Infrared Spectroscopic Characterization of the Interaction of Cationic Lipids with Plasmid DNA*

Sirarat Choosakoonkriang‡, Christopher M. Wiethoff‡, Thomas J. Anchordoquy§, Gary S. Koh¶, Janet G. Smith†, and C. Russell Middaugh‡‡

From the ‡Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047, the §Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, Colorado 80262, and ¶Valentis, Inc., Burlingame, California 94010

Fourier transform infrared spectroscopy was used to characterize the interaction of the cationic lipids 1,2-dioleoyl-3-trimethylammonium-propane and dioctadecyldimethylammonium bromide with plasmid DNA. The effect of incorporating the neutral colipids cholesterol and dioleoylphosphatidylethanolamine on this interaction was also examined. Additionally, dynamic and phase analysis light scattering were used to monitor the size and ζ potential of the resulting complexes under conditions similar to the Fourier transform infrared measurements. Results suggest that upon interaction of cationic lipids with DNA, the DNA remains in the B form. Distinct changes in the frequency of several infrared bands arising from the DNA bases, however, suggest perturbation of their hydration upon interaction with cationic lipids. A direct interaction of the lipid ammonium headgroup with and dehydration of the DNA phosphate is observed when DNA is complexed with these lipids. Changes in the apolar regions of the lipid bilayer are minimal, whereas the interfacial regions of the membrane show changes in hydration or molecular packing. Incorporation of helper lipids into the cationic membranes results in increased conformational disorder of the apolar region and further dehydration of the interfacial region. Changes in the hydration of the DNA bases were also observed as the molar ratio of helper lipid in the membranes was increased.

Complexes of cationic lipids with DNA are currently being employed as nonviral gene delivery vehicles for a variety of therapeutic applications (1, 2). Complexes shown to be most effective for transfection typically contain neutral colipids, termed helper lipids, such as Chol1 or DOPE (3–5). Information, however, regarding the physical properties of cationic lipid-DNA complexes necessary for efficient transfection of cells both in vitro and in vivo is incomplete. Characterization of the size and electrokinetic potential of these complexes has resulted in some understanding of their colloidal properties as they relate to the efficiency of transfection (6, 7). The fluorescence properties of extrinsic membrane probes have been used to characterize changes in the electrostatic surface potential (8, 9), membrane fluidity (10), thermotropic phase behavior (11), and degree of hydration (12) of cationic lipids upon DNA binding. Probably most instructively, the longer range order of these complexes has been characterized by small angle x-ray scattering (13, 14) and cryo-electron microscopy (15, 16). Results from these studies suggest the formation of multilamellar, liquid crystalline phases of the lipid, with DNA sandwiched in between. Additional studies using small angle x-ray scattering have shown the interaxial spacing of DNA to be highly dependent on membrane charge density (17). Monitoring changes in the secondary structure of DNA upon interaction with cationic lipids has been attempted using circular dichroism, but interpretation is complicated by large changes in peak position and intensity from the induction of chiral supramolecular structures (18, 19) and potential differential scattering and absorption flattening artifacts (20).

FTIR and Raman scattering have the potential to provide significant detail about the fine structure of both cationic lipids and DNA upon complexation without the use of extrinsic probes. In the case of lipids, these vibrationally based spectroscopic techniques have been used to characterize the degree of conformational disorder of the apolar region of membranes (21, 22), the hydration state of carbonyl groups in interfacial regions (23, 24), as well as headgroup orientation as a function of various thermotropic and lyotropic perturbations (25). They have also been used to analyze changes in duplex DNA under various environmental conditions and to determine the effect of binding of various drugs and toxins on DNA structure (26, 27). In general, the potential utility of vibrational methods to characterize complexes of cationic lipids with DNA is probably greater than other spectroscopic techniques because of reduced effects of light scattering, applicability to heterogeneous systems, and the plethora of resolvable peaks from each component. No such studies, however, have yet been reported. Therefore, we have used FTIR spectroscopy to characterize the interaction of cationic and helper lipids with DNA.

**EXPERIMENTAL PROCEDURES**

**Materials**

DOTAP, DDAB, DOPE, and Chol (Fig. 1) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and used without further purification. Plasmid pMB113 (95% supercoiled) encoding the wild-type cystic fibrosis transmembrane conductance regulator was obtained from Valentis, Inc. (Burlingame, CA). The concentration of DNA was determined by UV absorbance at 260 nm using an extinction coefficient of 0.02 (mg/ml)−1 cm−1.

