In contrast, large differences are found between the more hydrophobic *trans*-**2** and *cis*-**2** surfactants; *trans*-**2** behaves similarly to CTAB while *cis*-**2** is clearly less efficient in inducing DNA condensation (Figure 1b), pointing out the influence of stereochemistry for these surfactants.

In all studied systems, the higher normalized intensity values have larger error bars than those with lower intensities. This fact can be attributed to several factors related to the different species present in the solution. At high intensities, DNA is not condensed and has many degrees of freedom presenting different coil conformations, each one with different accessibility for the GelStar. In addition, the cooperative effect of the binding process, highlighted by the sigmoid shape, also accounts for the larger errors; further binding of the compacting agent to the partially compacted DNA is preferred, and the coexistence of more compact and more extended DNA−surfactant complexes is likely to contribute to the large error bars.[24]

Since the decrease in fluorescence intensity is associated with the lower accessibility of GelStar, due to the formation of surfactant aggregates at the surface of DNA, it is tempting to estimate the CAC values from the concentration of surfactant at which the intensity starts to decrease. These are presented in Table 1 using both surfactant molar concentration and charge ratio between surfactant headgroups and DNA phosphate groups, here designated by $Z_{hp\text{-}1}$. The CAC of CTAB in the presence of DNA has been reported to be between 4 and 10 μM,[51,55−57] depending on the used technique and conditions, which is in good agreement with the obtained result. Since the hydrophobic part of the surfactant is shorter, DTAB shows a higher CAC than CTAB, but the value (50 μM) is somewhat lower than the reported value of 80 μM obtained using fluorescence microscopy.[51]

Table 1. Summary of the Obtained CAC and C_{CI} Values, Corresponding to the Charge Mixing Ratios $Z_{hp\text{-}1}$ and $Z_{hp\text{-}2}$, Respectively, Their Difference (ΔZ_{hp}), and the CMC Values for the Surfactants under Study

surfactant	CAC[a] ($Z_{hp\text{-}1}$)	C_{CI}[a] ($Z_{hp\text{-}2}$)	ΔZ_{hp}	CMC[b]
cis-**1**	7 (1)	100 (15)	14	0.87
trans-**1**	7 (1)	100 (15)	14	0.42
DTAB	50 (7.5)	2000 (300)	292.5	16
cis-**2**	60 (9)	300 (45)	36	0.14
trans-**2**	7 (1)	20 (3)	2	0.15
CTAB	7 (1)	50 (7.5)	6.5	0.98

[a]μM. [b]mM. CMC values, in mM at natural pH, were taken from refs 45 (for *cis*- and *trans*-**1** and -**2**), 58 (for DTAB), and 59 (for CTAB).

Table 1 also includes the surfactant concentration at which the normalized fluorescence intensity reaches its minimum, defined here as the concentration of critical intensity, C_{CI}, as well as the corresponding charge ratio, $Z_{hp\text{-}2}$. C_{CI} is thus defined as the lower surfactant concentration in which all (or most) DNA molecules are condensed. In addition, ΔZ_{hp} values, calculated as $Z_{hp\text{-}2} - Z_{hp\text{-}1}$, are also listed in Table 1 as they allow one to evaluate the cooperativity of the surfactant-induced DNA condensation, that is, the tendency of the surfactant to associate to a DNA molecule already occupied by surfactants.[36] Thus, the lower the ΔZ_{hp}, the higher will be the cooperativity and the strength of the interaction between the DNA and the surfactant aggregates. Finally, the CMC of the surfactants is also presented.

