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SURFACE POTENTIAL EFFECTS ON METAL ION BINDING TO PHOSPHATIDYLCHOLINE MEMBRANES

^{31}P NMR STUDY OF LANTHANIDE AND CALCIUM ION BINDING TO EGG-YOLK LECITHIN VESICLES *

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Summary

^{31}P NMR of phosphatidylcholine (lecithin) from egg-yolk in sonicated vesicles has been measured in the presence of various ions. Addition of Ln^{3+} ‡ or Ca^{2+} shifted the ^{31}P resonance of the phosphate groups of the outer surface of the vesicles. These shifts were measured at varied lanthanide or Ca^{2+} concentration at different ionic strengths obtained by addition of NaCl. The shifts induced by Tb^{3+} and Ca^{2+} have been analyzed using the theory of the diffuse double layer. Corrections were introduced for the effect of the ionic strength on the activities of the ions. The binding efficiency is shown to be controlled by the electrostatic potential produced by the bound cations at the membrane surface. This potential is slightly modified due to weak chloride binding. Binding constants have been derived.

Introduction

The binding of metal cations to phospholipid bilayers plays a conspicuous role in determining the physical properties and functions of biological membranes. Information on binding sites and the mechanism and strength of binding is thus of great importance. Because of its biological significance Ca^{2+} is of particular interest. In biological membranes the association of metal ions with charged phospholipids is probably of special importance. Interaction

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‡ Ln^{3+} refers to ions of the lanthanide series (At. Nos. 57—70).

between metal ions and zwitterionic phospholipids may also exist. The two types of lipid always appear together, the zwitterionic lipid generally being the more abundant. Hence, they might well compete effectively with the charged lipids for binding of the metal cations. Results of previous work carried out on the binding of metal ions to the neutral phosphatidylcholines are somewhat contradictory. For instance, Dervichian [1] concluded from potentiometric titration studies that phosphatidylcholine (lecithin) from egg-yolk did not bind Ca^{2+} in the pH range 2—8, whereas Träuble [2] reported a lecithin-Ca^{2+} binding constant of $10^3\ M^{-1}$ as deduced from fluorescence work. Levine et al. [3] studying dipalmitoyl lecithin vesicles measured the paramagnetic lanthanide-induced shift of the proton NMR lines from the outward facing $-N^+(Me)_3$ groups and concluded that the concentration of binding sites is approx. 1 per 10 lecithin molecules. They proposed that the upper limit of binding sites is determined by charge repulsion from the occupied binding sites. Nolden and Ackermann [4] have studied the influence of bound Mn^{2+} on the longitudinal relaxation rate, $1/T_1$, of the solvent water protons. They concluded that approximately one Mn^{2+} is bound per 210 dipalmitoyl lecithin molecules with an association constant of $1.5 \cdot 10^4\ M^{-1}$, and suggested that the small number of binding sites be due to the shielding effect of the positively charged choline groups. They prefer to speak not of specific binding but of more general dynamic interactions. Hauser et al. [5—7]have recently studied the effect of Ln^{3+} and Ca^{2+} on the ^1H and ^{31}P NMR spectra of egg-yolk lecithin. For the lanthanides a 1 : 2 complex with lecithin was suggested without presenting any direct proofs. It has been suggested [8] that a "high affinity binding site" of 5—10% of the total lecithin could account for the Ln^{3+}-induced choline shift as observed by ^{13}C NMR.

It has been shown [5—7] that the Ln^{3+} binds to the phosphate group of vesicular lecithin. Chemical shifts of the ^{31}P NMR signal in lecithin induced by different Ln^{3+} ions are composed of contact and pseudocontact contributions. Low concentrations of these ions apparently induce no alteration in the conformation of the lecithin polar group as reported from ^1H NMR studies [5]. The recorded shifts therefore measure the concentrations of phosphate $\cdot$ Ln^{3+} complexes and can be used to evaluate binding constants. Hence, high resolution ^{31}P NMR spectroscopy is a useful experimental technique for the study of the mechanism of ion-lipid interaction at a membrane/water interface.

The purpose of the present investigation is to give a consistent analysis of ion binding to the membrane taking into account the surface potential produced by binding of charged cations to the neutral zwitterionic egg-yolk lecithin bilayer. As will become clear, several of the apparently contradictory results in the literature will be resolved by this approach.

