Biochimica et Biophysica Acta, 468 (1977) 364—377

BBA 77770

THE INTERACTION OF IONS WITH PHOSPHATIDYLCHOLINE BILAYERS

H. HAUSER *, C.C. HINCKLEY **, J. KREBS, B.A. LEVINE, M.C. PHILLIPS *** and R.J.P. WILLIAMS

Inorganic Chemistry Laboratory, University of Oxford, South Parks Rd, Oxford (U.K.)

(Received December 27th, 1976)

Summary

The interaction of lanthanides and other cations with phosphatidylcholine bilayers present as single bilayer vesicles in ^2H_2O has been investigated in terms of stoichiometry, apparent binding constants and environmental conditions.

Lanthanides are shown to form 2 : 1 (molar ratio) phosphatidylcholine to metal ion complexes.

The apparent binding constant K_b varies as a function of the metal ion bound and as a function of the Cl^- concentration. The apparent binding constant at "zero loading" is $K_0 = 1.25 \cdot 10^4\ L^2 \cdot M^{-2}$ at 0.15 M KCl. It decreases exponentially with increased "loading" expressed as the molar ratio of metal ion bound to effective phosphatidylcholine concentration and increases exponentially with Cl^- concentration.

The interaction of lanthanides and divalent cations such as Ca^{2+} and Mg^{2+} is independent of pH in the pH range 3—7† and 3—10 respectively, but is sensitive to the nature of the anion. The presence of anions enhances the interaction with polyvalent cations, the chaotropic anions showing the largest effect. The order of enhancement is $Cl^- < Br^- < NO_3^- < SCN^- < I^- < ClO_4^-$. The nature of the monovalent counterion (cation) has little effect on the enhanced binding of lanthanides in the presence of the above anions.

The affinity of other polyvalent cations for phosphatidylcholine bilayers has been determined by competition with lanthanides. The physiologically important divalent cations Ca^{2+} and Mg^{2+} both bind less strongly (by about an order of magnitude) to the lipid surface. The order of binding of cations

* Present address: Laboratorium für Biochemie, ETH Zurich, Universitätstrasse 16, 8092 Zurich, Switzerland.

** Present address: Department of Chemistry, University of South Illinois, Carbondale, Ill. 62901, U.S.A.

*** Present address: Unilever Research Laboratory Colworth/Welwyn, The Frythe, Welwyn, Herts, U.K.

† Lanthanides are insoluble at pH > 7.

reflects direct binding to the phosphodiester group, with UO_2^{2+} showing the highest affinity.

Introduction

The uptake of ions by biological membranes is likely to be due in part to binding sites on the phospholipid bilayer [1] which is known to be an integral structure of biological membranes. As phosphatidylcholine is generally a major fraction of the total phospholipid occurring in natural membranes it is important to study the mode of ion binding to this phospholipid in bilayer vesicles. The affinity of cations for phosphatidylcholine, whose polar group is zwitterionic over the physiological pH range [2], is of course much lower than that of the negatively charged phospholipids [3—8]: its biological significance is not known for, as we shall show, the binding of ions such as calcium to phosphatidylcholine membranes has not been analysed in sufficient detail.

Here we describe the stoichiometry and strength of binding of lanthanide cations with the phosphate group of phosphatidylcholine present in bilayer vesicles. The cation binding can be followed by NMR methods, and "competition" experiments then enable a comparison of the affinity of the trivalent lanthanides with Ca^{2+} and other cations.

Besides the mode of cation binding to phosphatidylcholine bilayers, the question of whether or not the interaction with ions induces conformational changes in the polar group of phospholipid is of biological interest. To answer this question the polar group conformation in the absence and presence of ions must be known. The NMR methods employed [9—12] allow the determination of the phosphatidylcholine polar group conformation in the presence of bound metal ion.

Experimental Methods

Materials. Egg phosphatidylcholine (grade I) and glycerophosphorylcholine (as the $CdCl_2$ complex) were purchased from Lipid Products and used without further purification; the purity of the phospholipids was checked by thin-layer chromatography [13]. Glycerophosphate (as the disodium salt) was purchased from Sigma. All other chemicals were AR grade.

Lanthanide chloride solutions were prepared by dissolving the anhydrous chloride (Research Chemicals, USA) in D_2O. The concentration of lanthanide present was checked by titration against EDTA.

Sonicated dispersions of egg phosphatidylcholine in D_2O were prepared as previously described [13]. The concentration of phosphatidylcholine was determined according to ref. 14. This was within 10% of the value obtained using a molecular weight of 750 and the concentration (mg/ml) of the phosphatidylcholine in chloroform/methanol solution. Unless otherwise stated the phosphatidylcholine concentration [L_T] used in the calculations involving mass equilibria was the effective concentration, i.e. two-third of the total phosphatidylcholine concentration; this is based on the geometry of the vesicle [15,16] surrounded by a single cation impermeable bilayer with approximately

two-third of the phosphatidylcholine molecules facing outward [17,7] and in contact with the cation.

Titrations of phosphatidylcholine with lanthanides were carried out by adding aliquots of the lanthanide chloride solutions to a known volume. The pH of the solutions was 5.3—5.7. Where necessary the ionic strength was kept constant using KCl to complement the lanthanide chloride present.

NMR methods. ^{1}H spectra were obtained at both 90 and 270 MHz on Bruker instruments both operating in the Fourier transform mode. ^{31}P spectra (at 36.43 MHz) were recorded on a Bruker WH 90 instrument under conditions of proton decoupling. All samples were run at 28 ± 2° C unless otherwise stated.

