Tb^{3+} and Ca^{2+} binding to phosphatidylcholine

A study comparing data from optical, NMR, and infrared spectroscopies

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ABSTRACT The paramagnetic and luminescent lanthanides are unique probes of cation-phospholipid interactions. Their spectroscopic properties provide the means to characterize and monitor complexes formed with lipids in ways not possible with biochemically more interesting cations, such as Ca^{2+}. In this work, Tb^{3+}-phosphatidylcholine complexes are described using the luminescence properties of Tb^{3+}, the effect of its paramagnetism on the ^{31}P NMR and ^{13}C NMR spectra of the lipid, and changes in the infrared spectrum of the lipid induced by the cation. There are two Tb^{3+}-phosphatidylcholine complexes with very different coordination environments, as evidenced by changes in the optical excitation spectrum of the lanthanide. The NMR experiments indicate that the two complexes differ in the number of phosphate groups directly coordinating Tb^{3+}. Tb^{3+} binding induces changes in the phosphodiester infrared bands that are most consistent with bidentate chelation of Tb^{3+} by each phosphate, whereas Ca^{2+}-induced changes are more consistent with monodentate coordination. The significance of this discrepancy is discussed.

INTRODUCTION

Even membranes composed of a single phospholipid species present two or three different types of cation binding sites, and mixed lipid systems can be expected to have a proportionately greater number. Characterization of the structures and relative affinities of these sites is important for any analysis of the effects of cations on the chemical or physical activity of membrane lipids. The luminescence properties of two lanthanides, Eu^{3+} (Saris, 1983; Herrmann et al., 1986) and Tb^{3+} (Conti et al., 1987; Halladay and Petersheim, 1988; Petersheim and Sun, 1989), have been shown to be useful in distinguishing binding sites on phospholipid membranes and monitoring exchange among these sites. The objective of the work presented here and elsewhere (Petersheim and Sun, 1989) is to characterize the structures of the complexes indicated by the luminescence studies and to compare these complexes with those formed by the less tractable Ca^{2+}. These details will be useful in understanding surface equilibria and events involving bound cations, such as formation of nonbilayer structures and membrane fusion. In particular, they provide structural information relevant to extending molecular modeling studies of lipid systems (Wendoloski et al., 1989; Scott and Pearce, 1989) to include the effects of cation binding.

In an early luminescence study of Tb^{3+} binding to phosphatidylcholine vesicles two bound states for the cation were observed which were related by a very cooperative phase transition induced in the lipid by the cation (Conti et al., 1987). Chrzeszczyk et al. (1981) also presented evidence for two bound states with Pr^{3+} and phosphatidylcholine using ^{31}P NMR. Several other NMR studies of lanthanide binding to phosphatidylcholines predated that work, but none mentioned more than one state (Hauser et al., 1975, 1976, 1977; Sears et al., 1976; Grasdalen et al., 1977). This lanthanide induced transition in phosphatidylcholine is most likely driven by the positive charge deposited by the bound cation (Akutsu and Seelig, 1981; Altenbach and Seelig, 1984) and was shown to involve the choline quaternary amine being repelled from the surface (McIntosch, 1980).

This transition in the phosphatidylcholine headgroup is interesting for two reasons. Given that phosphatidylcholine, or its analogue sphingomyelin, is the most prevalent phospholipid species in most membrane systems the transition may be relevent to changes in lipid packing in response to changes in membrane potential. The second reason is that the Tb^{3+}-phosphatidylycholine complex that exists at low levels of bound lanthanide has a very unusual coordination. An allowed 4f to 5d Tb^{3+} excitation band shifts from ~217 nm in aquo-Tb^{3+} to 300 nm in this first Tb^{3+}-phosphatidylcholine complex. In the second form of the complex the allowed transition returns to higher energy. This work is directed toward describing these two Tb^{3+}-phosphatidylcholine complexes and comparing them with the complex formed by Ca^{2+}.