**Methods**

**Preparation of Liposomes and Complexes**—Cationic lipids were deposited on the wall of glass vials from chloroform solutions by evapo-
Cationic Lipids

DOTAP

DDAB

Helper Lipids

DOPE

Chol.

Fig. 1. The structure of the cationic lipids, DOTAP and DDAB, and helper lipids, cholesterol and DOPE, used in this study.

rating the solvent under a stream of nitrogen gas. The film obtained was then placed under vacuum for 2 h prior to being hydrated in 10 mM Tris buffer, pH 7.4, at a temperature above the Tc of the lipid for 30–60 min (Room temperature for DOTAP and 70 °C for DDAB). Vesicles were then extruded 10 times through 100 nm Nuclepore membranes (Whatman, Clifton, NJ). Liposomes were stored at 4 °C and used within 3 days of preparation.

Lipid-DNA complexes were prepared in a total volume of 0.6 ml using various weight ratios of lipid to DNA. Complexes were prepared by mixing equal volumes of solutions containing lipid or DNA (2 mg/ml), with the solution containing the lesser molar concentration of charge (DNA phosphate or cationic lipid) being added to the solution in charge excess. The resulting complexes were continuously stirred for 10 min and equilibrated at room temperature for 20 min before measurement.

Fourier Transform Infrared Spectroscopy—FTIR spectra were obtained with a Nicolet Magna-IR 560 spectrometer equipped with a mercury-cadmium-telluride detector (Nicolet, Madison, WI). Samples were measured by an attenuated total internal reflectance method in which the sample in solution was placed directly in a zinc selenide trough (Thermal A.R.K., SpectraTech, Shelton, CT). Although the use of attenuated total internal reflectance opens up the possibility that the material observed near the surface of the attenuated total internal reflectance element may be structurally perturbed by surface adsorption, spectra obtained in a conventional transmittance cell were found to be very similar. Spectra were obtained under dry air purge by coaddition of 256 interferograms. The interferograms were apodized using the Happ-Genzel function, with no zero filling, to give a final resolution of 4 cm

1. For attenuated total internal reflectance experiments, the association band of water around 2200 cm

2 was used as a reference for subtraction of water from the spectra (28). Line correction (1804 to 904 cm

1) and seven point Savitsky-Golay smoothing were applied to all spectra. Peak positions were assigned using a peak finding algorithm provided with the OmniC™ software. Spectra were obtained from several, independently prepared sample preparations (including at least three different lots of DNA and lipid) and averaged. Error bars reflect the standard error of the mean (n = 6–9 samples).

Dynamic Light Scattering—A Brookhaven (Holtsville, NY) BI-200SM system with a 50 mW HeNe laser was used for all measurements. Measurements were taken at 90° to the incident light. Samples were prepared by diluting the samples prepared for FTIR measurements 10-fold with Tris buffer that had been exhaustively filtered through a 0.2-μm filter (Acrodisc™). Data were collected over a period of 3 min. An electronic “dust filter” was used to exclude 2-s intervals of data whose average intensity differed by 30% of the average for the 3-min interval. The mean diffusion coefficients were obtained from the autocorrelation function using the method of cumulants (29). The effective diameter was calculated using the Stokes-Einstein equation.

Phase Analysis Light Scattering—A ZetaPALS instrument (Brookhaven) was used to measure the electrophoretic mobility of the complexes using samples prepared as above. Measurements were obtained using an electric field strength of 14–16 V/cm, which was reversed at a frequency of 3–5 Hz. Data were averaged over 30 cycles. The ζ potential of the particles was calculated from the electrophoretic mobility using the Smoluchowski approximation.

RESULTS

Spectral Properties of CLDC Components—Approximately 35 well defined DNA absorption bands occur in the region from 1800–700 cm

1 (30). A representative spectrum of the plasmid used in these studies is shown in Fig. 2. Several bands in this spectrum are representative of B-form DNA. The guanine/thymidine carbonyl stretching band at 1715 cm

1, indicative of interstrand base pairing, the asymmetric phosphate stretching band at 1223 cm

1, and the band at 970 cm

1, resulting from a 2’-endo deoxyribose conformation, are the most prominent and consistent indicators of B-form DNA in our hands. Additionally, bands at 1328, 1281, and 897 cm

1 (the latter not shown) further support the conclusion that the plasmid remains in the B-form (30). Despite water subtraction, the spectral regions between 1700 and 1600 cm

1 and below ~850 cm

1 are still partially obscured by the presence of the O-H bending vibrations of water. Although some data regarding base pairing/stacking is unavailable in the spectral regions dominated by water, a significant amount of data is still available in the region above 1700 cm

1 and between 1600 and 850 cm

1. We have found most useful the changes in three DNA vibrational bands related to the base carbonyls (1715 cm

1), the imidazole nitrogen of guanine (1492 cm

1), and the phosphate backbone (1223 cm

1).

