Influence of the Spacer of Cationic Gemini Amphiphiles on the Hydration of Lipoplexes

Paola Luciani,[†,‡] Cecilia Bombelli,[†] Marisa Colone,[§] Luisa Giansanti,[†,‡] Samppa J. Ryhänen,[||] V. Matti J. Säily,[||] Giovanna Mancini,*[,†] and Paavo K. J. Kinnunen[||]

CNR, Istituto di Metodologie Chimiche−Sezione Meccanismi di Reazione, and Dipartimento di Chimica, Universitá degli Studi di Roma "La Sapienza" P. le A. Moro 5, 00185 Roma, Italy, Centro di Eccellenza per i Materiali Innovativi Nanostrutturati per Applicazioni Chimiche, Fisiche e Biomediche, CEMIN, Via Elce di Sotto, 06123 Perugia, Italy, Dipartimento di Tecnologie e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy, and Helsinki Biophysics and Biomembrane Group, Institute of Biomedicine/ Biochemistry, University of Helsinki, Haartmaninkatu 8, FIN-00014 Helsinki, Finland

Received February 19, 2007; Revised Manuscript Received March 30, 2007

The impact of the length of gemini surfactant spacer on complexation and condensation of calf thymus DNA by cationic mixed phospholipid/gemini liposomes was investigated by monitoring the conformational changes of DNA by circular dichroism and the lipid hydration level by the emission characteristics of the fluorescent probe laurdan included in the lipid bilayer. The length of the spacer was shown to influence, on one hand, the hydration level and the organization of the corresponding liposomes and, on the other, the variation of lipid hydration level and the DNA conformation upon complexation. In fact, in correspondence with the longest spacer we observed more hydrated liposomes, probably organized in domains, a higher extent of dehydration promoted by the addition of DNA, and a minor extent of DNA conformational change. The physicochemical features of lipoplexes were shown to depend on the [cationic headgroup]/[DNA single base] ratio.

Introduction

Since the discovery of the potential of cationic lipids in delivering genes to cells, lipid/DNA complexes have became one of the most promising tools for a safe gene therapy. Different formulations can lead to different transfection results, and it has been established that changes in the physicochemical features of the complexes affect the transfection efficiency. Efficient transfection requires optimal phospholipid and surfactant architecture and the best potential nonviral vectors to date were obtained empirically by modulating phospholipid and surfactant structures. The most studied systems are lipid/cationic surfactant mixtures with the natural phospholipids 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine with *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride, and *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTAP).[1] Lately, besides these conventional amphiphiles, gemini surfactants have been taken into consideration as promising transfecting agents.[2] One of the advantages of using gemini surfactants is that they feature a wider possibility of structural modification with respect to single head/single tail surfactants, because it is possible to modulate the various segments of the surfactant, that is, one or both headgroups, one or both hydrophobic chains, and the spacer. Modulation of the molecular structure of a gemini yielded a transfecting agent that, once internalized by endocytosis, allows release of DNA into the cytoplasm because of pH-induced phase transition of the lipoplex with destabilization of endosome.[3] Recently we reported that the stereochemistry and substitution of the spacer of a class of gemini surfactants strongly affect their capability to condense calf thymus (CT) DNA[4] and plasmid DNA[2c] and, in the latter case, of conveying DNA into cells.

A full comprehension of the parameters responsible for interactions governing the assembly of components is critical, because a successful contact between the components may yield a compact structure that protects DNA from enzymatic degradation.

The colloidal behavior of gemini surfactants is controlled by the length of the spacer, because it controls head group charge separation and chain packing.[5] Hence it is anticipated that the length of the structure between the two charged headgroups also might control the complexation of DNA driven by electrostatic interactions.

Here we report a study aimed at investigating the influence of the spacer length of a gemini series in the complexation of calf thymus (CT) DNA by liposomes formulated with DMPC[6] and any of the three cationic gemini surfactants (**G)**, *N*,*N*′-dihexadecyl-*N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diammonium bromide (**G2**), *N*,*N*′-dihexadecyl-*N*,*N*,*N*′,*N*′-tetramethylpropane-1,3-diammonium bromide (**G3**), and *N*,*N*′-dihexadecyl-*N*,*N*,*N*′,*N*′-tetramethylbutane-1,4-diammonium bromide (**G4**). The conformational variations of DNA, induced by complexation, were followed by circular dichroism (CD). Because, usually, lipoplex formation decreases the extent of hydration of the lipid double layer,[7] we followed this process by fluorescence spectroscopy utilizing laurdan (6-dodecanoyl-2-dimethylaminonaphthalene), a membrane probe sensitive to the polarity of the environment. Gel electrophoresis experiments allowed us to evaluate the extent of DNA complexation by the different cationic liposomes.