Materials and Methods

Egg-yolk lecithin (Grade I) in chloroform/methanol solution was purchased from Lipid Products (South Nutfield, Surrey, U.K.) and used without further purification. The organic solvent was removed from the lipid by a stream of N_2 gas and subsequent prolonged evacuation. The thin lipid film was dispersed by vortexing in 4 ml of deuterium oxide (90% atomic, Norsk Hydro)

containing the desired amount of NaCl to fix the ionic strength. The sample was sonicated under N_2 in an ice-cooled glass tube using a probe type sonicator (Heat System-Ultrasonics, Inc., Model S-350, standard microtip) at 20 kHz. To avoid increased temperature the sonication was carried out intermittently for 0.5 h effective time at the relatively low power level setting of 4. Titanium metal released from the tip of the soniprobe was removed by centrifugation. The purity of the phospholipids after sonication was checked by thin-layer chromatography. Small amounts of degradation products were detected after the sonication. One of the main degradation products produced by sonication is fatty acids [9]. As determined by gas chromatography less than 0.5% free fatty acids were present. No disturbing effects from these degradation products were observed in the experiments which were carried out in the pH range 4—5. This was also controlled by incorporating known amounts of stearic acid. The pH was adjusted by addition of hydrochloric acid or sodium hydroxide. No buffer was employed in order not to interfere with the ions.

The concentration of phospholipids was varied by dilution with ^2H_2O containing the same concentration of NaCl. The lecithin concentration was determined by phosphorous analysis [10]. $TbCl_3$ and $EuCl_3$ (99.9%) were obtained from Koch and Light Ltd. $CaCl_2 \cdot 4H_2O$ and NaCl were of suprapure quality (Merck). Calcium stock solution was analyzed for impurities by mass spectrometry. No significant amounts of paramagnetic elements were detected. Other chemicals were obtained from commercial sources and used without further purification.

^{31}P NMR spectra were measured at 30—31°C on a Varian XL-100-12 spectrometer operating at 40.48 MHz and equipped with a VFT-100 Fourier transform accessory. A deuterium field-frequency lock was used. Spectra were obtained under conditions of proton noise decoupling using 12-mm sample tubes (1.5—2.0 ml sample). Aqueous dispersions of lipids ranged from 60 to 0.9 mM. To obtain satisfactory spectra 40 000 transients were collected on the weaker sample. For measuring the initial slopes of the Ln^{3+}-induced shift curves, the appropriate lanthanide chloride was added as a concentrated ^2H_2O solution in volumes up to 80 μl. When necessary volume corrections due to these additions were made. For some experiments at higher ratios of Ln^{3+} to lecithin, the chloride was weighed directly into the NMR tube and dissolved in the added vesicle suspension. The shifts reported are between the interior and exterior signals of the vesicles when otherwise is not indicated. Some experiments were also carried out with trimethylphosphate as internal standard.

Published values for the mean acticity coefficients $\gamma^{\pm}$ for $LnCl_3$ and $CaCl_2$ in aqueous solutions [11—13] were used to evaluate the activity of the individual ions. For the Cl^- ion the single-ion activity coefficient was employed [14]. From intensity measurements of the ^{31}P and choline ^1H NMR signals it was found that the vesicles contained approximately two-thirds of the total number of lipids on the outer layer of the vesicles. Hence, all the lipid concentrations discussed later refer to this exterior contribution.

Theory

The theory of the diffuse double layer is well known and has been treated in many texts [see e.g. 15]. It has been applied to membrane phenomena, e.g. by

McLaughlin et al. [16] in a study of the influence of divalent ions on the surface potential of charged phospholipid membranes. Our approach is somewhat different and it is necessary for the following to give a brief outline of the formalism applied to our particular problem.

The interaction between a metal ion, M, and the membrane surface may be conceived as taking place in a heterogeneous system. The simplest way to treat the ion binding to the membrane would be to consider a direct adsorption of an ion in the aqueous interface (I; the Stern layer), to a spatially fixed site of one or more phosphate moieties, P, according to

$$M(I) + P_n \leftrightarrows MP_n$$

Disregarding any interaction between the sites, this may be described by a Langmuir adsorption isotherm. In our experiments the phospholipid membrane is in a liquid-crystalline state characterized by a high degree of lateral mobility, but also of order. The vesicle is a fluid mosaic of lipid molecules. In the Langmuir isotherm no translational motion in the surface is implied. Therefore we chose here to describe the primary event as a partitioning of the ions between the aqueous interface and the polar head group regions in the membrane surface. This distribution may be described by a distribution coefficient (cf. a perfect two-dimensional gas)

$$K_I = \frac{\{M\}_I}{\{M\}_s} \tag{1}$$

where $\{M\}_I$ is the volume activity (mol/l) of the binding ions in the immediate vicinity of the surface and $\{M\}_s$ is the surface activity (mol/Å^2) of the same ions in the surface. For simplicity we include in K_I the effect of any constant electrostatic potential difference between those two regions and neglect any variation in this potential difference.