Results

Interaction with lanthanides

The interaction between phosphatidylcholine and lanthanides is investigated by monitoring the induced chemical shift of the proton and phosphorus NMR resonances of phosphatidylcholine as a function of lanthanide concentration. The induced shifts are frequency independent over all concentration ranges used. Exchange of metal ion between complexed and free phosphatidylcholine

$$M + nL \rightleftharpoons ML_n$$

must therefore be fast on the NMR time scale; the apparent binding constant K_b under any given conditions is:

$$K_b = \frac{[ML_n]}{[M][L]^n} = \frac{[ML_n]}{[M_T - ML_n][L_T - nML_n]^n} \quad (1)$$

where [M], [L] and [ML_n] are the concentrations of free metal ion, free phosphatidylcholine and phosphatidylcholine · lanthanide complex, respectively; M_T and L_T are the total metal ion and the effective lipid concentration, respectively, and n is the number of phosphatidylcholine molecules complexing one metal ion.

Under fast exchange conditions the observed shifts of NMR lines (δ_i) are the weighted average of the free and bound environments. Titration curves (Fig. 1), i.e. chemical shift changes $\Delta\delta_i$ as a function of the total lanthanide concentration (at constant phosphatidylcholine concentration) reflect the growing fraction of complexed phosphatidylcholine

$$\Delta\delta_i = \frac{n\Delta_i[ML_n]}{[L_T]} \quad (2)$$

where Δ_i is the shift change of the ith nucleus observed at saturation; it is a function of the particular lanthanide cation used.

Typical titration curves for phosphatidylcholine (10 mg/ml = 0.014 M) and $EuCl_3$ under conditions of varying ionic strength are shown in Fig. 1. The value of the observed shift ($\Delta\delta_i$) of all the resonances, and therefore the extent of complex formation, at any given metal ion concentration is found to be strongly dependent on the counterion Cl^- concentration. This is further demonstrated in Fig. 2 which shows the shift changes $\Delta\delta_i$ as a function of $EuCl_3$ at varying Cl^- concentrations. At a fixed lanthanide concentration, $\Delta\delta_i$

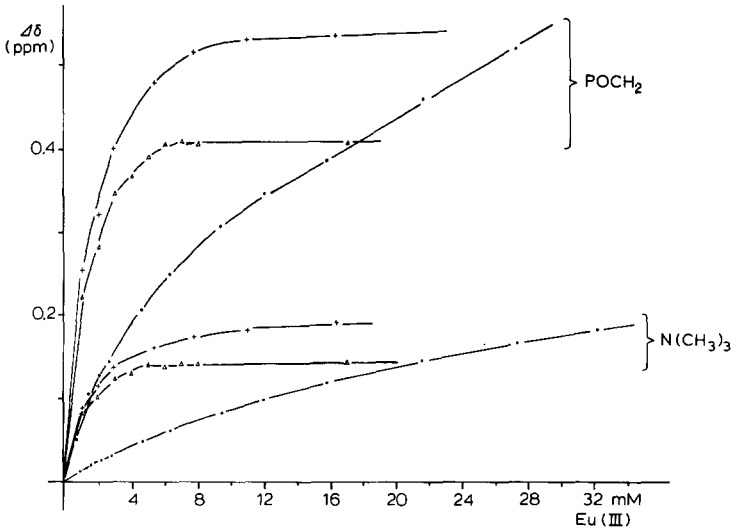

Fig. 1. Changes in chemical shift Δδ (ppm) of the $POCH_2$ (choline) and $N(CH_3)_3$ proton resonances of egg yolk phosphatidylcholine vesicles in ^2H_2O induced upon addition of $EuCl_3$. The effective phosphatidylcholine concentration, 0.014 M, was held constant during titration. ●———●, $EuCl_3$; △———△, $EuCl_3$ keeping the ionic strength constant at 0.1; +———+, $EuCl_3$ keeping the ionic strength constant at 0.2. The ionic strength was adjusted using KCl.

increases with increasing Cl^- concentration indicating that the presence of the counterion (anion) produces an enhancement of the degree of complex formation. Titrations at constant ionic strength (I = 0.1 and 0.2 using $EuCl_3$ and KCl,

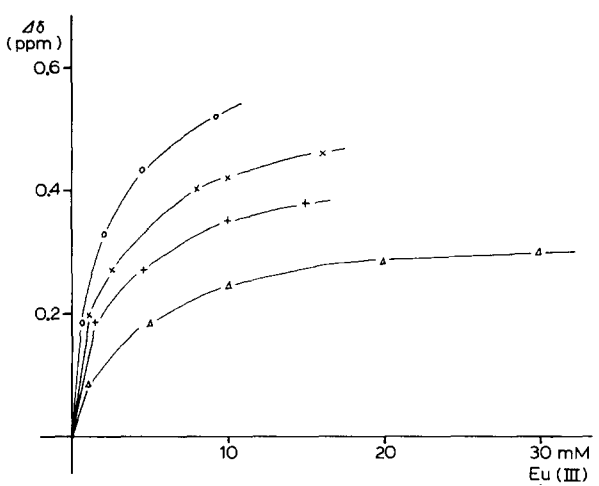

Fig. 2. Changes in chemical shifts Δδ (ppm) of the $POCH_2$ (choline) proton resonance as a function of the total $EuCl_3$ concentration under varying Cl^- concentrations. The effective phosphatidylcholine concentration, 0.014 M, was held constant. The total Cl^- concentration was held constant by adding the appropriate amount of KCl. +———+, 0.045 M Cl^-; X———X, 0.076 M Cl^-; ○———○, 0.15 M Cl^-; △———△, in the absence of Cl^-. The latter curve was obtained as follows: The shift changes observed at a given Eu^{3+} concentration were plotted as a function of [Cl^-] and extrapolation of these plots to zero [Cl^-] yielded the points on the curve. For the plots of Δδ vs. [Cl^-] the data of Fig. 2 were used together with those of similar titration curves at [Cl^-] ranging from 1 to 45 mM not included in Fig. 2. The monovalent counterion of Cl^- had no specific effect because similar results were obtained using LiCl or NaCl instead of KCl.