MATERIALS AND METHODS

Dimyristoylphosphatidylcholine (DMPC) and lysomonomyristoylphosphatidylcholine (lysoMPC) were purchased from Avanti Polar Lipids,

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Pelham, AL, and used as received. Trimethylphosphate (gold label) was purchased from Aldrich Chemical Co., Milwaukee, WI, and was used to prepare the sodium salt of dimethylphosphate by refluxing in NaI/acetone (Zervas and Dilaris, 1951). Analytical grades of $Ca(NO_3)_2$ and $NaNO_3$ were used and $TbCl_3$ was purchased from Aldrich Chemical Co. as being 99.9% pure.

The dry phospholipids were suspended in a 0.5 M $NaNO_3$ solution or the same containing an appropriate concentration of $Ca(NO_3)_2$ or $TbCl_3$. This high level of nitrate was introduced to obtain maximum binding of the cations in the infrared experiments. In absence of a counterion with a high affinity for the choline headgroup, the deposited charge of the cations greatly inhibits full saturation of the lipid lattice with bound cation. Nitrate has a high affinity for phosphatidylcholine membranes (Tatulian, 1983; Hahn et al., 1983) and it has no infrared bands that overlap with the phosphodiester bands of the lipid. The DMPC samples were sonicated in small glass vials using a 300-W ultrasonic dismembrator with a 25-ml jacketed bath attachment held at 50°C.

Excitation spectra of the Tb^{3+}-phosphatidylcholine complexes were collected on a Fluorolog II spectrofluorometer (Spex Industries, Inc., Edison, NJ), monitoring the 545-nm emission band of Tb^{3+} (Halladay and Petersheim, 1988). ^{31}P (121.5 MHz) and ^{13}C (75.5 MHz) NMR spectra were collected on a model QE 300 spectrometer (General Electric Co., Wilmington, MA) using a 10-mm broadband probe for the ^{31}P experiments and a 5-mm $^1H/^{13}C$ probe for ^{13}C. The pulse sequence consisted of a 3-s delay with the decoupler off for thermal equilibration, a 2-s interval with the decoupler on for nuclear Overhauser enhancement, and ^1H decoupling during acquisition.

Infrared spectra were collected on a Cygnus 25B Fourier transform spectrometer (Mattson Instruments, Madison, WI) using a total-internal-reflectance (TIR) prism liquid cell with a ZnSe crystal (Harrick Scientific Corp., Ossining, NY). The TIR cell was thermally regulated using a refrigerated circulating bath and all experiments were performed at 20.0 ± 0.2°C, as measured with a digital thermometer probe at the cell. The optical chamber of the instrument was purged with nitrogen to remove interference from carbon dioxide and water vapor and the TIR cell was assembled with a syringe and narrow-bore Teflon tubing so that background and sample spectra could be collected without opening the chamber. All spectra were collected with a spectrometer resolution of 2 cm^{-1} and involved coadding 24,000 scans. A solvent spectrum collected under identical conditions was subtracted from the final sample spectrum.

Resolution of overlapping infrared bands was accomplished by both Fourier self-deconvolution and nonlinear regression of selected spectral regions. The deconvolution software was provided with the infrared instrument. Pure Lorentzian bands were assumed with the enhancement factors and line widths employed depending on the particular spectrum. Of the apodization functions available with the software, the Bessel function (Kauppinen et al., 1981) generated the fewest artifacts in the deconvolved spectrum. The nonlinear regression, or curve-fitting, program was adapted from Bevington (1969). Selected regions of the spectrum were fit with up to 16 Lorentzian peaks including two parameters for a linear baseline correction. Residuals from the curve-fitting were inspected and used to establish the minimum number of peaks required to represent the data.