The ions in the surface (s) are able to react with adjacent phosphate ligands due to lateral diffusion and a series of complexes may be formed

$$M(s) + nP \rightleftarrows MP_n$$

with

$$K_{s,n} = \frac{\{MP_n\}_s}{\{M\}_s \{P\}_s^n} \tag{2}$$

Hence the overall complex formation may be characterized by

$$K_n = K_{s,n}/K_I = \frac{[MP_n]_s}{\{M\}_I [P]_s^n} \tag{3}$$

In the last step we treat the membrane phase as ideal and therefore use surface concentrations (mol/Å^2) instead of surface activities. When $n = 1$, Eqn. 3 has the form of a Langmuir isotherm, but this is only apparent since mobile sites are here allowed.

The surface concentration of free phosphatidylcholine, $[P]_s$, and complexes, $[MP_n]_s$, are related to the total surface concentration of outer lipids, $[P]_{os}$, by

$$[P]_{os} = [P]_s + n[MP_n]_s \tag{4}$$

Here and in the following we have assumed that only one complex, MP_n, is formed. Necessary changes for the case of more than one complex are easily introduced, but will complicate the calculations considerably. We have neglected eventual changes in the lateral dimensions due to ion binding. The cross-sectional area of an lecithin molecule in the membrane is estimated to be approx. 70 Å^2. $[P]_{os}$ has a constant value independent of the lecithin concentration:

$[P]_{os}$ = (70 × Avogadro number)$^{-1}$ mol/Å^2

The mixed constant in Eqn. 3 can hence be transformed according to

$$K_n^* = K_n[P]_{os}^{n-1} = \frac{f_n}{\{M\}_I(1 - nf_n)^n} \tag{5}$$

where $f_n = [MP_n]_s/[P]_{os}$. The expression for K_n^* has now the dimension of l/mol.

We have retained activities when dealing with the metal ions in the aqueous interface. At equilibrium $\{M\}_I$ is related to the bulk activity $\{M\}_b$ by the equation

$$\{M\}_I/\{M\}_b = \exp\left(-\frac{z_i F\psi}{RT}\right) \tag{6}$$

where ψ (mV) is the potential difference between the interface and the bulk encironment. Here ψ accounts for the potential difference across the diffuse double layer and is referred to as the surface potential. For a non-ideal solution we assume that the cation bulk activities and concentrations are related by

$$\{M\}_b = \gamma_b^{\pm}[M]_b \tag{7}$$

where the mean activity coefficient $\gamma^{\pm}$ is evaluated from published values for the total ionic strengths applied [11—13]. We further assume that the mean activity coefficients in the two phases are identical, i.e. $\gamma_I^{\pm} = \gamma_b^{\pm} = \gamma$. Eqn. 6 is then transformed into the usual Boltzmann distribution of the concentrations in a dilute system.

The zwitterionic lecithin is electrically neutral in the pH range 4—5. The electrophoretic mobility is reported [5] to be nearly zero indicating a negligible zeta- or surface potential at the membrane interface. However, binding of cations and/or anions will modify this potential. We have here treated the vesicle surface, seen by the ions, as being approximately flat. Considering the relatively small size of the vesicles (≈300 Å o.d.) this assumption may not be fully justified. Nevertheless, a more general and elaborate treatment of the spherical case will probably also require severe approximations before numerical solutions are obtained. We therefore apply the Grahame equation [15,16]

$$\sigma = \pm\left\{2\epsilon\epsilon_0 RT \sum_i C_i\left(\exp\left(\frac{-z_i F\psi}{RT}\right) - 1\right)\right\}^{1/2} \tag{8}$$

where σ is the surface charge density in electronic charges per Å^2, C_i is the concentration of the i'th ionic species in the bulk solution in mol/l, z_i is its