In more detail and starting with the surfactants with the shortest alkyl chain, it can be observed in Table 1 that both *cis*- and *trans*-**1** present a similar behavior (CAC of 7 μM, C_{CI} of 100 μM) and that they are much more efficient in condensing DNA than DTAB (CAC of 50 μM, C_{CI} of 2000 μM). Since the alkyl chain of the surfactants is the same, the difference must originate in the headgroup. The lower CAC indicates that the attractive interactions between surfactant headgroups are stronger for the β-amino acid-based surfactants than for the tetramethylammonium surfactants. This could be due to the formation of H-bonds between the headgroups (more favorable than those with water) and/or the more hydrophobic nature of the headgroups due to the chiral cyclobutane. This reasoning is supported by the fact that the CMC of the *cis*- and *trans*-**1** surfactants is much lower than that of the DTAB. It is also interesting to note that the CAC of the *cis*- and *trans*-**1** is apparently the same even though the CMC of *trans*-**1** is half of that of *cis*-**1**. It has been shown that the CMCs of *cis*- and *trans*-**1** are about the same at low pH conditions[45] where most of the surfactants are protonated, which suggests that the interaction with DNA enhances the ionization of the surfactants upon interaction. This is induced by the decrease in the electrostatic repulsions between the headgroups of the surfactants on the aggregates, as has been shown for protein− and nanoparticle−polyelectrolyte systems using experiments and molecular modeling.[60−62] The longest surfactants (**2**), on the other hand, show a different behavior with the *cis* isomer having a larger CAC and C_{CI} (estimated to be 60 and 300 μM, respectively). The CAC of *trans*-**2** was found to be the same as surfactants **1** and CTAB, while the C_{CI} was even lower than CTAB, highlighting its strong cooperativity and rendering it the most efficient surfactant for DNA condensation of those studied in this work, that is, low concentrations of surfactant are required to both start DNA condensation and to render it inaccessible to small molecules. An increase in the hydrophobic surfactant chain is known to increase the cooperativity of the binding to DNA.[63] The *cis*-**2** surfactant, on the other hand, shows the largest CAC of all surfactants under study, including all three possessing a shorter chain length. The C_{CI} and ΔZ_{hp} are however lower than that of DTAB, showing a stronger cooperative binding than the commercial surfactant. It should be mentioned that *cis*-**2** has a low solubility, and surfactant crystals were observed at concentrations of 2.5 mM using cryogenic transmission electron microscopy,[45] which could be the reason behind the surprisingly high CAC.

Electrophoretic Mobility Experiments. The condensation of DNA induced by the cationic surfactants was additionally studied using EMSA experiments, and the results are shown in Figure 2. The complexation of the surfactant to the DNA can be seen by the decrease in the intensity of the band corresponding to the naked DNA (due to the lower accessibility of the dye), the lower mobility of the bands, and/

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ACS Appl. Bio Mater. 2021, 4, 7034−7043

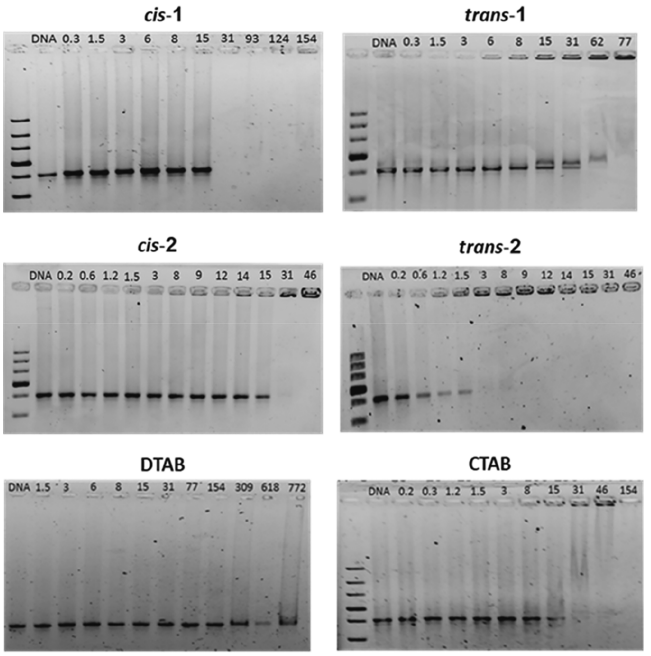

Figure 2. Electrophoretic mobility shift assay of 25 μg/mL DNA with increasing surfactant concentrations. The first lane in each gel contains the ladder, and the lanes marked with DNA refer to DNA in the absence of surfactant. The surfactant concentration for each sample is shown in the gel image as charge ratios (Z_{hp}).

or complete retention of the bands in the wells as the surfactant concentration increases. The impact of the association of cationic surfactants to DNA on its mobility is not trivial. The decrease in the dimensions of the DNA molecules (see AFM results below) increases its electrophoretic mobility. On the other, both the neutralization of the DNA charges and the increase in the molecular weight of the complexes (due to the presence of the surfactant) lead to a decrease in the electrophoretic mobility. The retention of the DNA in the wells at high surfactant concentrations occurs due to the neutralization of the negative charge of DNA and/or the formation of DNA−surfactant complexes that are larger than the mesh size of the gel.[64]