RESULTS

Optical evidence for two Tb^{3+}-phosphatidylcholine complexes

Complexes of Tb^{3+} with phospholipids have distinctive excitation spectra for each headgroup type and unique spectra for different forms of the complex with a given lipid (Halladay and Petersheim, 1988; Petersheim and Sun, 1989; Halladay, 1989; Petersheim et al., 1989). The gross spectral differences observed for these Tb^{3+} complexes are due to two 4f to 5d absorption bands (Carnall et al., 1968). One of these is a strong allowed band, at 217 nm for aquo-Tb^{3+}, and the second is a weak, spin-forbidden band at 260 nm. The positions of both of these bands are very sensitive to changes in ligand field.

The allowed excitation band for aquo-Tb^{3+} shown in Fig. 1 *A* has a maximum at 224 nm rather than 217 nm. This is a consequence of the highly structured profile of the pulsed Xe lamp used for these experiments. Although the emission from continuous Xe lamps has fewer features, the intensity drops precipitously below 230 nm. A pulsed Xe lamp was used in this work to extend the studies down to 200 nm. As a result, several of the sharp features present in the spectra of Fig. 1 are due to convolution of the lamp profile with broader absorption bands.

Fig. 1 *A* also shows the effect of binding to lysoMPC on the Tb^{3+} excitation spectrum (*dashed line*). This Tb^{3+}-lysoMPC spectrum represents three different states for the Tb^{3+}: free Tb^{3+}, as indicated by the band at 224 nm, and two different complexes with lysoMPC indicated by a broad band at 300 nm and the manifold of sharp bands near 260 nm. Again many of these sharp bands are from the lamp profile; these two regions are actually dominated by single broad bands. All of the micelle and bilayer forming phosphatidylcholines form these same two Tb^{3+} complexes, which are more clearly shown by the Tb^{3+}-DMPC spectra of Fig. 1 *B*. The two complexes are related by a Tb^{3+}-induced phase transition that is electrostatically driven (Conti et al., 1987; Halladay and Petersheim, 1988). The complex that dominates at low levels of bound Tb^{3+} has a very strong excitation band at 300 nm (Fig. 1 *B*) which is ~500 times greater than the forbidden bands usually observed for Tb^{3+} >230 nm. Some of these forbidden bands are just barely discernable between 340 and 380 nm in the solid line spectrum of Fig. 1 *B*. This strong 300-nm band is apparently the allowed 4f to 5d absorption found at 217 nm in aquo-Tb^{3+}. At high levels of bound Tb^{3+}, i.e., one Tb^{3+} per 20 lipid molecules, this allowed band moves from 300 nm back to ~217 nm. The remaining bands at higher wavelengths are weak forbidden 4f to 4f transitions or the spin forbidden 4f to 5d band.

More detailed discussion of Tb^{3+} optical properties for complexes with phosphatidylcholine and other phospholipids is presented elsewhere (Conti et al., 1987; Halladay and Petersheim, 1988; Petersheim et al., 1989; Halladay, 1989). These optical spectra are presented here to emphasize that there are two very different coordination states for Tb^{3+} with aggregated forms of phosphatidylcholine.