valence, and $RT/F = 26.1$ mV and $(2\epsilon\epsilon_0 RT)^{-1/2} = 274.0$ at $T = 30°C$. The surface charge density, σ, is related to f_n by

$$\sigma = \frac{z_i}{70} f_n + \sigma_o \tag{9}$$

where z_i is the valence of the bound ion and σ_o is an eventual inherent initial charge density (e/Å^2). We obtain by combination of Eqns. 6, 8 and 9:

$$(274)^2\left[\frac{z_i}{70} f_n + \sigma_o\right]^2 = C_{NaCl} \cdot 4 \sinh^2\left(\frac{\psi}{52.2}\right) + [M]_o\left[|z_i| \exp\frac{\psi}{26.1}\right.$$

$$\left. + \exp\left(-\frac{z_i\psi}{26.1}\right) - |z_i| - 1\right] - [P]_o\left[\exp\left(-\frac{z_i\psi}{26.1}\right) - 1\right] f_n \tag{10}$$

and by combination of Eqns. 3, 5, 6 and 7:

$$\exp\left(\frac{z_i\psi}{26.1}\right) = \gamma K_n^* [P]_o\left[\frac{[M]_o}{[P]_o} - f_n\right] \cdot [1 - n \cdot f_n]^n f_n^{-1} \tag{11}$$

Here $[M]_o$ is the total concentration of ions added and $[P]_o$ is the total concentration of lecithin phosphorous in the outward-facing layer of the vesicle bilayer, both measured as volume concentration (mol/l). Those equations can be solved numerically, for a given set of parameters, C_{NaCl}, K_n, z_i, $[P]_o$ and $[M]_o$. From the f_n value the NMR shift δ is obtained by

$$\delta = n \cdot \Delta_n \cdot f_n \tag{12}$$

where Δ_n is the bound shift for a cation liganded phosphate group of lecithin in a complex with a stoichiometry $1 : n$. Eqn. 12 is valid where the exchange rate of the ions between the outward facing phosphate groups is faster then Δ_n, which is readily achieved by diffusion of ions as well as lipids.

In the simulations of the binding curves it was found necessary to include binding of anions to the lecithin choline group. The anion binding was treated with the same formalism as introduced for the cations, replacing M with X for the anion. In the equilibirum it is most reasonable to assume $n = 1$ (Eqn. 3). Contribution from the chloride binding to the surface charge density was included in Eqn. 9 by a term $\sigma_x = -[XP]_s/70[P]_{os}$, where $[XP]_s$ is the surface concentration of bound anions. Neglecting the amount of bound anions in comparison with the amount in the bulk solution, $[X]_b$, this term may be written

$$\sigma_x = -\frac{1}{70} \frac{K_x \gamma_x^- [X]_b \exp(F\psi/RT)}{1 + K_x \gamma_x^- [X]_b \exp(F\psi/RT)} \tag{13}$$

where K_x is the anion binding constant and γ_x^- is the single ion activity coefficient [14]. For simplicity it is assumed that cation and anion may bind simultaneously to the same lecithin molecule.

For very small concentrations, $[M]_o$, of added ions $f_n << 1$, and the variation in the surface potential ψ can be neglected. Starting with Eqns. 11 and 12 it is

then straight-forward to deduce the Eqn.

$$k = -\frac{\exp\left(\frac{z_i \psi_{in}}{26.1}\right)}{\gamma K_n^*} \cdot \frac{k}{[P]_o} + n \cdot \Delta_n \qquad (14)$$

where $k = \partial\delta/\partial([M]_o/[P]_o)$ is the initial slope of the shift curves and ψ_{in} is the initial value of the surface potential, including any contribution from bound anions. A plot of k against $k/[P]_o$ yields a straight line with slope and intercept according to Eqn. 14.

Results and Discussion

Ln^{3+} lecithin interaction

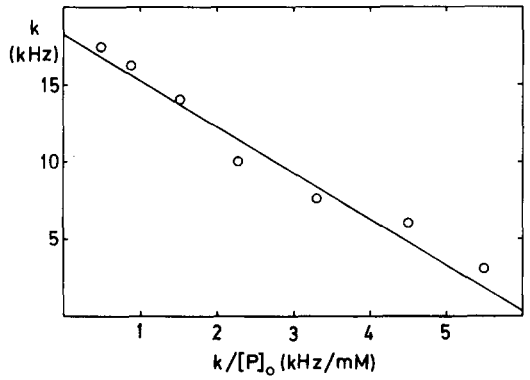
We have used ^{31}P NMR for the study of lanthanide ion binding to egg-yolk lecithin vesicles. The interior signal remained unshifted whereas the exterior signal was shifted and broadened when lanthanide ions were added. The ^{31}P linewidth for the inward facing groups remained constant (approx. 15 Hz) and independent on the metal ion concentration. Hence, practically no ion permeability exists and no significant aggregation or fusion of the vesicles occurred.