The images show very clearly the difference in the DNA condensation ability of the different cationic surfactants. While it is difficult to assess when condensation begins, the concentration at which no free DNA is observed can be compared to the Z_{hp-2} value obtained from the DEA. Except for the DTAB, the concentration of free DNA is reduced to 0 within the studied surfactant concentration ranges, showing total DNA complexation induced by the surfactant aggregates. We can see that *trans*-**2** is the most efficient surfactant showing complete DNA complexation for Z_{hp} = 3 in excellent agreement with the results of the DEA. CTAB, *cis*-**1**, and *cis*-**2** show similar complexation profiles with the band corresponding to the bare DNA molecules disappearing for Z_{hp} values between 15 and 31. While *cis*-**1** is in reasonable agreement with the DEA, CTAB required a much larger surfactant concentration to reach full DNA condensation and *cis*-**2** required less. It is not clear to us why this is so. The discrepancy between the results obtained using EMSA and DEA has been observed previously for the DNA−CTAB system and justified by the difference in the experimental setup.[47,64] In short, these are the presence of the electric field and the impact of the gel network on the complexes, the

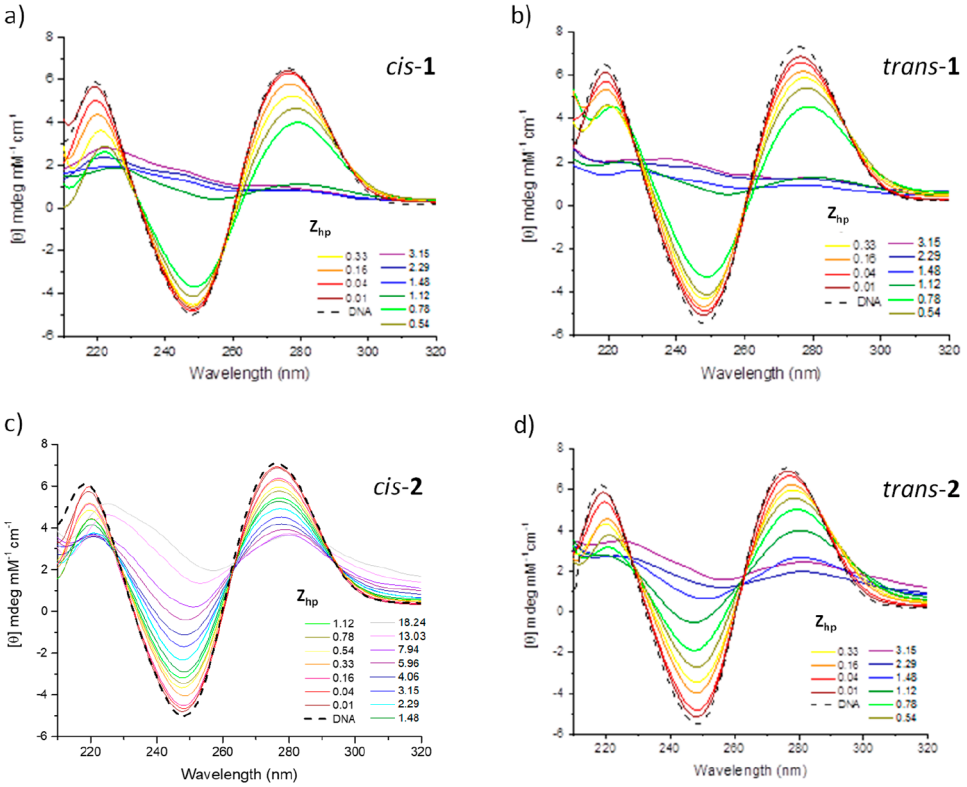

Figure 3. Circular dichroism spectra of (a) salmon sperm DNA-(*cis*-**1**), (b) DNA-(*trans*-**1**), (c) DNA-(*cis*-**2**), and (d) DNA-(*trans*-**2**) complexes at different Z_{hp} values.