The spectrum of the cationic lipid DOTAP is also shown in Fig. 2 (A and B). As seen from the structure of DOTAP in Fig. 1, the main structural features include an unsaturated hydrocarbon side chain resulting in asymmetric and symmetric methylene C-H stretching bands at 2924 and 2854 cm

1 (Fig. 2A) and various methylene bending bands between 1470 and 1100 cm

1 (Fig. 2B). The ester portion of DOTAP gives rise to a carbonyl stretching band at 1739 cm

1, while the trimethylammonium headgroup produces a weak band at 1489 cm

1 (asymmetric C-N stretching). Because these were key structural changes, the methylene C-H and carbonyl stretching bands were followed upon formation of complexes with DNA.

Similarly, DDAB, the cationic lipid found in the transfection reagent LipofectACE™ was also studied. As predicted from the structure of DDAB (Fig. 1), a much simpler spectrum is observed because of the absence of C-O vibrations (compare Fig. 2, B–D). Additionally, DDAB contains saturated alkyl chains that increase its Lc to Ld phase transition to between 40 and 50 °C. DDAB vesicles show the methylene C-H stretching vibrations at 2918 (asymmetric) and 2850 cm

1 (symmetric) (Fig. 2C). Other major bands observed include methylene bending vibrations from 1470 to 1100 cm

1 and a characteristic C-N asymmetric stretching band at 1489 cm

1 from the ammonium group (Fig. 2D). Again, because of their sensitivity to DNA binding, methylene C-H stretching bands were followed in monitor complex formation between DDAB and DNA.

FTIR of DOTAP-DNA Complexes—Given the distinct assignments of the signals from the DNA and lipid discussed above, several bands were monitored as cationic lipids were added to a fixed amount of DNA at various weight ratios. Representative spectra of several complexes at various weight ratios are shown in Fig. 2 (A and B). Changes in the vibrational bands of DNA were followed as the ratio of DOTAP to DNA was varied. The
carbonyl stretching band, reflecting interstrand hydrogen bonding, manifests a quasi-linear increase from 1715 to 1719 cm\(^{-1}\) with a discontinuity in the trend observed around a 0.5 weight ratio (Fig. 3D). The guanine/cytosine vibration at 1492 cm\(^{-1}\) shows a linear decrease to 1489.5 cm\(^{-1}\), saturating at a weight ratio of 0.6 as increasing amounts of DOTAP are added (Fig. 3E). The DNA asymmetric phosphate stretching band manifests a saturable increase in frequency as DOTAP is added, going from 1223 to 1226.5 cm\(^{-1}\) with the spectral shift complete at a ratio of 0.5 (Fig. 3F). In contrast, the frequency of the symmetric phosphate stretching vibration remains constant at \(1089\) cm\(^{-1}\) (data not shown). Although distinct changes in several of the DNA vibrations are noted, the presence of bands around 1715 and 1223 and at 1328, 1281, and 970 cm\(^{-1}\) all suggest that DNA remains in a B conformation.

Several reproducible spectral changes were also observed in DOTAP vibrational modes upon complexation with DNA (Figs. 2, A and B, and 3, A–C). Both the asymmetric and symmetric methylene C-H stretching bands show identical trends exhibiting subtle increases in frequency compared with that of DOTAP alone (2923.7 and 2854 cm\(^{-1}\), respectively; Fig. 3, A and B). Less systematic but reproducible variations in peak positions are also seen at lower charge ratios. The carbonyl stretching vibration of DOTAP shows a gradual decrease from 1743.5 to 1737 cm\(^{-1}\) with increasing lipid, which saturates above a 0.7 weight ratio (Fig. 3C).