By keeping the pH values in the solutions low (pH 4—5) we have tried to avoid hydrolysis of the metal ions leading to ionic species other than Ln^{3+}. In this study we have predominantly used Tb^{3+} as shift probe. The small upfield shift observed for diamagnetic La^{3+} was less than 2% of the paramagnetic shift for Tb^{3+}, hence no correction for diamagnetic contribution was employed in the case of Tb^{3+}.

One estimate of the bound shift, Δ_n, the stoichiometry factor, n, and the binding constant, K_n^*, was obtained by measuring the initial slope, k, of the Tb^{3+} induced ^{31}P NMR shifts as a function of lecithin concentration ($[M]_o/[P]_o < 0.01$). As shown in Fig. 1 the data yields a straight line (correlation coefficient 0.97) as predicted by Eqn. 14. A best fit was obtained for $\gamma K_n^* \cdot \exp(-z_i \psi_{in}/26.1) \cong 335\ M^{-1}$ and a product $n \cdot \Delta_n = 18\,200$ Hz. From these results we cannot directly discriminate between the possible values of n within the range 1—3 for Ln^{3+}.

Further information on the binding and shift parameters may be obtained from the experimental shift curves and simulations in Fig. 2, treated at three different NaCl concentrations. The effect of the potential of the bound cations is indicated by the shape of the curves showing the most effective ion binding at small additions of Tb^{3+} and a markedly less efficient binding at larger additions. Independent demonstration of the potential effect was obtained by preliminary experiments with charged vesicles (not shown). Diminished lanthanide-induced shifts were observed for vesicles containing 5—10 mol of the amphiphile cetyltrimethylammoniumbromide.

The pronounced effect of ionic strength on the shift values, i.e. the ion binding, is well explained by the screening effect (cf. Eqn. 8). It was checked that mere variations of the activity coefficient and dielectric constant could not explain the effect. It was further found that NaCl itself did not give any ^{31}P



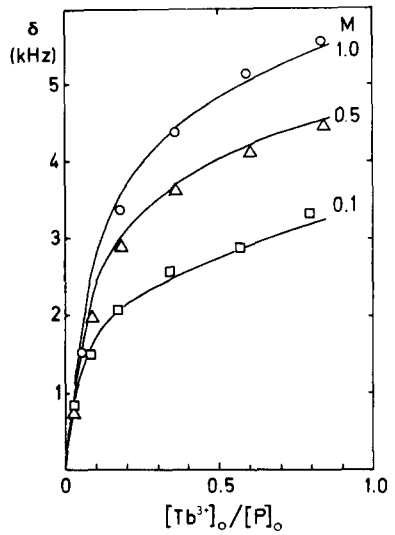


Fig. 1. Plot according to Eqn. 14 of the initial slopes, k, for the Tb^{3+}-induced chemical shifts of the ^{31}P NMR signal from egg lecithin vesicles. The straight line is a least-square fit to the experimental points. 90% ^2H_2O solution containing 0.1 M NaCl at p^{2}H 4—5. Temperature, 30°C.

Fig. 2. ^{31}P NMR chemical shift changes of egg lecithin vesicles as a functionn of the molar ratio $[Tb^{3+}]_o/[P]_o$ for three different NaCl concentrations. Points represent measured values. The lines are calculated for $n = 3$ with the parameters in Table I. Concentrations of lipids from outer bilayer $[P]_o$ were analyzed to be close to 39 mM. 90% ^2H_2O with p^{2}H 4—5. Temperature, 30°C.

shift. Nevertheless, in order to obtain a satisfactory fit between experimental and simulated curves it was found necessary to include a specific but weak binding of the anion to the lecithin choline group consistent with published proton NMR work [17]. In case of Tb^{3+} binding the specific effect of the anion was checked by comparing Cl^- and Br^-. The latter ion should have a stronger binding and it was found to enhance the measured ^{31}P shifts more than Cl^-.