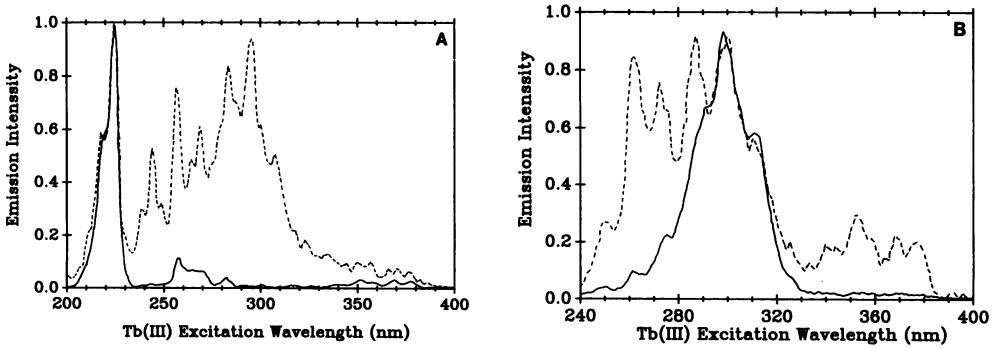


FIGURE 1 Tb^{3+} excitation spectra. (*A*) Emission from aquo-Tb^{3+} (*solid line*) and Tb^{3+}-lysoMPC (*dashed line*) was monitored at 545 nm. The allowed 4f to 5d transition for aquo-Tb^{3+} appears at 224 nm rather than at 217 nm because of the lamp profile. Likewise, the amplitude of this band should be 40–50 times greater than the absorption bands at higher wavelengths. The spin-forbidden 4f to 5d band is located at 260 nm. The manifold of sharp bands from 230 to 330 nm in the Tb^{3+}-lysoMPC spectrum actually represent two broad bands at ~250 and 300 nm, with the features representing the lamp profile. (*B*) Tb^{3+}-DMPC in the first bound state (*solid line*) obtained with 0.035 mM $TbCl_3$ and 2 mM DMPC in 0.1 M NaCl. This spectrum is dominated by the allowed 4f to 5d absorption that has moved to 300 nm. The second bound state for Tb^{3+}-DMPC is dominant at 0.40 mM $TbCl_3$ and 2 mM DMPC in 0.1 M NaCl (*dashed line*). There is some Tb^{3+} present in the first bound state at 0.40 mM Tb^{3+} but it represents <1% of the total bound lanthanide. It is evident at 300 nm because the extinction coefficient for this band is two orders of magnitude greater than that for the second bound state in this region of the spectrum. The emission intensity is in arbitrary units.

NMR evidence for two Tb^{3+}-PC stoichiometries

These two states are also apparent in the effect of paramagnetic lanthanides on the ^{31}P NMR spectrum of phosphatidylcholines. Fig. 2 shows that the Tb^{3+}-induced shift changes in magnitude with the amount of bound Tb^{3+}. For a single bound state this plot would be a horizontal line. Both Tb^{3+}-DMPC and Tb^{3+}-lysoMPC exhibit an inherent ^{31}P shift for the first bound state in the range of -200 to -300 ppm; the final bound state has a shift of ~ -600 ppm relative to the lipid alone. Comparing Tb^{3+}-induced shifts with those by Yb^{3+} and Nd^{3+} reveals that these changes are dominated by Fermi contact interaction between phosphorous and the paramagnetic lanthanide (Halladay, 1989), which requires that the phosphate is covalently attached to the lanthanide (Hauser et al., 1975, 1976, 1977; Grasdalen et al., 1977; Chrzeszczyk et al., 1980).

The transition to the second state appears more fascile for Tb^{3+}-lysoMPC than Tb^{3+}-DMPC. This is not consistent with earlier Tb^{3+}-DMPC optical studies in which it was found that the transition was essentially complete at one Tb^{3+} per 20 DMPC molecules (Conti et al., 1987; Halladay and Petersheim, 1988). This discrepancy is an indication that Tb^{3+} in the second state is not fully in fast exchange until the lipid transition is complete. Thus the observed ^{31}P resonance position may not represent a true population weighted average in the midpart of the Tb^{3+}-DMPC titration. By the same argument, Tb^{3+} appears to be in fast exchange at all levels in the lysoMPC experiment.

Although any change in the ligand field will alter the magnitude of the contact interaction, there is roughly a factor of two difference in the shift for the two complexes. The simplest interpretation of this result is that in the first bound state only a single phosphate coordinates the lanthanide and in the second state there are two coordinating phosphates. ^{13}C NMR studies of lysoMPC complexes with Tb^{3+}, Yb^{3+}, and Nd^{3+} are consistent with this interpretation. The choline and glycerol carbons undergo predominantly dipolar shifts in the presence of the lan-