* Corresponding author: tel 00390649913078; fax 003906490421; e-mail giovanna.mancini@uniroma1.it.

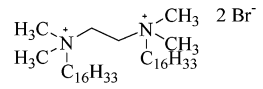
[†] CNR Istituto di Metodologie Chimiche−Sezione Meccanismi di Reazione, Universitá degli Studi di Roma "La Sapienza", and CEMIN.

[‡] Dipartimento di Chimica, Universitá degli Studi di Roma "La Sapienza".

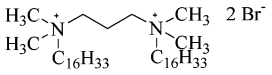
[§] Istituto Superiore di Sanità.

[||] Institute of Biomedicine/Biochemistry, University of Helsinki.

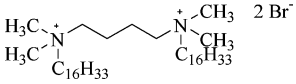
10.1021/bm070202o CCC: $37.00
Published on Web 05/23/2007



G2



G3



G4

Experimental Section

Materials. CT DNA, DMPC, ethylenediaminetetraacetic acid (EDTA), *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), ethidium bromide, and laurdan were purchased from Sigma. The gemini surfactants **G**, *N*,*N*′-dihexadecyl-*N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diammonium bromide (**G2**), *N*,*N*′-dihexadecyl-*N*,*N*,*N*′,*N*′-tetramethylpropane-1,3-diammonium bromide (**G3**), and *N*,*N*′-dihexadecyl-*N*,*N*,*N*′,*N*′-tetramethylbutane-1,4-diammonium bromide (**G4**) were prepared by quaternization of *N*,*N*,*N*′,*N*′-tetramethyl-1,4-ethanediamine, *N*,*N*,*N*′,*N*′-tetramethyl-1,4-propanediamine, and *N*,*N*,*N*′,*N*′-tetramethyl-1,4-butanediamine (Aldrich) with 1-bromohexadecane in 2-propanol, at room temperature;[8] the surfactants were crystallized from methanol/diethylic ether. Freshly deionized filtered water (Milli RO/Milli-Q, Millipore Inc., Jaffrey, NH) was used in all experiments and for preparing HEPES buffer (5 mM HEPES + 0.1 mM EDTA, at pH 7.4).

Sample Preparation. DNA concentrations (expressed in micromolar single base) were determined by absorbance at 260 nm (ϵ = 6600 L/mol·cm).

Monodispersed 100 nm liposomes were prepared by extrusion,[9] mixing appropriate amounts of the lipid stock solutions in chloroform (DMPC/**G** in a 1/1 ratio).[10] Thereafter the solvent was removed by evaporation under a stream of nitrogen. For removal of residual amounts of solvent, the samples were further maintained under high vacuum for at least 8 h; lipid films were then hydrated with HEPES buffer to obtain a 1.7 mM total lipid concentration, freeze−thawed and then extruded by a LiposoFast small volume homogenizer (Avestin, Ottawa, Canada) extruder by subjecting them to 19 passes through polycarbonate filter (100-nm pore size; Nucleopore, Pleasanton, CA).

DNA/DMPC/**G** complexes (lipoplexes) for electrophoresis, CD, and fluorescence experiments were prepared by addition of known volumes of an aqueous 2 mM solution of DNA in HEPES buffer to monodispersed suspensions of liposomes at the concentrations reported in Table 1. At a constant 85.0 μM concentration of CT DNA, both neutral (ρ = [cationic headgroup]/[DNA single base] = 1) and cationic (ρ = [cationic headgroup]/[DNA single base] = 2) complexes were obtained.

For fluorescence experiments, known volumes of laurdan stock solution in chloroform (1:1000 molar ratio of probe to lipid) were included in the indicated lipid mixtures.