The computer simulations of the whole set of curves of Fig. 2 were thus made by means of Eqns. 10—12 with due consideration of the effects introduced by Eqn. 13. Also, dilution and variation of activity coefficients were considered when necessary. The parameters giving the best fit are listed in Table I.

The curve fitting is not very sensitive to changes in the stoichiometry factor n as long as K_n and $n \cdot \Delta_n$ are kept constant. It is not possible to be definite about the stoichiometry as long as the value of Δ_n is unknown. There are no pertinent Δ_n values for ^{31}P in the literature, and Hauser et al. [6] have indicated the unique ^{31}P shift for Ln^{3+} complexes in lecithin bilayers. Among the values of n tested in our simulations we like to favour $n = 3$ for the lanthanides. Lower n values lead to somewhat inferior fit and furnish values for Δ_n which seem too high. Without accounting for the potential effect Hauser et al. [5—7] have suggested that $n = 2$. Further investigations will be necessary to settle this point.

The results of the curve fitting simulations rest on an overall agreement for a set of titration curves over the titration range $0.02 < [Tb^{3+}]_o/[P]_o < 0.85$.

TABLE I

PARAMETERS USED TO SIMULATE THE ^{31}P NMR CHEMICAL SHIFT CURVES FOR THE Tb^{3+} AND Ca^{2+} INTERACTIONS WITH EGG-YOLK LECITHIN VESICLES AT 30°C AND p^2H 4—5*

Method	Metal ion	[NaCl] (M)	$[P]_o$ from outer bilayer (mM)	$K_n^* = K_n \cdot [P]_{os}^{n-1}$ (M^{-1})	Stoichiometry factor n	Bound shift Δ_n (Hz)
Initial slope	Tb^{3+}	0.1	0.6—37	565	3	6 070
Curve fitting	Tb^{3+}	0.1				
		0.5	39	715	3	16 500
		1.0				
Curve fitting	Ca^{2+}	0.1				
		0.5	18	2.2	1	170
		1.0				

* The calculations were made with $K_X = 0.065\ M^{-1}$ for $X = Cl^-$, and $\sigma_o = 0$

This should be compared with the results from the initial slopes in the range $[Tb^{3+}]_o/[P]_o < 0.01$ with $\gamma K_n^* \exp(-z_i\, \psi_{in}/26.1) = 335\ M^{-1}$. From the simulations we could estimate $K_x\gamma_x^- = 0.05\ M^{-1}$ for the Cl^- binding. For 0.1 M NaCl, the ionic strength of the initial slope experiment, we obtain $\psi_{in} = -1.5$ mV by means of Eqns. 8, 9 and 13. With $\gamma = 0.5$ we obtain for the initial slope experiment $K_n^* = 565\ M^{-1}$, compared with $715\ M^{-1}$ for the full simulation.

The value for the bound shift $\Delta_3 = 18\,200/3 = 6070$ Hz obtained from Fig. 1 for the initial slope in 0.1 M NaCl is substantially lower than $\Delta_3 = 16\,500$ Hz applied for the overall simulations in Fig. 2. This discrepancy cannot be eliminated just by selecting a lower stoichiometry, since for $n = 1$ or 2 in the simulations the bound shift must be chosen quite high in order to meet the experimental curves.

It is not evident that a single complex is dominating over the total concentration range studied. However, the straight line in Fig. 1 indicates that, initially, mainly one single complex species exists. Preliminary comparisons of ^{31}P shifts induced by Tb^{3+} and Eu^{3+} suggest that the complexes at high and low cation binding might not be identical. It is here too early to speculate whether this possible difference could be due to different geometries and/or stoichiometries.