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ACS Appl. Bio Mater. 2021, 4, 7034−7043

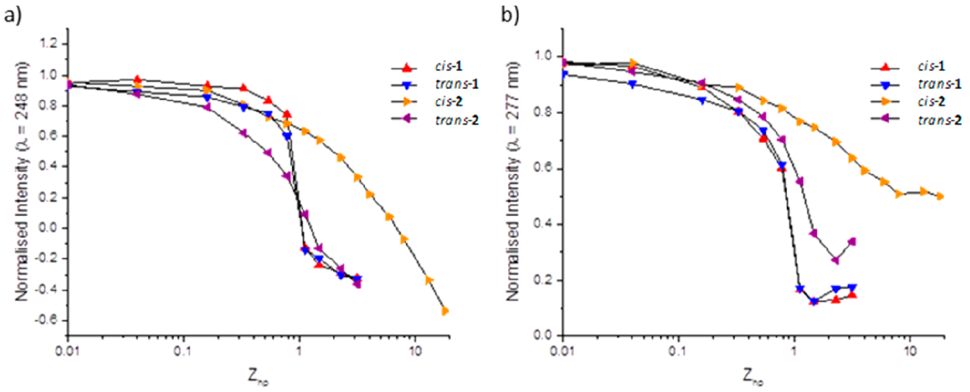

Figure 4. Intensity of CD spectra of the DNA−surfactant complexes at (a) 248 nm and (b) 277 nm normalized to the intensity of the band for DNA alone.

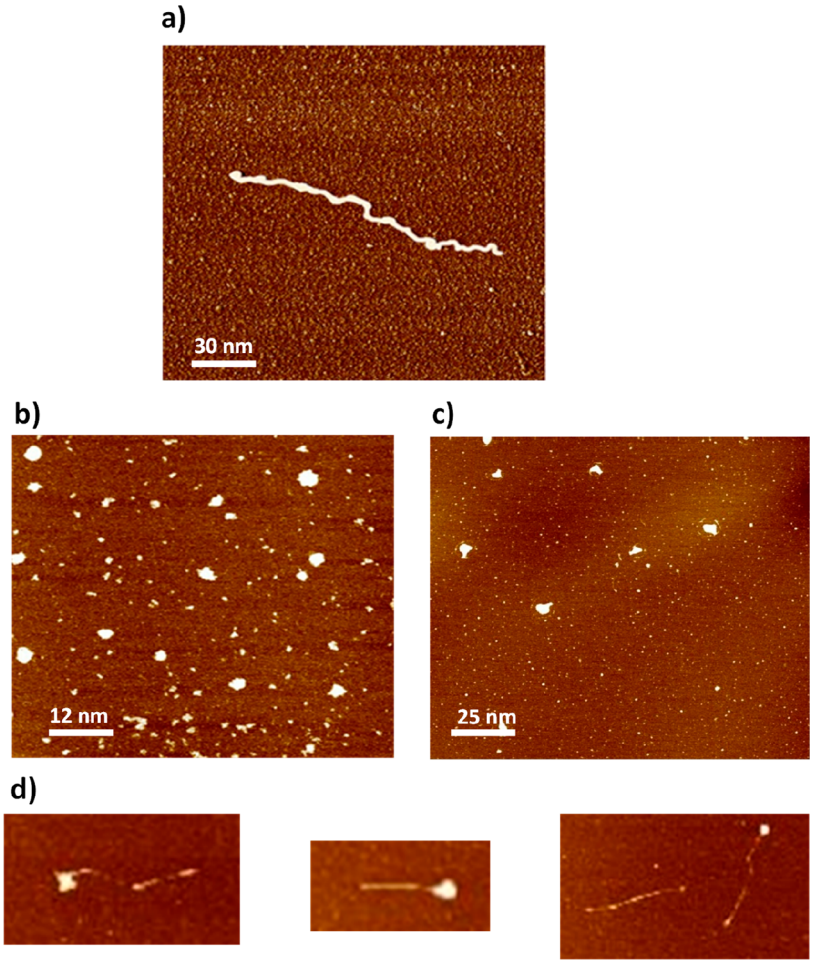

Figure 5. AFM images of (a) free DNA and DNA complexed with (b) *cis*-**1** and (c) *trans*-**1** during the compaction process at $Z_{hp} \sim 6$. Panel (d) shows extended and partially compacted DNA chains obtained at $Z_{hp} \sim 6$.

dilution of the complexes when these are loaded in the gel, and the different buffer used in the gel electrophoresis (to keep the buffering capacity at higher temperatures). However, they do not seem to affect all samples equally. It was also surprising that a Z_{hp} above 62 was required to induce full complexation of DNA using *trans*-**1**, making it the least efficient surfactant in this assay with the exception of DTAB. While these results were in lesser agreement with the DEA data, some of the trends are the same. Namely, the effect of stereochemistry is more pronounced in the case of the longer-chained surfactants, and these also show a stronger ability to complex DNA.