Complexation Evaluation. Neutral (ρ = 1) and cationic (ρ = 2) lipoplexes were run on 1% agarose gel with ethidium bromide (0.25 mg/mL) for 45 min at 65 eV, both 10 min (t = 0) and 24 h (t = 24 h) after preparation.

Circular dichroism measurements were carried out by a Jasco spectropolarimeter J-715 (Jasco, Easton, MD). Spectra were acquired in a 1-cm path length quartz cuvette placed in the sample compartment thermostated at 303 K, above the transition temperature, T_m, of the studied formulations (the T_m of DMPC/**G**, 1/1, being 300 K for the formulation containing **G3** and 301 K for those containing either **G2** or **G4**). Spectra were measured as the average of four scans from 220 to 350 nm at a scan rate of 50 nm/min. The spectra were performed 10 min after the addition of DNA to the liposome suspensions (t = 0) and after 24 h of incubation (t = 24 h). Between the acquisitions the samples were kept at 303 K.

Table 1. Concentration of the Lipid Components of Lipoplexes Used for CD and Fluorescence Experiments[a]

samples	total lipid concn, μM	DMPC, μM	**G**, μM
DMPC/**G2**	85.0	42.5	42.5
DMPC/**G2**	170.0	85.0	85.0
DMPC/**G3**	85.0	42.5	42.5
DMPC/**G3**	170.0	85.0	85.0
DMPC/**G4**	85.0	42.5	42.5
DMPC/**G4**	170.0	85.0	85.0

[a] CT DNA = 85.0 μM.

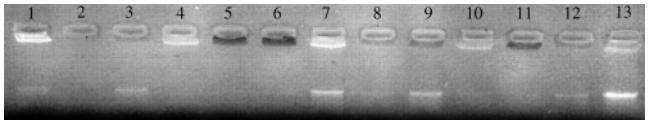


Figure 1. CT DNA complexation by cationic liposomes assessed by gel electrophoresis. Lanes correspond to lipoplexes formulated with **G2** (lanes 1, 4, 7, and 10), **G3** (lanes 2, 5, 8, and 11), and **G4** (lanes 3, 6, 9, and 12) at ρ = [cationic headgroup]/[DNA single base] = 1 and t = 0 (lanes 1−3), at ρ = 2 and t = 0 (lanes 4−6), at ρ = 1 and t = 24 h (lanes 7−9), and at ρ = 2 and t = 24 h (lanes 10−12). Lane 13 corresponds to free CT DNA.

Fluorescence spectroscopy measurements were carried out by a Perkin-Elmer LS 50B spectrofluorometer interfaced to a Pentium PC. Two milliliters of DMPC/**G** solution were placed into a magnetically stirred four-window quartz cuvette held in the sample compartment thermostated at 288 and 303 K. The spectra were performed 10 min after the addition of DNA to the liposome suspensions (t = 0) and after 24 h of incubation (t = 24 h).

Laurdan is a membrane probe highly sensitive to environment polarity, and it displays a large red shift of the emission in polar solvents with respect to nonpolar solvents.[11] It is possible to follow the interfacial water changes in the cationic liposomes upon their complexation with DNA by means of the spectral variations of the laurdan,[7] calculating the generalized polarization function (GP)[12] of each investigated sample as follows:

$$\mathrm{GP} = (I_{440} - I_{490})/(I_{440} + I_{490}) \quad (1)$$

where I_{440} and I_{490} are the intensities of the emission at wavelengths 440 and 490 nm, respectively.

The effect of DNA on GP for various cationic liposomes is described as

$$\Delta\mathrm{GP} = \mathrm{GP\ of\ lipoplexes} - \mathrm{GP\ of\ liposomes} \quad (2)$$

Emission spectra were obtained at two different excitation wavelengths, i.e., 340 and 410 nm.

Results and Discussion

Lipoplex Characterization by Gel Elecrophoresis. CT DNA was incubated with the indicated liposomes at two different charge ratios (ρ = 1 and ρ = 2) for 10 min and 24 h and run on agarose gel in the presence of ethidium bromide in order to evaluate the complexing capability of liposomes. Free DNA runs through the gel and bright bands are visible in the corresponding lanes (as shown in lane 13 of Figure 1, corresponding to CT DNA in the absence of liposomes). The more DNA is associated with the liposomes, the less its fragments run on the gel. When DNA is completely complexed, the lipoplexes remain in the starting well, at the top of the gel.