Ca^{2+}-lecithin interaction

We also observed an upfield ^{31}P NMR shift for the outward-facing phosphate groups of egg-yolk lecithin in the presence of Ca^{2+}, as shown in Fig. 3, for various electrolyte concentrations. At $[Ca^{2+}]_o/[P]_o \approx 10$ the shift is approx. 13 Hz in the presence of 1 M NaCl, and the shifted line is somewhat broadened. The shifts were derived from the overlapping spectral lines by curve fitting. In Fig. 3 the very highest Ca^{2+} concentrations studied have not been included. However, no anomalies were observed at a $[Ca^{2+}]_o/[P]_o$ ratio as high as 50. This finding is in contrast to results recently reported by Hauser et al. [5] who found no effect of Ca^{2+}. The magnitude of the Ca^{2+}-induced shift depends on the total electrolyte concentration in the way predicted by Eqn. 8, i.e. raising

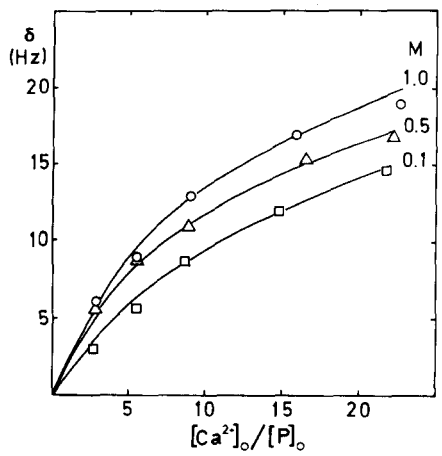


Fig. 3. ^{31}P chemical shift changes of egg lecithin vesicles as a function of the molar ratio $[Ca^{2+}]_0/[P]_0$ for different NaCl concentrations. Points represent measured values. The lines are calculated for $n = 1$ with the parameters in Table I. $[P]_0$ around 18 mM. 90% ^2H_2O with pD 4—5. Temperature, 30°C.

NaCl concentrations enhances the shift. Since relatively high Ca^{2+} concentrations were employed at the exterior of the vesicles we have also tried to keep the ionic strength equal at the two sides of the bilayer. Even with this precaution identical shifts were observed. Effects due to the variation in the magnetic susceptibility may be neglected since the field was monitored by the field frequency lock. The interaction scheme for calcium seems to be similar to that for the lanthanides, except that in the case of Ca^{2+} the interaction is approximately two orders of magnitude smaller as far as the bound shift and binding constant are concerned (Table I). The shifts are small and probably mainly due to electrostatic interaction between the diamagnetic Ca^{2+} and the phosphate. The shifts reveal that the Ca^{2+} binds to the phosphate groups in the case of a neutral zwitterionic lipid like egg-yolk lecithin. As the interactions of Ln and Ca^{2+} with the phosphates are mainly of electrostatic nature, a much smaller binding constant is expected for the divalent Ca^{2+}. The shift curves have been simulated by Eqns. 10—12 using the parameters listed in Table I. The values of σ_x were calculated from Eqn. 13 with the same binding constant for Cl^- as applied for Tb^{3+}. It was found that the introduction of chloride binding was more important in case of Ca^{2+} than for Tb^{3+}. As a matter of fact, in case of Ca^{2+} it was not possible to obtain the spread between the shift curves for different ionic strengths without introduction of the chloride binding. The simulated curves are shown in Fig. 3 for a 1 : 1 complex, but a stoichiometry factor of $n = 2$ cannot be completely excluded.

Conclusions

The results presented here demonstrate that the simplest form of diffuse double-layer theory is capable of accounting for the most dominant features of the binding mechanism of di- and trivalent cations to vesicle membranes formed from the neutral phospholipid egg-yolk lecithon. Unfortunately, the analysis is not sensitive enough to unambiguously discriminate between the

possible stoichiometries which might exist. Whatever the stoichiometry is, our results show the importance of potential effects in the control of ion-membrane interaction.

As mentioned before there are assumptions made in the derivation of the equations, and in this study the relevance of the following might be questioned: (1) a uniform distribution of the surface charge density, (2) the dielectric constant and the ion-activity coefficient in the aqueous interface are assumed to be equal to their bulk values and (3) the lipid molecules are behaving independently.

In view of the approximations indicated above the derived values for the binding constants and the bound shifts may have substantial systematic error. Among the experimental sources of error the most critical in this context is the uncertainty in $[P]_o$. Both the phosphorous analysis and the vesicle size determination contribute and might give a total error as high as ±5—10%. This makes it difficult to get a perfect fit of the simulated curves to the experimental points. The figures of the parameters of Table I are, however, believed to be of the correct order of magnitude.

In general, our results clearly demonstrate that membrane surface charge and potential must be considered for a correct and detailed treatment of ion binding to membrane lipids, and probably other membrane components as well, which is of considerable importance for the understanding of many biological systems and functions. The potential effect appears to clarify most of the contradictions in previous NMR work on ion-membrane interaction.

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