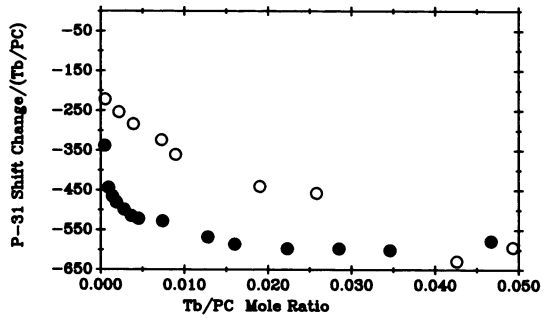


FIGURE 2 ^{31}P NMR evidence for a change in Tb^{3+}-phosphatidylcholine stoichiometry. The ordinate is the inherent shift per bound Tb^{3+} and the abscissa is the mole ratio of Tb^{3+} to lipid phosphate. Both DMPC (*open circles*) and lysoMPC (*solid circles*) exhibit two bound states for the Tb^{3+} with roughly the same factor of two change in the inherent shift. LysoMPC and DMPC are 0.1 M with 0.5 M $NaNO_3$ to assure complete binding of Tb^{3+} and 10% D_2O for the lock. The shift changes are relative to the Tb^{3+}-free lipid solutions, using trimethylphosphate as an external reference in a coaxial tube to partially compensate for bulk suceptibility changes from the added paramagnetic Tb^{3+}.

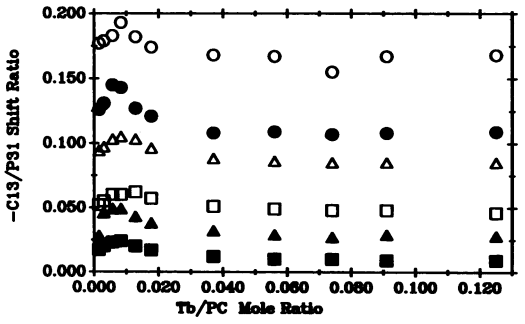
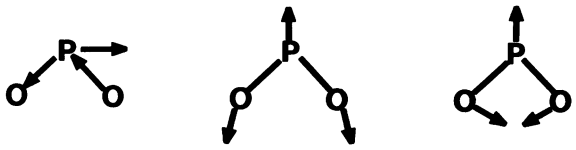


FIGURE 3 ^{13}C NMR evidence for a stoichiometry change for the Tb^{3+}-lysoMPC complex. The ordinate is the change in the ^{13}C band position relative to the ^{31}P band for the same level of added Tb^{3+}. The ^{13}C data displayed is for the choline phosphoester carbon (*open circles*), the glycerol phosphoester (*solid circles*), the CH_2-N (*open triangles*), the N-CH_3 (*open squares*), the glycerol CH-OH (*solid triangles*) and the glycerol ester (*solid squares*).

thanides (Halladay, 1989). Comparing the ^{13}C and ^{31}P shifts gives evidence of a change in lipid packing before transition to the second bound state (Fig. 3). The bimodal behavior of these plots, along with the near factor of two change in ^{31}P shift, suggests (*a*) the first bound state involves a single lipid molecule, (*b*) the initial increase in the ^{13}C shift relative to ^{31}P is due to the choline and glycerol groups of a nearest neighbor molecule approaching the lanthanide without the second phosphate forming a bond, and (*c*) the subsequent decrease in the ratio is a consequence of forming bonds to the second phosphate with and accompanying increase in the phosphate shift. Similar bimodal behavior was observed with the other two lanthanides. Thus, the NMR studies are consistent with a change in stoichiometry from one to two coordinating phosphodiesters for the two bound states.

Comparison of Ca^{2+}- and Tb^{3+}-DMPC by infrared spectroscopy

There are three normal modes of vibration for the nonester oxygens of phosphodiesters:



Antisymmetric stretch | Symmetric stretch | Symmetric bend

The ester oxygens and other groups perturb the energies and directions for these normal vibrations but do not alter the multiplicity of the nonester bands. The symmetric bending vibration is found below 600 cm^{-1} and cannot be observed in these experiments because of the absorption band of water below 850 cm^{-1}. The other two modes fall in the spectral region shown in Fig. 4.