The results from gel electrophoresis experiments are reported in Figure 1. On the basis of what is explained above, the lanes

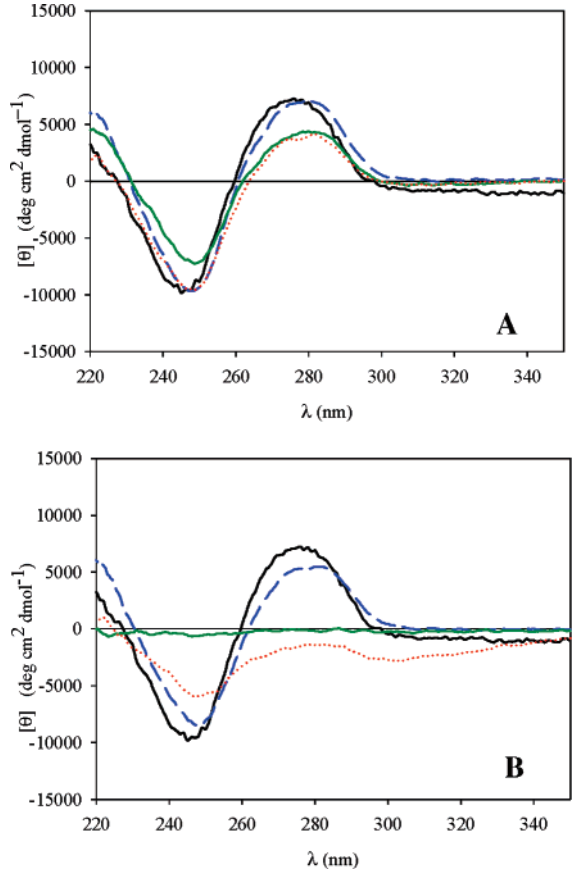


Figure 2. CD spectra of CT DNA/DMPC/**G** lipoplexes (**G2**, – blue dashed line; **G3**, – green solid line; **G4**, – red dotted line) at a charge ratio $\rho = 1$, at $t = 0$ (A) or $t = 24$ h (B). The solid black line is the CD spectrum of uncondensed DNA (DNA in HEPES-buffered solution). Measurements were carried out, at 303 K, on samples at 85 μM total lipid and 85 μM DNA single base concentration.

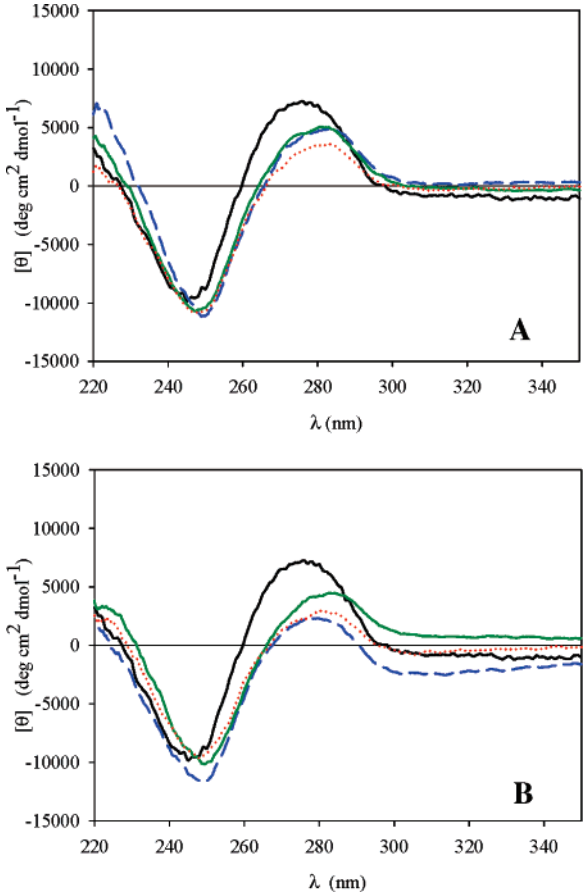


Figure 3. CD spectra of CT DNA/DMPC/**G** lipoplexes (**G2**, – blue dashed line; **G3**, – green solid line; **G4**, – red dotted line) at a charge ratio $\rho = 2$, at $t = 0$ (A) or $t = 24$ h (B). The solid black line is the CD spectrum of uncondensed DNA (DNA in a HEPES-buffered solution). Measurements were carried out, at 303 K, on samples at 170 μM total lipid and 85 μM DNA single base concentration.