Fig. 1) show a saturation effect; the apparent 'fully bound' shift values are inconsistent with each other and the degree of complex formed upon titration of phosphatidylcholine with $EuCl_3$ alone where the $[Cl^-]$ is determined simply by the amount of $EuCl_3$ added.

Analysis of the observed shift data in terms of shift ratios, i.e. relative shifts on different nuclei, shows that the variation in shift values at saturation could not be due to the presence of a mixture of different complexes between phosphatidylcholine and Eu^{3+}. Shift ratios, which are directly related to the geometry of the complex, are independent of both ionic strength and metal ion concentration. The most probable conclusion is that only one type of complex exists.

The titration curves for phosphatidylcholine (Fig. 1) cannot be fitted using the mass equation (Eqn. 1) and assuming that 1 : 1 or 2 : 1 complexes are formed. It is necessary to examine the interaction with lanthanides in terms of a variable apparent binding constant K_b which is a decreasing function of the amount of metal ion bound per phosphatidylcnoline vesicle. Eqn. 3 meets this requirement [19,29].

$$K_b = K_0 \exp\left(-a\frac{[ML_n]}{[L_T]}\right) = \frac{[ML_n]}{[M_T - ML_n][L_T - nML_n]^n} \tag{3}$$

K_0 is the binding constant at conditions approaching zero loading (i.e. $[ML_n] \rightarrow 0$), a is the decay constant and the concentration of vesicles is proportional to the total lipid concentration $[L_T]$ with the proportionality constant being included in a. The apparent binding constants K_b for the interaction of lanthanides with phosphatidylcholine can be obtained by either of the two following methods.

(1) By monitoring the amount of metal ion bound to phosphatidylcholine through competition with acetate. The titration of acetate with $EuCl_3$ in the

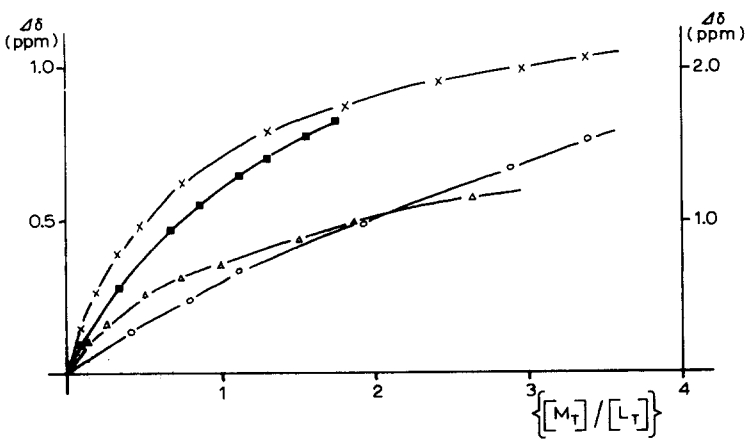

Fig. 3. Changes in chemical shifts Δδ (ppm) of proton resonances as a function of the metal ion ($EuCl_3$) to ligand molar ratio. X———X, glycerophosphate, CH_2OP signal, at 0.028 M and pH 2.1 (right hand ordinate); o———o, glycerophosphorylcholine · $CdCl_2$ complex, $POCH_2$ (choline) signal, at 0.023 M and pH 6.1; Δ———Δ, egg phosphatidylcholine, $POCH_2$ (choline) signal at 0.014 M and pH 5.7; ■———■, phosphorylcholine (as the Ca^{2+} salt), CH_2OP signal, at 0.12 M and pH 1.8, $Eu(NO_3)_3$ was used (right hand ordinate).

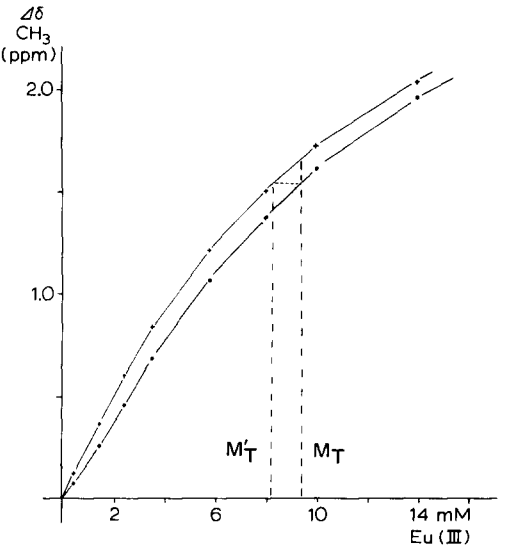

Fig. 4. Changes in chemical shift $\Delta\delta$ (ppm) of the CH_3-proton signal of acetate induced by $EuCl_3$ added at pH 5.6. Total acetate concentration 5 mM, the total chloride concentration was kept constant at 0.15 M using KCl. +———+, acetate; •———•, acetate in the presence of phosphatidylcholine (effective concentration = 0.014 M). The amount of metal ion bound $[ML_n]$ to phosphatidylcholine at a certain total metal concentration M_T is given by the difference $M_T - M'_T$ as shown in the diagram. The values $[ML_n]/[L_T]$ thus obtained were independent of acetate concentration between 5 and 15 mM.

absence and presence of phosphatidylcholine (Fig. 4) enables the determination of the amount of metal ion bound to phosphatidylcholine (see legend of Fig. 4) and hence of the free metal ion concentration. From such an analysis it is seen that the apparent binding constants for the interaction of phosphatidylcholine and lanthanides decrease with increasing metal ion concentration; the amount of metal ion bound to phosphatidylcholine approaches saturation at total metal ion concentrations above $2 \cdot 10^{-2}$ M but increases with increasing Cl^- concentration.