**FTIR of DDAB-DNA Complexes**—When DDAB is added to DNA at various weight ratios, discrete changes are also observed in the vibrational frequencies of several bands from both the lipid and DNA (Figs. 2, C and D, and 3, G–K). As seen with DOTAP, the spectral features of the DNA around 1715, 1223, 1328, 1281, and 970 cm\(^{-1}\) suggest that the DNA remains in the B-form at all ratios of DDAB to DNA. Several trends for the DNA signals parallel those seen for complexes of DOTAP and DNA (Fig. 3, I–K). The frequency of the base pairing sensitive carbonyl stretching band shows a slight increase as DNA is titrated with increasing amounts of DDAB (Fig. 3I), albeit to a much lesser extent than observed for DOTAP complexes (Fig. 3D). The most distinctive feature is a small optimum in frequency shift of \(-1\) cm\(^{-1}\) seen at low weight ratios (\(-0.5\); Fig. 3I). The band at 1492 cm\(^{-1}\) is strongly altered at low lipid-DNA weight ratios, dropping 3 cm\(^{-1}\) to 1489 cm\(^{-1}\) at a 0.5 ratio, followed by a more gradual decline (Fig. 3J). The phosphate asymmetric stretching band shows a trend similar to that observed for DOTAP complexes, in which it gradually increases from 1223 to 1225 cm\(^{-1}\) as DDAB is added up to a ratio of 0.5–0.7 (Fig. 3K). Both the asymmetric and symmetric C-H stretching bands of DDAB show a decrease in frequency beginning at low lipid ratios (0.2 to 0.25) from 2921 to 2919 cm\(^{-1}\) and 2852 to 2851 cm\(^{-1}\), respectively (Fig. 3, G and H).

**The Influence of Cholesterol on the Interaction of Cationic Lipids with DNA**—Helper lipids such as Chol and DOPE have
been shown to significantly improve the transfection efficiency of vectors employing cationic lipids (3–5). This effect is presumably due to the altered membrane properties upon addition of helper lipid. Therefore, the effect of these lipids on the cationic membrane structure itself, as well as on the interaction of these binary lipid bilayers with DNA, was monitored using FTIR spectroscopy by varying the amount of helper lipid in:

For DOTAP-DNA complexes, with both shifts saturating at 1720 cm$^{-1}$.

The Influence of DOPE on the Interaction of Cationic Lipids with DNA—Of the bands arising from DNA, only the frequency of the guanine/thymidine carbonyl stretching vibration of the DOPE is observed at 1736 cm$^{-1}$ as it is a unique vibration of the DOPE molecule.

An upward shift in the frequency of the lipid carbonyl stretching band was observed with increasing amounts of DOPE incorporated into the DOTAP vesicles (Fig. 5C). These results are somewhat confounded by the fact that DOPE possesses a carbonyl stretching band itself. This band is located around 1737 cm$^{-1}$ for a dispersion of DOPE alone, which is 5 cm$^{-1}$ lower than the frequencies observed here. Upon complexation of the DOTAP-DOPE vesicles with DNA, a consistent decrease in the frequency of this band of 1–2 cm$^{-1}$ was observed at all ratios.

The effect of incorporating DOPE into DDAB vesicles is shown in Fig. 5 (G–I). The methylene C-H stretching bands of the lipid show increases in frequency to 2853 and 2924 cm$^{-1}$ (data not shown), suggesting a slight increase in the mobility of the alkyl groups of the lipids. As observed previously with Chol, the addition of DNA did not cause any further shifts for all ratios of DOPE to DOTAP examined with the exception of the complex formed in the absence of DOPE (compare solid and dashed lines in Fig. 5, A and B).

An abrupt increase in frequency was seen at ratios above 0.5, indicating lipid mobility comparable with that seen for DOPE alone (1737 cm$^{-1}$). When complexed to DNA, the DOPE carbonyl stretching band shows an overall decrease in frequency relative to free lipid as the ratio of DOPE to DOTAP, and hence DOPE to DNA phosphate, is increased (Fig. 5I). This decrease in frequency is similar to the trend observed for the lipid

Incorporating Chol into DDAB vesicles also results in frequency changes in lipid C-H stretching bands as shown in Fig. 4 (G and H, dashed lines). Upon addition of as little as 10 mol% of Chol into the vesicles, an increase of $\sim 1$ cm$^{-1}$ in the C-H stretching bands was observed, similar to that seen for DOTAP (Fig. 4, A and B). Further incorporation of Chol results in an additional increase in the frequency of the asymmetric C-H stretching band from 2919 to 2921 cm$^{-1}$, whereas the symmetric stretching band shows a minimal increase of 0.5 cm$^{-1}$ up to a Chol/DDAB molar ratio of 1. The trends are comparable when the vesicles are complexed with DNA, with the notable exception of complexes formed in the absence of Chol.