The symmetric stretch is assigned to a band within a few wavenumbers of 1,085 cm^{-1} for phosphatidylcholine and other lipids with phosphodiesters. With DMPC there is a strong band at 1,088 cm^{-1} located by both deconvolution of this region and curve-fitting the spectrum with Lorentzian bands. This peak from curve-fitting is shown in Fig. 4 *A*, along with three others relevant to the following arguments.

The antisymmetric band has not been as consistently assigned, having been located near 1,250 cm^{-1} (Wallach et al., 1979; Casal and Mantsch, 1984), 1,240 cm^{-1} (Tsai et al., 1987), 1,230 cm^{-1} (Umemura et al., 1980), and 1,220 cm^{-1} (Dluhy et al., 1983). All of these assignments are for fully hydrated phosphatidylcholines. Deconvolution and curve-fitting reveal two strong bands in this region for DMPC, one at 1,232 cm^{-1} and the other at 1,220 cm^{-1}. The latter is assigned here as the antisym-

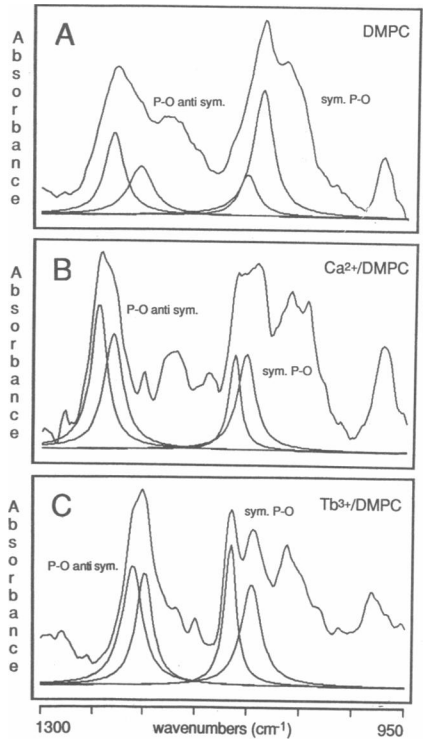


FIGURE 4 Phosphate vibrational spectra of DMPC with and without Ca^{2+} and Tb^{3+}. (*A*) DMPC (0.15 M DMPC, 0.5 M $NaNO_3$) where the four bands drawn beneath the spectrum are from nonlinear regression of the data and are located at 1,232, 1,220, 1,102, and 1,088 cm^{-1}. (*B*) Ca^{2+}-DMPC (1 M $Ca(NO_3)_2$, 0.12 M DMPC) where the four bands are 1,211, 1,199, 1,115, and 1,094 cm^{-1}.

metric O—P—O stretch based on the behavior of the bands when the cations bind.

When Ca^{2+} binds to DMPC only two bands shift appreciably, all of the other bands become narrower but are located within two to three wavenumbers of their original positions. The symmetric O—P—O stretch moves from 1,088 to 1,115 cm^{-1}, and the band at 1,220 cm^{-1} is replaced by a band at 1,248 cm^{-1}.

When Tb^{3+} binds to DMPC, the O—P—O symmetric stretch moves from 1,088 to 1,115 cm^{-1}, as it did with Ca^{2+}-DMPC (Fig. 4 *C*). The O—P—O antisymmetric stretch moves from 1,220 to either 1,211 or 1,199 cm^{-1} and three other bands move by several wavenumbers: (*a*) The 1,232 band also moves to either 1,211 or 1,199 cm^{-1}. (*b*) A band at 1,102 moves to 1,094 cm^{-1}. (*c*) The NCH_3 antisymmetric stretch moves from 970 to 979 cm^{-1}. The 1,232 cm^{-1} band of DMPC appears to be correlated with the 1,102 cm^{-1} band and the 970 cm^{-1} $N(CH_3)_3$ stretch because all three bands move only when Tb^{3+} binds and not Ca^{2+}; the 1,232 and 1,102 cm^{-1} bands are assumed to be headgroup vibrations, possibly rocking modes of the choline CH_3.