that show a high extent of DNA complexation are those corresponding to neutral lipoplexes formulated with **G3** (both at $t = 0$ and $t = 24$ h; lanes 2 and 8, respectively) and all cationic lipoplexes at $t = 0$ (lanes 4, 5, and 6, corresponding to lipoplexes formulated with **G2**, **G3**, and **G4**, respectively) and at $t = 24$ h (lanes 10, 11, and 12, corresponding to lipoplexes formulated with **G2**, **G3**, and **G4**, respectively). These results underline that the length of the gemini spacer as well as the charge ratio may substantially modulate the capability of DMPC/**G** liposomes to bind DNA.

CD Investigations. In Figure 2 the CD spectra of lipoplexes formed by CT DNA/DMPC/**G** at a $\rho = 1$ charge ratio are reported, at $t = 0$ and $t = 24$ h (panels A and B, respectively). In each panel of Figure 2 we also reported the spectrum (solid line) of a buffered solution of CT DNA that shows the typical chiroptical features of DNA in the B form (B-DNA).[13] The spectra of neutral lipoplexes formulated with **G3** and **G4** show at $t = 0$ very small variations with respect to the features of B-DNA (Figure 2A, solid line); the same formulations precipitate either completely (**G3**) or partially (**G4**) after 24 h (Figure 2B). CD spectra relative to neutral lipoplexes ($\rho = 1$) containing **G2** did not show any relevant variation at either $t = 0$ or $t = 24$ h, as expected on the basis of the results obtained in the gel electrophoresis experiments that showed, in this case, a limited complexation.

In Figure 3 we reported the CD spectra of lipoplexes at a $\rho = 2$ charge ratio, at $t = 0$ and $t = 24$ h (panels A and B, respectively). CD spectra of all cationic lipoplexes show a decrease in the intensity of the positive band and a modest increase of the negative band, that is, the typical features of the C form of DNA (C-DNA),[13] both at $t = 0$ (panel A) and at $t = 24$ h (panel B). Conformational variations induced by **G2** are more significant than those induced by the other two geminis. Therefore, both neutral and cationic **G2**-containing lipoplexes behave differently with respect to the others: the neutral ones are less effective in inducing conformational variations of DNA, whereas cationic ones are the most effective.

Fluorescence Measurements. In principle, fluorescence emission spectra should be independent of excitation wavelength; however, this may not be true for fluorescent probes included in systems containing two or more components. In fact, it was shown that laurdan excitation spectrum obtained in gel-phase vesicles features a higher intensity in the red edge, with respect to the excitation spectrum obtained in the liquid crystalline phase. The behavior of this fluorescent probe in the pure phase allows, in the presence of domains, a preferential excitation of laurdan molecules in the gel phase, by excitation at the red edge.[14] We have therefore used two excitation wavelengths (λ_{exc}), 340 or 410 nm, for revealing the possible presence of domains in our mixed liposomes.

Because laurdan is a membrane probe sensitive to the phase of the lipid bilayer, and the transition from gel to liquid crystalline phase depends on temperature, we carried out our experiments at two temperatures, that is, at 303 K, under the same conditions of CD experiments (above the T_m of DMPC/

Table 2. GP Values of DMPC/**G** Liposomes at 303 K[a]

samples	total lipid concn, μM	$GP_{liposomes}$ ($t = 0$)	$GP_{liposomes}$ ($t = 24$ h)
DMPC/**G2** liposomes	85.0	0.260 ± 0.026	0.314 ± 0.015
DMPC/**G2** liposomes	170.0	0.273 ± 0.032	0.310 ± 0.019
DMPC/**G3** liposomes	85.0	0.002 ± 0.010	0.139 ± 0.021
DMPC/**G3** liposomes	170.0	-0.002 ± 0.009	0.103 ± 0.010
DMPC/**G4** liposomes	85.0	-0.083 ± 0.021	-0.003 ± 0.009
DMPC/**G4** liposomes	170.0	-0.076 ± 0.018	-0.003 ± 0.008

[a] Excitation wavelength was 340 nm; CT DNA = 85 μM.