The effect of DOPE on the C-H stretching bands for DOTAP vesicles is seen as a distinct 2-cm$^{-1}$ increase in frequency to 2853 and 2854.5 cm$^{-1}$ (Fig. 5, A and B). DOPE alone displays these vibrational modes at 2924 and 2853.4 cm$^{-1}$ (data not shown), suggesting a slight increase in the mobility of the alkyl groups of the lipids. As observed previously with Chol, the addition of DNA did not cause any further shifts for all ratios of DOPE to DOTAP examined with the exception of the complex formed in the absence of DOPE (compare solid and dashed lines in Fig. 5, A and B).

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The effect of incorporating DOPE into DDAB vesicles is shown in Fig. 5 (G–I). The methylene C-H stretching bands of the lipid show increases in frequency to 2853 and 2924 cm$^{-1}$ once DOPE is added above a molar ratio of 0.25 (Fig. 5, G and H). The presence of DNA at a 1:1 weight ratio with DDAB does not affect these bands significantly. The carbonyl stretching vibration of the DOPE is observed at 1736 cm$^{-1}$ when it is added to DDAB vesicles at a molar ratio of 0.1 (Fig. 5I). An abrupt increase in frequency was seen at ratios above 0.5, indicating lipid mobility comparable with that seen for DOPE alone (1737 cm$^{-1}$). When complexed to DNA, the DOPE carbonyl stretching band shows an overall decrease in frequency relative to free lipid as the ratio of DOPE to DOTAP, and hence DOPE to DNA phosphate, is increased (Fig. 5I). This decrease in frequency is similar to the trend observed for the lipid

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carbonyl stretching band; $E$

solid lines represent lipid-DNA complexes. $B$

dylene stretching band; $B$

DOPE to cationic lipid. $D$

complexes. The peak positions were plotted for DOTAP-DNA ($A$–$F$)

and $F$
cytosine; $D$

DDAB-DNA complexes were also determined (Fig. 6, $A$

at around 220 nm as lipid is added up to a 0.6 weight ratio (Fig.

The size of DDAB-DNA complexes remains relatively constant

up to a weight ratio of 1.5 (Fig. 6$A$)). This size increase appears

to occur in two phases with the second phase initiated at a weight ratio of 0.5. Beyond this point, the complexes have an estimated size much greater than 1 $\mu$m, making it impossible to accurately determine the hydrodynamic radius by dynamic light scattering. Fortunately, complexes prepared at ratios above 1.5 could be examined by phase analysis light scattering to determine $\zeta$ potential. Phase analysis light scattering analysis of the complexes indicates a fairly constant negative $\zeta$ potential between $-40$ and $-60$ mV up to a weight ratio of 2 (Fig. 6$B$). Beyond this ratio, the $\zeta$ potential gradually increases to values of $+20$ mV at a ratio of 4. These data demonstrate that the DOTAP-DNA complexes studied by FTIR spectroscopy all possess negative $\zeta$ potentials. The size and $\zeta$ potential of DDAB-DNA complexes were also determined (Fig. 6, $A$ and $B$).

The size of DDAB-DNA complexes remains relatively constant at around 220 nm as lipid is added up to a 0.6 weight ratio (Fig.

6A). Above this point, aggregation of the complexes occurs resulting in particles greater than 1 $\mu$m in diameter. The $\zeta$ potential shows a similar trend to that observed for DOTAP complexes. It is interesting that despite the larger hydrodynamic size of the DDAB-DNA complexes, their $\zeta$ potentials are very similar to that of DOTAP-DNA complexes.

The colloidal properties of complexes upon incorporation of the helper lipids Chol and DOPE are shown in Fig. 6 (C–$F$). For DOTAP complexes, addition of Chol results in only a minor increase in size from 185 to 215 nm (Fig. 6$C$). DDAB-Chol complexes demonstrate an average diameter that is roughly twice as large as the DOTAP-Chol complexes (compare Fig. 6, $E$ and $C$). As the amount of Chol increases, the colloidal stability of the DDAB complexes is greatly increased, i.e. in the absence of Chol, DDAB complexes form particles greater than 1 $\mu$m in diameter. Above a Chol to DDAB molar ratio of 0.5, the size appears to decrease from 400 nm to around 300 nm (Fig. 6$E$). When DOPE-DOTAP vesicles are complexed with DNA, a smaller particle size is observed (Fig. 6$C$). The size remains fairly constant up to a DOPE to DOTAP ratio of 0.75, above which the diameter gradually increases. In contrast, the size of DDAB-DOPE complexes remains between 150 and 180 nm for all ratios of DOPE to DDAB, again showing a marked stabilizing effect of helper lipid on the DDAB-DNA complexes.