(2) By the method of corresponding samples, i.e. by titrating phosphatidylcholine samples differing in concentration $[L_T]$ with $EuCl_3$, as shown in Fig. 5. At a given value of $\Delta\delta$ observed the degree of complex formation or the extent of metal ion bound must be the same for each of the three phosphatidylcholine samples (corresponding samples) according to Eqn. 2. Eqns. 1 and 2 may then be solved simultaneously for values of $n > 0$ (see Appendix) in order to obtain the amount of lanthanide ion bound $[ML_n]$.

To derive apparent binding constants using either method, assumptions about the stoichiometry of the complex, i.e. the number n of phosphatidylcholine molecules bound to one metal ion have to be made. Some ambiguity with regards to the value of n is resolved by the constraint that the calculated values of the bound metal ion concentration $[ML_n]$ must not exceed the total metal ion concentration $[M_T]$; thus $n = 1$ or 2 are the only possible solutions. Derived values for $[ML]$ or $[ML_2]$ are substituted into Eqn. 3 to obtain apparent binding constants K_b. Expressing Eqn. 3 in the form

$$\log_e K_0 - a\frac{[ML_n]}{[L_T]} = \log_e \frac{[ML_n]}{[M_T - ML_n][L_T - nML_n]^n} \qquad (4)$$

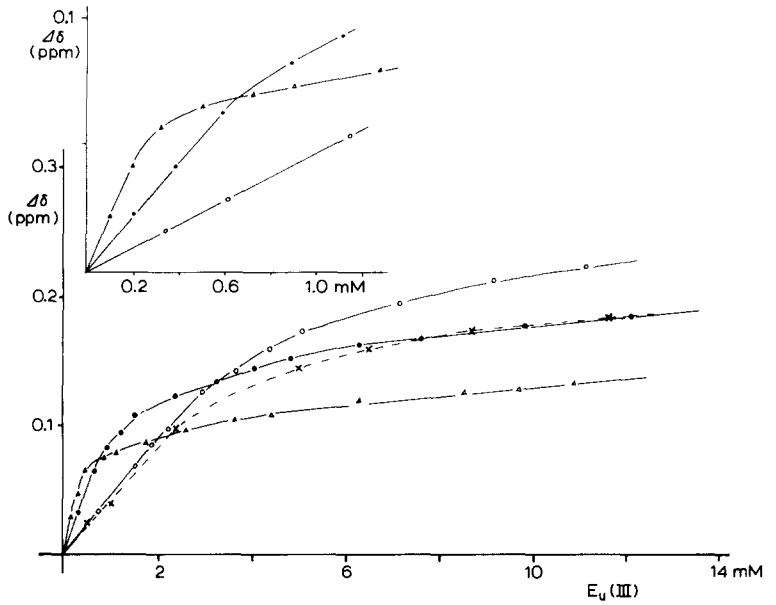

Fig. 5. Changes in chemical shifts $\Delta\delta$ (ppm) of the proton $N(CH_3)_3$ resonance of phosphatidylcholine as a function of the total $EuCl_3$ concentration. o———o, effective phosphatidylcholine concentration $[L_T]$ = 0.04 M keeping the $[Cl^-]$ constant at 0.45 M; $[Cl^-]/[L_T]$ molar ratio = 10.7; X- - - - - X, $[L_T]$ = 0.04 M; $[Cl^-]$ = 0.15 M; $[Cl^-]$/[lipid] molar ratio = 3.6; •———•, $[L_T]$ = 0.014 M; $[Cl^-]$ = 0.15 M; $[Cl^-]$/[lipid] = 10.7; Δ———Δ, $[L_T]$ = 0.004 M; $[Cl^-]$ = 0.045 M; $[Cl^-]/[lipid]$ = 10.7. The inset is an expansion of the titration curves in the concentration range up to approx. 1 mM $EuCl_3$.

and plotting $\log_e K_b$ according to Eqn. 4 as a function of $[ML_n]/[L_T]$ gives the two curves for $n = 1$ and $n = 2$ shown in Fig. 6. It is clear that the initial binding to the vesicles is essentially independent of the loading expressed as $[ML_n]/[L_T]$. At higher degrees of loading (>0.02) $\log_e K_b$ decreases linearly with $[ML_n]/[L_T]$, the slope being independent of n (Fig. 6) but sensitive to the Cl^- concentration (cf. Fig. 7). The derived apparent binding constant at zero loading, i.e. $[ML_n]/[L_T] \to 0$ and $K_b = K_0$, is $K_0 \approx 1.25 \cdot 10^4\ L^2 \cdot M^{-2}$ at 0.15 M KCl; this value decreases to $3 \cdot 10^3\ L^2 \cdot M^{-2}$ at zero KCl concentration.

The variation of the apparent binding constant at zero loading K_0 with $[Cl^-]$ is shown in Fig. 7. Also shown as a function of $[Cl^-]$ is the decay constant a; $\log_{10} a$ decreases linearly with increasing $[Cl^-]$. The effect of Cl^- is further illustrated in Fig. 5 which shows the titration curves of three phosphatidylcholine samples differing in lipid concentration. The Cl^- concentration in each of the samples is kept constant during titration and is chosen such that in all three samples the $[Cl^-]/[L_T]$ molar ratios are identical (cf. legend to Fig. 5). The initial binding at low metal ion concentration expressed as $[ML_n]/[L_T]$ (or $\Delta\delta$) is stronger the smaller the phosphatidylcholine concentration (Fig. 5), as would be expected from the mass action law. Increasing metal ion concentration leads to a reversal of the order of the relative magnitudes of the observed shifts because of the chloride dependence (cf. Fig. 2). This indicates that, at higher metal ion concentrations, the induced shift changes and thus the amount of ion bound is determined by the total Cl^- concentration. The dashed curve in Fig. 5 represents the titration curve of a phosphatidylcholine sample ($[L_T]$ =