Circular Dichroism of DNA−Surfactant Complexes. The variation of the DNA conformation upon surfactant addition was monitored by CD spectroscopy.[65−68] As shown in Figure 3, the CD spectrum of DNA is affected by the addition of different amounts of surfactant.

The black dashed curves in Figure 3 refer to DNA control samples (without surfactant) and show the characteristic features of the B form of DNA given by a negative signal at 245

https://doi.org/10.1021/acsabm.1c00683
ACS Appl. Bio Mater. 2021, 4, 7034−7043

nm, a positive band at 274 nm, and a crossover near 260 nm.[69] When one starts with *cis*- and *trans*-**1** surfactants (Figure 3a,b, respectively), it is seen that, up to Z_{hp} = 0.78, the intensity of both positive and negative bands slightly decrease without an appreciable shift in their position. At Z_{hp} of 1.12 and above, significant changes are observed in the CD spectra of the DNA. Besides the large decrease in the signal, the negative band at around 246 nm, attributed to the helicity of the DNA chain,[66] becomes positive; the positive band at 274 nm shifts to slightly larger wavelengths, and a small increase in the intensity of the band at 220 nm is observed with a further increase in surfactant concentration. Similar, albeit less extreme, changes have been observed for intermediate concentrations of gemini cationic surfactants.[66]

cis- and *trans*-**2** surfactants seemingly induce analogous changes to the CD spectrum of DNA, but the variations are more gradual (Figure 3c,d). In addition, a larger concentration of *cis*-**2** surfactant is required to induce the large variations in the CD spectrum of DNA.

To best visualize the differences between the surfactants, the normalized intensity of the peaks at 248 and 277 nm is shown as a function of the ratio of charge, Z_{hp}, in Figure 4.

It is interesting to note that the abrupt variation in intensity observed for both *cis*-**1** and *trans*-**1** surfactants occurs at $Z_{hp} \sim 1$, identified as the CAC values ($Z_{hp\text{-}1}$) for these surfactants based on the DEA experiments. On the other hand, *trans*-**2**, which was deemed to be the surfactant with the most cooperative binding to DNA, is found to give rise to a smoother conformational transition, though seemingly starting at lower surfactant concentrations, at least in what concerns the negative band. Finally, *cis*-**2** shows, again, a very smooth decrease in intensity. Interestingly, the smoother conformational transitions observed with CD were obtained for the longer-chained surfactants (*cis*- and *trans*-**2**) although the reason for this remains unclear.

Although it is difficult to compare the results of the different techniques directly, the results obtained using CD spectroscopy are consistent with those of DEA and EMSA experiments. These results suggest that the length of the DNA chain (2 or 4 kbp) has a low influence on the condensation process and on the CAC value.

Atomic Force Microscopy. It has been observed previously that *cis*- and *trans*-**1** surfactants form, above the CMC and at neutral pH, vesicles and mixtures of vesicles and fibers, respectively. The longer-chained surfactants, on the other hand, were found to form precipitates and crystals.[45] Even though these studies are performed with surfactant concentrations below the CMC, it was interesting to verify the impact of the stereochemistry of the surfactant in the morphology of the formed complexes. To this end, DNA−surfactant complexes formed with *cis*- and *trans*-**1** were analyzed by AFM. To verify the impact of surfactant concentration on the complex geometry, a concentration of surfactant of $Z_{hp} \sim 6$, between $Z_{hp\text{-}1}$ and $Z_{hp\text{-}2}$, was considered.