In general, complexes of cationic lipid and DNA (in the absence of helper lipid) at a 1:1 weight ratio possess negative $\zeta$ potentials (Fig. 6$B$). The $\zeta$ potential of complexes containing both cationic and helper lipid all show similar trends regardless of the cationic or helper lipid used (Fig. 6, $D$–$F$). Surprisingly, as the amount of helper lipid in the complex is increased, the $\zeta$ potential decreases despite constant quantities of cationic
lipid. The greatest decrease occurs for DDAB-DOPE complexes that exhibit a 2-fold decrease in the magnitude of the $\zeta$ potential.

**DISCUSSION**

Attempts to study the helical conformation of DNA upon complexation with cationic lipids using ultraviolet circular dichroism have led to the suggestion by several authors that upon complexation with cationic lipids, DNA adopts a form (e.g., C conformation) rather than the native B conformation (31). Possible spectral contributions of chiral supramolecular aggregates (18) as well as artifacts such as the differential scattering of circularly polarized light and absorption flattening phenomena (20) inhibit unambiguous physical interpretation of these results. By using FTIR spectroscopy, we are able to provide strong evidence that DNA remains in the B-conformation when complexed with cationic lipids. This observation is supported by the relatively minor changes in several B-form-specific bands upon titration of DNA with cationic lipid as well as the absence of other bands indicative of non-B forms of DNA (30).

Although the DNA appears to remain in the B-form in cationic lipid-DNA complexes, distinct changes in several DNA vibrational bands are observed. Typically, changes in frequency of the vibrations from polar groups such as those studied here (C = O, N-C-H, and O = P-O-) result from alterations in external noncovalent interactions such as the formation or loss of hydrogen bonds. For example, the frequency shifts observed in the bands assigned to the C6 carbonyl and N7 of guanine suggest alterations in the hydration of these two groups. Both the carbonyl and imidazole nitrogen are located in the major groove of B-form DNA, with the carbonyl located further away from the phosphate backbone (32). Although the N7 band shows an equivalent decrease in frequency for both cationic lipids investigated, the carbonyl band shows a much greater increase when DNA is complexed to DOTAP than to DDAB. Based upon the lamellar model proposed for the complexes formed between cationic lipids and DNA (13) it seems that the observed differences in the effect of DOTAP and DDAB on the carbonyl vibration likely reflect the orientation of DNA relative to the lipid bilayer as well as the interfacial conformation and mobility of the lipid ammonium headgroup. Furthermore, we suggest that the more extended headgroup of DOTAP allows it to interact more strongly with the DNA carbonyl, resulting in the greater frequency shift observed here. Unfortunately, the band from the lipid ammonium headgroup appears to dominate the spectrum near 1492 cm$^{-1}$ at higher weight ratios, making interpretation of the effects of cationic lipids on the imidazole nitrogen difficult. However, the frequency decrease observed at lower weight ratios seems to indicate a dehydration of guanine. A previous study of the interaction between a bis(arginyl) derivative of a tricationic porphyrin and duplex DNA found a similar trend in these bands as the porphyrin was titrated into DNA (33). This was interpreted as a direct interaction of the arginine residues with guanine. It was postulated that these shifts reflect an alteration of the hydrogen bonding of the carbonyl and imidazole nitrogen of guanine, consistent with our interpretation. Additional studies regarding the specificity of this interaction are warranted.

Because the interaction between cationic lipids and DNA is thought to be largely electrostatic, the asymmetric phosphate stretching band was followed as DNA was titrated with cationic lipids. Changes in the vibrational frequency of the DNA phosphate group upon titration with various cationic species have been previously reported (34–36). In most cases, an increase in frequency was observed similar to that reported here. Thus, our findings are consistent with a direct interaction of the lipid headgroup with the phosphate and the concomitant displacement of hydrogen-bonded water. This observation is also consistent with previous studies suggesting that the release of bound water and counterions is the driving force for complex formation (37, 38). Additionally, the release of bound water from the lipid interface upon complexation of DNA with cationic lipids has been reported using both fluorescence techniques and differential scanning calorimetry (12). It is noteworthy that changes observed in all of the DNA vibrations are greatly attenuated above a 0.25–0.35 charge ratio of cationic lipid to DNA phosphate, much earlier than expected based on charge neutralization.