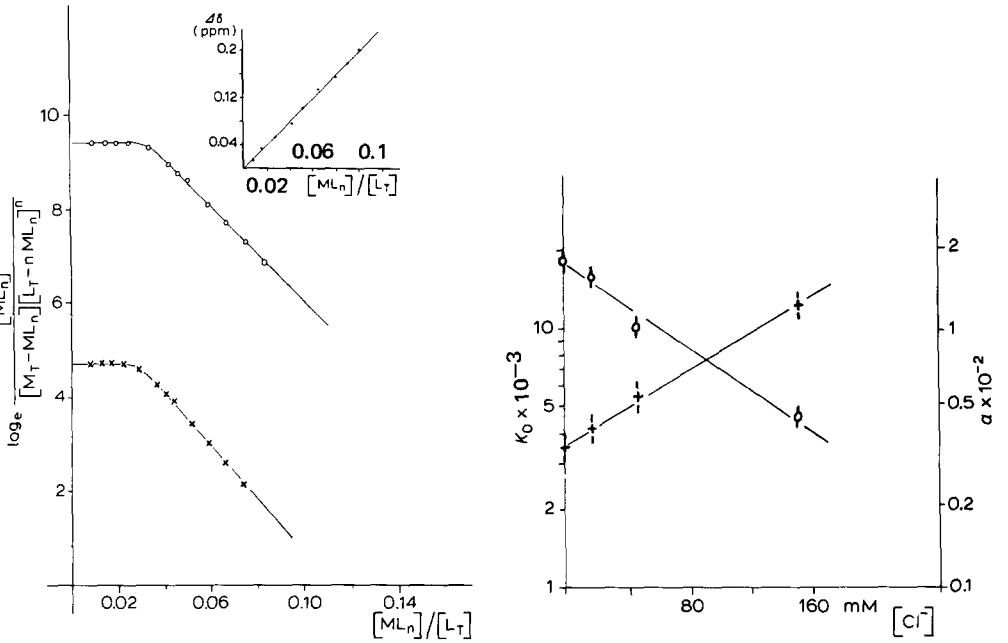

Fig. 6. $\log_e \frac{[ML_n]}{[M_T - ML_n][L_T - nML_n]^n}$ as a function of $[ML_n]/[L_T]$ plotted according to Eqn. 4. Effective phosphatidylcholine concentration $[L_T]$ = 0.014 M keeping the $[Cl^-]$ constant at 0.15 M. X———X, n = 1; o———o, n = 2. The inset is a plot relating $\Delta\delta$ (ppm) of the $N(CH_3)_3$ proton resonance of phosphatidylcholine to $[ML_n]/[L_T]$; $[ML_n]$ = concentration of metal ion bound; the slope of the curve is 0.5, i.e. $\Delta\delta$ = 1 corresponds to $[ML_n]/[L_T]$ = 0.5.

Fig. 7. Dependence of K_0 and the decay constant a as defined in Eqn. 3 on Cl^- concentration. $\log_{10} K_0$ and $\log_{10} a$ are given on the left hand and right hand ordinate, respectively. +———+, K_0; o———o, decay constant a. The bars represent the standard deviation.

0.04 M) at constant $[Cl^-]$ = 0.15 M with the $[Cl^-]/[L_T]$ molar ratio = 3.6 which is approximately one-third of that chosen for the other titration curves in Fig. 5. At low metal ion concentration there is good coincidence with the titration curve of that phosphatidylcholine sample which has the same lipid concentration $[L_T]$ = 0.04 M while at higher metal ion concentrations the dashed curve matches that obtained in the presence of the same Cl^- concentration $[Cl^-]$ = 0.15 M. This again shows that the amount of metal ion bound at higher metal ion concentrations is determined by the total chloride concentration.

The two methods of analysing the binding of lanthanides to phosphatidylcholine give apparent K_b values which are in good agreement. The quantity $[ML_n]/[L_T]$ derived from the competition experiment with acetate is consistent with that derived from the second method of corresponding samples with n assumed to be 2 (see Appendix). The binding of lanthanides to phosphatidylcholine is independent of pH values >3. At pH = 3 the value of K_0 decreases to $7.5 \cdot 10^3$ at $[Cl^-]$ = 0.15 M, indicating that protons compete effectively with the trivalent cations.

For comparison, the binding of lanthanide ions to phosphorylcholine, glycerophosphate and glycerophosphorylcholine, which are all constituents of the phosphatidylcholine polar group, has been investigated (Fig. 3). As with phosphatidylcholine the derived ratios are independent of both ionic strength and metal ion concentration. However, in contrast to the results for phosphatidylcholine in bilayer vesicles, the absolute shifts $\Delta\delta$ at saturation are insensitive to ionic strength, and the binding by these monomeric ligands is readily characterized by a 1 : 1 complex formation. The binding constants K_b determined for the 1 : 1 complexes using the mass equation are summarized in Table I. Similar to the approach described above binding constants K_b can also be derived from the competition for Eu^{3+} of glycerophosphorylcholine and acetate. The values thus obtained are in good agreement with the results of Table I.