Although it is not certain which Tb^{3+}-DMPC band is the new high energy phosphate stretch, that at 1,211 or at 1,199 cm^{-1}, it is clear that the phosphate bands have moved closer together with this complex. This has been shown to be indicative of bidentate coordination with oxyanions having equivalent stretching modes. The antisymmetric and symmetric O—S—O sulfinato bands (Vitzthum and Lindner, 1971) and the equivalent O—C—O acetato bands (Nakomoto, 1978) move closer together in bidentate metal complexes and move farther apart in monodentate complexes. A more rigorous analysis of this change in band position, i.e., a normal mode analysis, is not reasonable at present because the lanthanide-oxygen bond lengths and the pucker of the bidentate ring are not known.

Separation of the two phosphate bands for Ca^{2+}-DMPC is essentially the same as that for DMPC alone, i.e., 133 cm^{-1} and 132 cm^{-1}, respectively. This gives no clear indication of the type of coordination. However, the position of the high energy band for Ca^{2+}-DMPC is very close to that of the P═O band of trimethylphosphate, e.g., 1,248 cm^{-1} vs. 1,242 cm^{-1}. Based on this observation it seems that Ca^{2+}-DMPC is a monodentate complex.

Very similar results were obtained for Tb^{3+}-cardiolipin and Ca^{2+}-cardiolipin (Conti et al., 1987; Halladay, 1989), suggesting there is an inherent difference in preferred phosphate coordination for the two cations.

CONCLUSIONS

These optical, NMR, and infrared studies provide a comprehensive view of lanthanide-phosphatidylcholine complexes, a view being extended to the other phospholipids (Halladay and Petersheim, 1988; Petersheim and Sun, 1989; Petersheim et al., 1989). The conclusions vary in agreement with earlier studies. As reported by Chrzeszczyk et al. (1981), there are clearly two different forms of the lanthanide-phosphatidylcholine complex, both of which were assumed to have two coordinating phosphates. However, the results presented here strongly suggest a change in the number of phosphodiester groups directly coordinating the cation. With bilayer forming DMPC, low levels of bound lanthanide appear to occupy sites in which only a single phosphate is available for coordination. This is probably a consequence of the choline quaternary amine being closely associated with the surface phosphates. Either the choline groups compete with the lanthanide for phosphates or headgroup packing causes the spacing of the phosphates to be too great to permit the lanthanide to be in contact with more than one. At high levels of bound lanthanide the choline groups are electrostatically repelled from the surface (McIntosch, 1980; Altenbach and Seelig, 1977) and the lanthanide is able to coordinate with two phosphates. In the optical studies with micelle forming lysoMPC, there is evidence that both forms of the complex are present at all levels of bound Tb^{3+}, although the relative amount of the two-phosphate species increases with Tb^{3+} (Halladay, 1989). This is consistent with weaker constraints on headgroup packing, as would be expected for micelles versus bilayers.

Hauser et al. (1976) performed a similar set of NMR experiments but reported only a single form for the lanthanide-phosphatidylcholine complex, which is undoubtedly the high Tb^{3+} state described here. They used the lanthanide-induced shifts of ^{1}H and ^{31}P NMR lipid resonances in a molecular modeling of the lanthanide-lipid complex, concluding that there were two phosphodiesters involved in monodentate coordination of the lanthanide. Although the data presented here is consistent with this stoichiometry, the behavior of the phosphate infrared bands in the presence of Tb^{3+} argues for bidentate rather than monodentate coordination.

The source of this discrepancy derives from the fact that there is no a priori knowledge of the magnitude and orientation of the lanthanide dipolar shielding tensor in the lipid complex. Hauser et al. (1976) assumed that the principal axis of the tensor falls along the lanthanide-oxygen bond. The lanthanide-lysoMPC ^{31}P and ^{13}C data mentioned here also indicates that monodentate coordination is most reasonable if the principal tensor axis is constrained to the Ln—O—P bond (Halladay, 1989). However, it was found that the NMR data could not distinguish between monodentate and bidentate forms of the complex if this constraint was relieved. It is possible to obtain the same orientation of the principal susceptibility

axis with respect to the phosphorous atoms for both forms of the complex. The magnitude of the dipolar shielding tensor must be estimated from the dipolar component of the ^{31}P shift by assuming a lanthanide-phosphorous distance in addition to an orientation for the tensor. Because it is reasonable to give the mono- and bidentate complexes the same lanthanide-phosphorous distances, both the magnitude and orientation of the dipolar shielding tensor can be made the same in both cases.