Information regarding the perturbation of the cationic lipids upon interaction with DNA was obtained from changes observed in the methylene C-H stretching bands and, in the case of DOTAP, the carbonyl band at 1739 cm$^{-1}$. A slight increase in the frequency of the methylene C-H stretching bands when either DOTAP or DDAB is bound to DNA suggests a small increase in disordered conformation of the lipid alkyl chains when lipid is complexed to DNA. Small angle x-ray scattering data have demonstrated that complexes of DOTAP with DNA form lamellar liquid crystalline phases in which DOTAP appears to exist as a bilayer (13). These observations suggest that complexation with DNA results in increased fluidity of the apolar region of the membrane. We propose that alignment of the lipid headgroups with the DNA phosphate may create an altered packing of the lipid, resulting in the observed fluidity increase.

The carbonyl stretching band of DOTAP shows a shift from higher frequencies down toward the frequency of DOTAP alone as the ratio of lipid to DNA is increased. This stretching vibration is composed of two complementary vibrations based on populations of carbonyl groups with differing degrees of hydration. Fourier self-deconvolution further resolves these bands into two peaks at 1744 and 1735 cm$^{-1}$, corresponding to dehydrated and hydrated carbonyls, respectively (data not shown). Comparing the intensities of these two bands shows that the frequency shift observed in Fig. 3C is due primarily to changes in the relative contributions of these two components rather than an overall frequency shift. Therefore, our data suggest tighter packing of the lipid molecules and dehydration at the interfacial region of the bilayer when lipid is present in low amounts. This is consistent with dehydration of the DNA phosphates upon complex formation. Although C-H and carbonyl stretching bands typically display parallel frequency changes during lipid phase transitions, the different trends presented here are in agreement with studies employing fluorescence anisotropy to probe the interaction of DOTAP with DNA (10).

Helper lipids such as DOPE and Chol are commonly included in cationic lipid-DNA complexes because they frequently enhance transfection efficiency (3–5). The most studied of the two, DOPE, is thought to increase the delivery of DNA to the nucleus by facilitating the release of DNA from endosomal compartments (5). This is presumably due to the ability of this lipid to facilitate the formation of inverted hexagonal phases (39). In fact, incorporation of significant amounts of DOPE into DOTAP vesicles has been shown by small angle x-ray scattering to result in the formation of the inverted hexagonal phase upon complexation with linear DNA (14). Our data show that incorporation of either helper lipid into vesicles with DOTAP or DDAB results in an increased conformational disorder of the lipid alkyl chains, even at relatively low mole fractions of these lipids. These findings are consistent with perturbations in the normal bilayer structure that have been correlated with increased transfection efficiency (40). The magnitude of the increased conformational disorder for the alkyl chains was
greater for DDAB than DOTAP, possibly because of the fact that DDAB is in the gel phase at room temperature, whereas DOTAP is in the more disordered liquid crystalline phase. The lack of an effect of DNA on the degree of conformational disorder in the alkyl chains in complexes as compared with liposomes alone suggests that the helper lipids are able to fluidize the bilayer (to an extent comparable to DNA binding) prior to complexation. This effect could result from the ability of DOPE and Chol to promote nonbilayer phases in lipid vesicles (39, 41).

In the absence of DNA, the increase in the frequency of the carbonyl stretching vibrations of DOTAP vesicles is similar to that observed for the C-H stretching bands when either Chol or DOPE is incorporated into the bilayer, suggesting dehydration of the bilayer interfacial region. When these vesicles are complexed to DNA, the carbonyl stretching band is decreased relative to vesicles alone, suggesting a more hydrated interfacial region in the complex. Although these data parallel results obtained for DOTAP liposomes including DOPE or Chol by titration of DNA with DOTAP, suggesting that the DOPE carbonyl is either tightly held or somewhat dehydrated when incorporated into DDAB complexes may reflect the increased fluidity and flexibility of the membrane, allowing for the formation of smaller, more stable structures.

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