Stoichiometry

The stoichiometry of the reaction is defined by n (Eqn. 1). As discussed above, the constraint $[ML_n] < [M_T]$ restricts the possible values of n to $n = 1$ or $n = 2$. Extrapolation of the linear relationships of Fig. 6 above $[ML_n]/[L_T] = 0.02$ to

$$\log_e \frac{[ML_n]}{[M_T - ML_n][L_T - nML_n]^n} = 0 , \qquad \text{i.e. } K_b = 1$$

yields values of $[ML_n]/[L_T] = 0.22$ and 0.11 for $n = 2$ and $n = 1$, respectively; these values effectively correspond to the situation of maximum loading under the given experimental conditions. Using the straight line relationship (inset of Fig. 6) between $\Delta\delta$ of the $N(CH_3)_3$ proton resonance and $[ML_n]/[L_T]$ it is seen that the shift change $\Delta\delta$ corresponding to the saturation value $[ML_n]/[L_T] = 0.11$ derived for $n = 1$ (Fig. 6) is too small compared with the observed shifts of that resonance at $[Eu^{3+}] \geqslant 0.01$ M (cf. Fig. 1). The derived value of the shift at apparent saturation is consistent with the data for a 2 : 1 phosphatidylcholine to metal ion complex, i.e. $n = 2$. Furthermore, the analysis of the stoichiometry is confirmed as the concentrations of metal ion bound to phosphatidylcholine (derived from the method of corresponding solutions using $n = 2$, cf. Figs. 5 and 6) are in agreement with the results obtained by competition with acetate (Fig. 4).

TABLE I

APPARENT BINDING CONSTANTS K_b DETERMINED FOR THE 1 : 1 LANTHANIDE COMPLEXES FORMED WITH VARIOUS PHOSPHATIDYLCHOLINE POLAR GROUP CONSTITUENTS

Compound	Binding constant ($L \cdot M^{-1}$) *
Glycerophosphate (Eu^{3+})	78 ± 12 (pH 2.1)
Phosphorylcholine · $CaCl_2$ (Pr^{3+})	5.2 ± 1.4 (pH 5.7)
Glycerophosphorylcholine · $CdCl_2$ (Eu^{3+})	7.8 ± 1.6 (pH 6.1)

* The mean value obtained using a least squares fit to the experimental binding data for the various ^1H resonances observed.

Binding of Ca^{2+} to phosphatidylcholine

The binding of Ca^{2+} to phosphatidylcholine is determined by measuring its effect on lanthanide binding. Fig. 8 shows that increasing amounts of Ca^{2+} displace more and more of the lanthanide ion bound to phosphatidylcholine. The amount of Ca^{2+} bound is obtained by monitoring the changes in chemical shift induced by Eu^{3+} in the presence of Ca^{2+} keeping the lipid to chloride molar ratio constant. Assuming that the stoichiometry of the Ca- · phosphatidylcholine complex is the same as for the lanthanide · phosphatidylcholine complex the following equilibria hold:

$$K_b\,(Ca^{2+}) = \frac{z}{[M_{Ca^{2+}} - z][L_T - 2(z + z')]^2} \tag{5}$$

and

$$K_b(Ln^{3+}) = \frac{z'}{[M_{Ln^{3+}} - z'][L_T - 2(z + z')]^2} \tag{6}$$

from which

$$K_b(Ca^{2+})/K_b(Ln^{3+}) = \frac{z[M_{Ln^{3+}} - z']}{z'[M_{Ca^{2+}} - z]} \tag{7}$$

is obtained. M^{3+}_{Ln} and M^{2+}_{Ca} are the total concentrations of lanthanide and Ca^{2+}, respectively, z and z' are the concentrations of Ca^{2+} and lanthanide ions bound, respectively. z' is obtained from the induced shift change and z is obtained by assuming that it equals the amount of lanthanide ions displaced from the phosphatidylcholine bilayer in the presence of Ca^{2+}. Using this treatment the amount of Ca^{2+} bound at $[CaCl_2] = 10^{-2}$ M is $1.5 \cdot 10^{-4}$ M which inserted into Eqn. 1 gives an apparent binding constant for the 2 : 1 lipid to Ca^{2+} complex of $K_b = 90\ L^2 \cdot M^{-2}$. At 10 mM $EuCl_3$ the apparent binding constant K_b for the 2 : 1 phosphatidylcholine · Eu^{3+} complex is about $910\ L^2 \cdot M^{-2}$ showing that

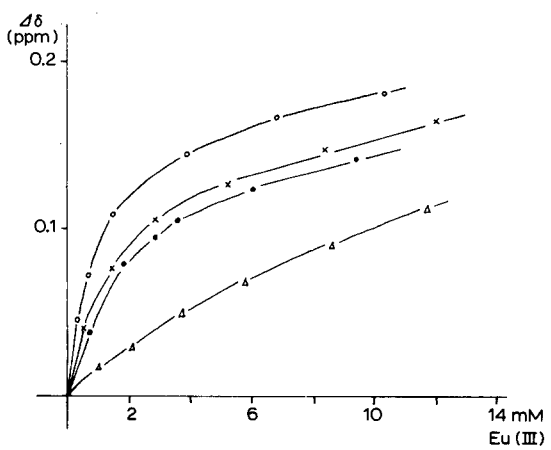

Fig. 8. Change in chemical shift $\Delta\delta$ (ppm) of the proton $N(CH_3)_3$ signal of phosphatidylcholine induced by $EuCl_3$ added in the presence of varying concentrations of $CaCl_2$ and $LaCl_3$. Effective phosphatidylcholine concentration $[L_T] = 0.014$ M, the total $[Cl^-]$ was kept constant at 0.15 M by adding KCl. ○———○, KCl alone; X———X, 30 mM $CaCl_2$; ●———●, 60 mM $CaCl_2$; △———△, 30 mM $LaCl_3$.

under these experimental conditions the affinity of Ca^{2+} for phosphatidylcholine is about 1/10 that of lanthanides. Similar to the interaction of lanthanides with phosphatidylcholine the Ca^{2+} binding is enhanced in the presence of Cl^-.