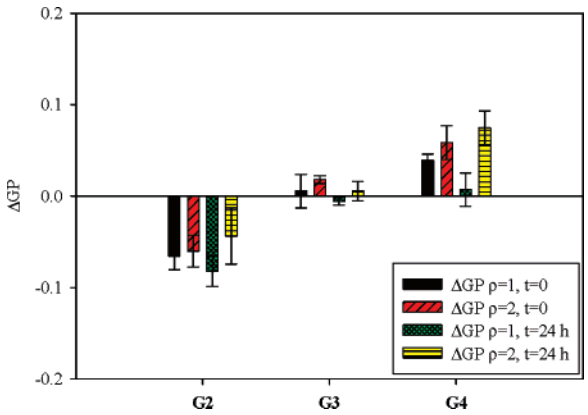


Figure 4. ΔGP values at 303 K. Excitation wavelength was 340 nm.

G, 1/1), and at 288 K, well below the T_m of our mixed liposomes, where all possible domains should be in the gel phase.

The emission spectrum of laurdan shows a dependence on the excitation wavelength in the measurements carried out at 303 K for the formulations containing **G3** and **G4**, whereas it is independent of excitation wavelength in the experiments carried out at 288 K on all formulations and at 303 K on those formulated with **G2** (as an example, the spectra relative to **G2**- and **G4**-containing formulations are reported as Supporting Information). Hence, the variation of laurdan emission spectrum as a function of excitation wavelength suggests the presence of domains in liposomes formulated with **G3** and **G4**. This confirms the different behavior of **G2** when compared to **G3** and **G4**, observed by CD. However, the results obtained at 303 K by excitation at either 340 or 410 nm show a similar trend; consequently, here we report only results relative to λ_{exc} 340 nm (Table 2 and Figure 4) while all other result are reported as Supporting Information.

On the basis of the definition of GP, a negative value corresponds to a higher intensity of the emission at 490 nm and thus to a more polar environment, whereas a positive value corresponds to a less polar environment. Results of fluorescence experiments reported in Table 2 (as the average of three independent experiments and three measurements for each experiment) show that the medium sensed by laurdan depends on the spacer length of the gemini used in the formulations; actually, the polarity of the medium increases in correspondence with a longer spacer of the gemini, demonstrating that hydration of the lipid double layer increases by increasing the length of the spacer.

Histograms relative to ΔGP values are shown in Figure 4. Upon addition of DNA, there is a clear correlation between the length of the spacer and ΔGP values. In fact the dehydration induced by the addition of CT DNA to liposomes containing **G4** is higher with respect to the dehydration shown by lipoplexes containing **G3**, whereas the addition of CT DNA to liposomes containing **G2** induces hydration.

Both CD and fluorescence experiments show that the length of gemini spacer influences the complexation process; in fact, **G2**-containing liposomes have different features with respect to liposomes containing geminis with a longer spacer (namely, **G3** and **G4**), and behave differently in the complexation of CT DNA. GP values reported in Table 2 show that **G2**-containing liposomes are less hydrated than the other mixed liposomes. This lower extent of hydration could be explained by the formation of a salt bridge between the phosphate group of DMPC and the quaternary nitrogen of the gemini. This is in analogy to what was suggested previously[7] for explaining the lower hydration observed in DOTAP liposomes formulated with either 1,2-dioleoyl-*sn*-glycero-3-phosphocholine or 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine with respect to 100% DOTAP liposomes. In principle, the ion pairing with the formation of a salt bridge could be possible in the presence of each of the geminis investigated, but it probably occurs only in the case of **G2** because it reduces the repulsion of the two ammonium head groups due to their vicinity. This ion interaction would reduce the coordination of water by charged groups; at the same time this would reduce, as well, the possibilities for DNA to be complexed at $\rho = 1$, because of the strong reduction of the available cations. The formation of a salt bridge between **G2** ammonium groups and DMPC phosphate group could also account for the absence of segregation in domains observed in liposomes formulated with this gemini. At the same time the high charge density featured by **G2** could be responsible for the highest conformational change toward a C-DNA observed upon complexation of the biopolymer by cationic liposomes containing **G2** at $\rho = 2$ (Figure 3). In fact, a recent investigation[15] has identified the C-form of DNA as a BII-rich B-form and indicated that BII nucleotides are stabilized by complexation of DNA phosphate groups via densely charged cations.