Figure 5a shows an AFM image of a DNA molecule with a contour length of about 1.35 μm as one would expect from a 4145 bp-long molecule. Panels (b) and (c) show images of the DNA−surfactant complexes for *cis*- and *trans*-**1**, respectively. Clearly, these surfactants compact DNA into very small complexes with diameters ranging from about 55 to 110 nm. This is in a similar size range to DNA−surfactant complexes precipitated from chloroform (diameters of 25−70 nm using 300−500 bp long DNA molecules)[70] and the hydrodynamic radius (~80 nm) of T2-DNA (164 kbp)−CTAB complexes.[71] DNA compaction by the 12 carbon-long surfactants under study is thus shown to be very effective and suitable for biological studies. Furthermore, extended DNA chains and partially compacted chains were also observed in these sample as shown in panel (d), confirming that the large error bars in the DEA experiments are related to the coexistence of different species in solution due to the cooperative binding of the surfactant molecules.

CONCLUSION

DNA condensation by β-amino acid-based surfactants possessing different chain lengths and headgroups with *cis*/*trans* stereochemistry was studied using a range of techniques and compared with the well-studied surfactants DTAB and CTAB. All considered surfactants were shown to induce DNA condensation, which is expected to provide DNA with protection against DNases.[64] The effect of the *cis*/*trans* stereochemistry was found to be most relevant for a surfactant with the longest alkyl chain with *cis*-**2** showing the highest CAC according to dye exclusion assays. We speculate that this may be related to the lower solubility of this surfactant. *trans*-**2**, on the other hand, was shown to be the most efficient of the studied surfactants with a CAC similar to that of CTAB and a very short coexisting region of condensed and extended DNA molecules. Surprisingly, the β-amino acid-based surfactants with the shortest chain length (C_{12}) were found to be as efficient in condensing the first DNA molecules as CTAB (C_{16}), considered a reference of efficiency. These results point out the singularity of the headgroups in these surfactants that show stronger attractive interactions than for the tetramethylammonium ones, thus favoring the formation of surfactant aggregates. This is probably due to hydrogen bonding between the headgroups and/or the enhanced hydrophobicity of these surfactants induced by the chiral cyclobutane unit. The relation between the CAC of the DNA−surfactant systems and the CMC of the surfactants was found to be less straightforward than usual. The CMC varies with the pH of the solution and, although the work was performed in a buffer solution at pH 7.4, the presence of DNA is likely to affect the protonation/deprotonation of the surfactants. Considering the potential of using pH-sensitive lipid formulation in the delivery of nucleic acids, a more in-depth study of these mechanisms is deemed important, as extrapolating the efficiency of a lipid formulation based on its CMC only may not be sufficient.

The very low concentrations of β-amino acid-based surfactant required to condense DNA (except for *cis*-**2**), the obtained DNA−surfactant aggregates with diameters of about 55−110 nm, and the absence of cytotoxicity[45] identify these cationic surfactants as promising candidates for efficient DNA delivery.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.1c00683.

Details for the DEA experiments: experimental parameters of the measurements of the emission spectra of the DNA−GelStar−surfactant mixtures (Table S1), emission spectra of DNA−GelStar complexes at different concentrations of *cis*-1 (Figure S1), and emission spectra of DNA−GelStar complexes at different surfactant

https://doi.org/10.1021/acsabm.1c00683
ACS Appl. Bio Mater. 2021, 4, 7034−7043

concentrations for all studied surfactants (Figure S2) (PDF)

AUTHOR INFORMATION

Corresponding Authors

Rita S. Dias − *Department of Physics, The Norwegian University of Science and Technology, NO-7491 Trondheim, Norway;* orcid.org/0000-0001-9600-0934; Email: rita.dias@ntnu.no

Rosa M. Ortuño − *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain;* orcid.org/0000-0001-7635-7354; Email: Rosa.Ortuno@uab.cat

Authors

Bernat Pi-Boleda − *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain;* Present Address: Kao Corporation, S.A., Puig dels Tudons, 10, 08210 Barberà del Vallès, Barcelona, Spain

Sravani Ramisetty − *Department of Physics, The Norwegian University of Science and Technology, NO-7491 Trondheim, Norway;* Present Address: University of Southern California Health Sciences Campus, Zilkha Neurogenetic Institute, 1501 San Pablo Street, Los Angeles, CA 90033, USA.

Ona Illa − *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain;* orcid.org/0000-0001-7390-4893

Vicenç Branchadell − *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain;* orcid.org/0000-0003-3480-1669

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.1c00683

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support from Ministerio de Economía y Competitividad (MINECO), Spain (CTQ2016-77978-R AEI/FEDER, UE), is gratefully acknowledged.

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