From competition between lanthanides and other cations for the phosphodiester group of phosphatidylcholine the following order of binding is derived:

$$UO_2^{2+} > Ln^{3+} > Ca^{2+} \simeq Mg^{2+} > [Co(NH_3)_6]^{3+} > K^+ \simeq Na^+$$

Thus uranyl cations bind more strongly than the trivalent lanthanides and Ca^{2+}; the latter behaves very much like Mg^{2+} which binds more strongly than the monovalent alkali metal ions. The above sequence reflects direct binding to the phosphate group (cf. ref. 18).

Discussion

Lanthanides form 1 : 1 complexes with the small molecules (Table I) which are all constituents of the phosphatidylcholine polar group; their binding is adequately described by the mass action law and the binding constants are typically in the range of 10—100 $L \cdot M^{-1}$ and independent of ionic strenth. In our earlier work [7,11] where changes in chemical shifts and relaxation behaviour indicated that the phosphodiester group was the binding site, we assumed that the binding to phosphatidylcholine bilayers could be described as above. On this basis, an order of magnitude estimate of the apparent binding constant was obtained for the interaction of lanthanides at concentrations >10 mM, $K_b \approx 30\ L \cdot M^{-1}$ *

Data presented here indicate that lanthanides form a 2 : 1 phosphatidylcholine to metal ion complex which is consistent with the conclusions arrived at from the conformational analysis of such complexes reported earlier [12] and which will be described in detail in later publications. Because of the different form of the mass equation (cf. Eqn. 1) used in the above treatment the experimental data, which are presented in this paper and which are consistent with results reported previously [7,11,12], give apparent binding constants different from those reported before [7], both in terms of magnitude and units, e.g. for comparison at lanthanide concentrations >10 mM $K_b \leqslant 10^2 (L^2 \cdot M^{-2})$ **. In summary, the binding of lanthanides to phosphatidylcholine bilayers depends on the "loading", i.e. the amount of lanthanide bound to the lipid surface, the ionic strength and the nature of the anion added, but is independent of pH between pH 3 and 7. The binding constant K_0 observed at zero loading (Fig. 6) is rather high compared to those of the ligands in Table I reflecting the chelation of one metal ion by two phosphate groups. The apparent binding constant K_b is invariant ($K_b = K_0$) up to a loading of the lipid surface of $[ML_n]/[L_T] \approx 0.02$, but decreases then exponentially with $[ML_n]/[L_T]$ at higher loadings (Fig. 6).

It is apparent (Fig. 8) that Ca^{2+} binding (which is similar to that of Mg^{2+} [8]) is weaker by at least one order of magnitude than that of lanthanides (cf.

* (liter/mol)
** $(liter/mol)^2$

refs. 18 and 24), whereas UO_2^{2+} is the cation most strongly bound to phosphatidylcholine bilayers (cf. refs. 18, 22, 25 and 26).

The affinity of the biologically important cations Ca^{2+} and Mg^{2+} to phosphatidylcholine bilayers is such that at concentrations of 5 mM typical for a large number of biological fluids, approx. 1% of all phosphatidylcholine polar groups are likely to be bound by these cations. It is possible that the cation-bound conformation of the polar group, and in turn the packing of the hydrophobic bilayer region, are somewhat different from the ion-free state of the bilayer. Given that the rate of cation binding (both on and off reaction steps) is high, a rapid fluctuation of the local surface potential (electric field) can be expected.

Anion effects

Fig. 7 demonstrates that the intrinsic binding constant increases with increasing $[Cl^-]$. This is presumably due to the zwitterionic nature of the phosphatidylcholine polar group, because, in the case of phosphatidylserine, which carries a net negative charge at physiological pH, the addition of KCl has the opposite effect; the monovalent cation competes with and displaces polyvalent cations from the binding sites of the lipid polar group [3,4,19,23]. The increase in binding of divalent cations such as Ca^{2+} to phosphatidylcholine bilayers in the presence of NaCl was predicted on theoretical grounds by Gillespie [21]; he calculated that the variation with electrolyte concentration of the electric field from a bilayer array of fixed dipoles is such that addition of 100 mM NaCl should enhance binding of Ca^{2+} over the concentration range 0.1—100 mM $CaCl_2$.

We have shown in experiments not described in this paper that other anions also enhance the interaction with lanthanides, the order of enhancement being $Cl^- < Br^- < NO_3^- < SCN^- < I^- < ClO_4^-$. This is also the order of effectiveness of these anions in reducing the positive ζ-potential of phosphatidylcholine bilayers to which lanthanide ions are bound (cf. ref. 7). The reduction in ζ-potential is now explicable in terms of binding of lanthanide ions plus anion to the phospholipid surface. The nature of the monovalent cation added together with the anion has little or no effect on the interaction of lanthanides with phosphatidylcholine bilayers; this indicates that the effect is solely due to anions. McLaughlin et al. [20] and Simon et al. [27] also observed that chaotropic anions such as SCN^-, ClO_4^- and I^- bind more strongly to phospholipid surfaces such as phosphatidylcholine and phosphatidylethanolamine bilayers. In further work (Hauser, H. and Howell, K., unpublished) using ζ-potential measurements we have shown that F^- anions can reverse the positive ζ-potential of phosphatidylcholine bilayers containing lanthanide cations. Slightly negative ζ-potential values ($\zeta \geqslant -8$ mV, cf. ref. 28) characteristic for phosphatidylcholine bilayers in the absence of lanthanides are measured in the presence of $[F^-] > 0.005$ M. The F^- anion is well known to have a high affinity for lanthanides, presumably due to its very high surface charge density and relatively small radius; following the ^1H shift changes induced by Pr^{3+} as a function of increasing $[F^-]$ it can be shown that excess $[F^-]$ ($[F^-]/[Pr^{3+}] > 1$) displaces lanthanides from the lipid surface.