Fluorescence experiments also indicate that the complexation of DNA induces dehydration of the double layer in the presence of **G4**, negligible variation in the presence of **G3**, and hydration of the lipid double layer in the presence of **G2**. It is probable that, in the case of **G2**, the complexation of the DNA by **G2** cationic head groups, by breaking part of the above-mentioned salt bridges, would render DMPC phosphate groups available for water coordination. We have previously reported that the surface charge density of binary membranes of cationic and zwitterionic lipids affects the molecular level organization of the air−water interface. At low surface charge density the $P^-{-}N^+$ dipoles of PC headgroups reorient after electrostatic pairing with cationic headgroups,[16] and subsequently, at higher surface densities, a transition to interdigitated phase was observed.[17]

Conclusions

A rationalization of the parameters controlling the complexation of DNA is fundamental to the success of lipid systems as nucleic acid vehicles, and we believe that knowledge of the physicochemical properties of liposomes and of the corresponding lipoplexes is imperative for understanding their behavior in the biological milieu.

We have investigated the role of the length of gemini surfactant spacer on the complexation of calf thymus DNA, a model system that can give valuable information on the physicochemical features of lipoplexes.[4,18] The obtained results demonstrate that the length of the spacer influences the

complexation of DNA and the physicochemical features of lipoplexes. A lower hydration level of liposomes, a higher hydration of lipid double layer upon addition of DNA, a higher extent of DNA conformation changes toward a C-form, and the absence of segregation in domains were observed in correspondence to the presence of the gemini featuring the shortest spacer.

The rationalization of these findings demonstrates for the first time, as far as we know, that ion pairing between cationic gemini and the phosphate of the PC headgroup is dependent on the spacer length of the gemini. This is an interesting finding because it clearly points out that interactions between lipid headgroups affect the interaction with DNA and should be taken into account when designing lipofection systems.

The results of our investigations underline the importance of a rational approach to the design of the major components of transfecting formulations and of exploring the physicochemical features of the resulting complexes.

Acknowledgment. We acknowledge contributions from CNR, Dipartimento di Progettazione Molecolare. Helsinki Biophysics and Biomembrane Group (HBBG) is supported by the Finnish Academy. S.J.R. acknowledges financial support from the Emil Aaltonen Foundation. Kristiina Söderholm and Kaija Niva are thanked for technical assistance.

Supporting Information Available. Emission spectra of laurdan included in **G4**- and **G2**-containing liposomes obtained by excitation at 340 and 410 nm at 303 K (Figures S1 and S3 for **G4** and **G2**, respectively) and at 288 K (Figures S2 and S4 for **G4** and **G2**, respectively); tables relative to liposome GP values λ_{exc} 410 nm + 303 K (Table S5); λ_{exc} 340 nm + 288 K (Table S6); and λ_{exc} 410 nm + 288 K (Table S7); and histograms relative to ΔGP values λ_{exc} 410 nm + 303 K (Figure S8), λ_{exc} 340 nm + 288 K (Figure S9), and λ_{exc} 410 nm + 288 K (Figure S10). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Simberg, D.; Weisman, S.; Talmon, Y.; Barenholz, Y. *Crit. Rev. Ther. Drug Carrier Syst.* **2004**, *21*, 257−317. (b) Pedroso de Lima, M. C.; Simões, S.; Pires, P.; Faneca, H.; Düzgüneş, N. *Adv. Drug Delivery Rev.* **2001**, *47*, 277−294. (c) Ren, T.; Song, Y. K.; Zhang, G.; Liu, D. *Gene Ther.* **2000**, *7*, 764−768.