There is never more than a percent of the total lipid involved in the first bound state (Conti et al., 1987). Consequently, with the high degree of band overlap in the phosphate stretch region of the infrared spectrum, it was not possible to reliably detect the spectral changes due to the first form of the complex. However, two factors from the NMR studies suggest that the complex at low bound Tb^{3+} involves a single bidentate phosphate: the ^{31}P shift changes by about a factor of two in forming the second complex, and the $^{13}C/^{31}P$ shift ratio is roughly the same at low and high levels of Tb^{3+} despite the bimodal behavior in that data (Fig. 3). Both of these observations indicate that the Tb^{3+}-phosphate interactions are similar for the two complexes.

Infrared spectroscopy provides one of the few ways in which Ca^{2+} coordination by the lipids can be compared with the more informative lanthanides. The results presented here indicate that Ca^{2+} forms monodentate complexes with phosphatidylcholines, whereas Tb^{3+} binding is bidentate. Similar band changes are observed for Ca^{2+} and Tb^{3+} complexes with cardiolipin (Conti et al., 1987; Halladay, 1989), suggesting there is a general difference in phosphodiester coordination of these two cations.

The significance of this difference can be determined only by comparing the phase transitions induced by Ca^{2+} and the trivalent lanthanides. This has been done in studies of fusion of artificial membranes. The lanthanides are more effective at inducing fusion than is Ca^{2+} (Liao and Prestegard, 1980; Bentz et al., 1988; Ohki, 1988) but this may be due to the greater charge density and intrinsic affinity of the lanthanides rather than a difference in cation coordination.

With regard to these cations binding to phosphatidylcholine, only Tb^{3+} affected the choline bands, suggesting a difference in headgroup packing for the two cation complexes. Both cations presumably force the quaternary amine away from the membrane surface as a consequence of the positive surface charge (McIntosch, 1980; Seelig et al., 1987). The final conformation of the choline may be different in the Tb^{3+} complexes either as a direct consequence of bidentate versus monodentate coordination or a difference in the number of coordinating phosphates. Although Ca^{2+} binding also appears to involve a stoichiometry of one cation per two phosphates (Akutsu and Seelig, 1981; Altenbach and Seelig, 1984) there is no evidence that two phosphates are directly coordinating Ca^{2+}. The Ca^{2+}-DMPC infrared spectrum does retain moderate to weak bands at 1,217 and 1,088 cm^{-1} which are not present in the Tb^{3+}-DMPC spectrum. These bands could be an indication that Ca^{2+} has a nearest neighbor but noncoordinating phosphate group. Thus, the effect of Tb^{3+} on the choline bands may result from more crowded packing of the cholines in the bis-complex.

The combination of luminescent and paramagnetic properties of the lanthanides provides a means for detecting and describing cation-phospholipid complexes at levels which would be unnoticeable by x-ray diffraction, infrared and Raman spectroscopies, or other more common methods for studying the physical properties of phospholipids. The distinctive luminescence properties allow monitoring of several coexisting complexes under isothermal conditions (Saris, 1983; Herrmann et al., 1986; Conti et al., 1987; Halladay and Petersheim, 1988; Petersheim and Sun, 1989; Petersheim et al., 1989; Halladay, 1989). This is crucial to the study of cation distributions on the surface sites and correlation of site occupation with particular phase changes in the lipid, e.g., cation-induced formation of nonbilayer structures and membrane fusion. Given the unique potential of these probes, further investigation of their properties and relation to more intractable ions is warranted.

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