Appendix

Calculation of the loading $[ML_n]/[L_T]$ of phosphatidylcholine vesicles with lanthanides and of apparent binding constants

From Eqn. 2, at a given $\Delta\delta_i$, $[ML_n]/[L_T]$ will be the same for different solutions; thus for the three titration curves of Fig. 5, with the total lanthanide concentrations corresponding to the effective phosphatidylcholine concentrations L_1, L_2, L_3 being M_1, M_2 and M_3, and the respective $[ML_n]$ being x_1, x_2, x_3, we have

$$\frac{x_1}{L_1} = \frac{x_2}{L_2} = \frac{x_3}{L_3} \tag{8}$$

Inserting into Eqn. 3:

$$K_0 \exp\left(-a\frac{x_i}{L_i}\right) = \frac{x_i}{[M_i - x_i][L_i - nx_i]^n} \tag{9}$$

There are three forms of Eqn. 9 with $i = 1,2,3$ corresponding to the three titration curves of Fig. 5. These three equations can be combined using Eqn. 8 to express x_1 and x_2 in terms of x_3; this yields

$$x_3 = \frac{M_1L_3L_1^{n-1} - M_3L_3^n}{L_1^n - L_3^n} = \frac{M_2L_3L_2^{n-1} - M_3L_3^n}{L_2^n - L_3^n} \tag{10}$$

A computer programme was used to solve Eqn. 10 with various values of n. With the constraint of $x_i \leqslant M_i$ (for any $\Delta\delta_i$ value chosen) the above equation yields $n = 1$ or 2. Fig. 6 is a plot of

$$\log_e \frac{x_i}{[M_i - x_i][L_i - nx_i]^n} \quad \text{vs.} \quad \frac{[ML_n]}{[L_T]} = \frac{x_i}{L_i}$$

Acknowledgements

H.H. and J.K. wish to acknowledge financial support by the Swiss National-fond (Kredit Nr. 3.4940.75) and by the German Academic Exchange Service (D.A.A.D.), respectively. We wish to thank Mr. Kevin Howell for his expert technical assistance. We also acknowledge an exchange of information and ideas with Professor A. Ehrenberg and his coworkers.

References

1 Hauser, H., Levine, B.A. and Williams, R.J.P. (1976) Trends Biochem. Sci. 1, 278—281
2 Bangham, A.D. and Dawson, R.M.C. (1959) Biochem. J. 72, 486—492
3 Rojas, E. and Tobias, J.M. (1965) Biochim. Biophys. Acta 94, 394—404
4 Hauser, H. and Dawson, R.M.C. (1967) Eur. J. Biochem. 1, 61—69
5 Seimiya, T. and Ohki, S. (1972) Nat. New Biol. 239, 26—27
6 Seimiya, T. and Ohki, S. (1973) Biochim. Biophys. Acta 298, 546—561
7 Hauser, H., Phillips, M.C., Levine, B.A. and Williams, R.J.P. (1975) Eur. J. Biochem. 58, 133—144
8 Hauser, H. and Phillips, M.C. (1977) Prog. Surf. Membrane Sci., in the press
9 Barry, C.D., Glasel, J.A., Williams, R.J.P. and Xavier, A.V. (1974) J. Mol. Biol. 84, 471—490
10 Dobson, C.M. and Levine, B.A. (1976) in New Techniques in Biophysics and Cell Biology (Pain, R. and Smith, B., eds.) Vol. 3, pp. 19—91, John Wiley and Sons
11 Hauser, H. (1976) J. Colloid Interface Sci. 55, 85—93

12 Hauser, H., Phillips, M.C., Levine, B.A. and Williams, R.J.P. (1976) Nature 261, 390—394
13 Hauser, H. (1971) Biochem. Biophys. Res. Commun. 45, 1049—1055
14 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756—1758
15 Huang, C. (1969) Biochemistry 8, 344—351
16 Hauser, H., Oldani, D. and Phillips, M.C. (1973) Biochemistry 12, 4507—4517
17 Bystrov, V.F., Dubrovina, N.I., Barsukov, L.I. and Bergelson, L.D. (1971) Chem. Phys. Lipids 6, 343—350
18 Barton, P.G. (1968) J. Biol. Chem. 243, 3884—3890
19 Hauser, H., Darke, A. and Phillips, M.C. (1976) Eur. J. Biochem. 62, 335—344
20 McLaughlin, S., Bruder, A., Chen, S. and Moser, C. (1975) Biochim. Biophys. Acta 394, 304—313
21 Gillespie, C.J. (1970) Biochim. Biophys. Acta 203, 47—61
22 Levine, Y.K., Lee, A.G., Birdsall, N.J.M., Metcalfe, J.C. and Robinson, J.D. (1973) Biochim. Biophys. Acta 291, 592—607
23 Kimizuka, H., Nakahara, T., Uejo, H. and Yamauchi, A. (1967) Biochim. Biophys. Acta 137, 549—556
24 Misiorowski, R.L. and Wells, M.A. (1973) Biochemistry 12, 967—975
25 Shah, D.O. (1969) J. Colloid Interface Sci. 29, 210—215
26 Furuya, K., Yamaguchi, T., Inoko, Y. and Mitsui, T. (1976) Acta Cryst. B32, 1811—1817
27 Simon, S.A., Lis, L.J., Kauffman, J.W. and MacDonald, R.C. (1975) Biochim. Biophys. Acta 375, 317—326
28 Hanai, T., Haydon, A. and Taylor, J. (1965) J. Theor. Biol. 9, 278—296
29 Davies, J.T. and Rideal, E.K. (1963) Interfacial Phenomena, 2nd edn., pp. 84—85, Academic Press, New York