(2) (a) Jennings, K. H.; Marshall, I. C. B.; Wilkinson, M. J.; Kremer, A.; Kirby, A. J.; Camilleri, P. *Langmuir* **2002**, *18*, 2426−2429. (b) Kirby, A. J.; Camilleri, P.; Engberts, J. B. F. N.; Feiters, M. C.; Nolte, R. J. M.; Söderman, O.; Bergsma, M.; Bell, P. C.; Fielden, M. L.; Garcia Rodríguez, C. L.; Guédat, P.; Kremer, A.; McGregor, C.; Perrin, C.; Ronsin, G.; van Eijk, M. C. P. *Angew. Chem., Int. Ed.* **2003**, *42*, 1448−1457. (c) Bombelli, C.; Faggioli, F.; Luciani, P.; Mancini, G.; Sacco, M. G. *J. Med. Chem.* **2005**, *48*, 5378−5382.

(3) (a) Ellens, H. M.; Monck, M. A.; Yeh, P.-Y. PCT Int. Appl. WO 2002036073, 2002; 44 pages. (b) Bell, P. C.; Bergsma, M.; Dolbnya, I. P.; Bras, W.; Stuart, M. C. A.; Rowan, A. E.; Feiters, M. C.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **2003**, *125*; 1551−1558.

(4) Bombelli, C.; Borocci, S.; Diociaiuti, M.; Faggioli, F.; Galantini, L.; Luciani, P.; Mancini, G.; Sacco, M. G. *Langmuir* **2005**, *21*, 10271−10274.

(5) Zana, R. *J. Colloid Interface Sci* **2002**, *248*, 203−220.

(6) DMPC was chosen because it features a relatively low T_c, though characterized, as well as the investigated surfactants, by saturated alkyl chains. The choice of a saturated lipid allowed us to avoid the introduction of an additional parameter.

(7) Hirsch-Lerner, D.; Barenholz, Y. *Biochim. Biophys. Acta* **1999**, *1461*, 47−57.

(8) Moss, R.; Sunshine, W. L. *J. Org. Chem.* **1974**, *39*, 1083−1089.

(9) Hope, M. J.; Nayar, R.; Mayer, L. D.; Cullis, P. R. In *Liposome Technology*, 2nd ed; Gregoriadis, G., Ed.; CRC Press: Boca Raton, FL, 1992; Vol. 1, pp 123−139.

(10) Liposomes prepared at higher DMPC/**G** ratios did not show relevant capability of complexing CT DNA.

(11) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, 1999.

(12) (a) Parasassi, T.; Giusti, A. M.; Gratton, E.; Monaco, E.; Raimondi, M.; Ravagnan, G.; Sapora, O. *Int. J. Radiat. Biol.* **1994**, *65*, 329−334, (b) Parasassi, T.; Gratton, E. *J. Fluoresc.* **1995**, *5*, 59−69.

(13) Zuidam, N. J.; Barenholz, Y.; Minsky, A. *FEBS Lett.* **1999**, *457*, 419−422.

(14) (a) Parasassi, T.; De Stasio, G.; d'Ubaldo, A.; Gratton, E. *Biophys. J.* **1990**, *57*, 1179−1186.; (b) Parasassi, T.; De Stasio, G.; Ravagnan, G.; Rusch, R. M.; Gratton, E. *Biophys. J.* **1991**, *60*, 179−189.

(15) van Dam, L.; Levitt, M. H. *J. Mol. Biol.* **2000**, *304*, 541−561.

(16) (a) Säily, V. M. J.; Ryhänen, S. J.; Holopainen, J. M.; Borocci, S.; Mancini, G.; Kinnunen, P. K. J. *Biophys. J.* **2001**, *81*, 2135−2143. (b) Ryhänen, S. J.; Säily, M. J.; Paukku, T.; Borocci, S.; Mancini, G.; Holopainen, J. M.; Kinnunen, P. K. J. *Biophys. J.* **2003**, *84*, 578−587. (c) Säily, V. M. J.; Alakoskela, J. A.; Ryhänen, S. J.; Karttunen, M.; Kinnunen, P. K. J. *Langmuir* **2003**, *19*, 8956−8963.

(17) Ryhänen, S. J.; Alakoskela, J. A.; Kinnunen, P. K. J. *Langmuir* **2005**, *21*, 5707−5715.

(18) Zhou, S.; Liang, D.; Burger, C.; Yeh, F.; Chu, B. *Biomacromolecules* **2004**, *5*, 1256−1261.

BM070202O