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THE BINDING OF ORGANIC IONS TO PHOSPHOLIPID BILAYERS

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Summary

The binding of organic anions and cations, mainly tetraphenylboride and tetraphenylarsonium, to phospholipid membranes has been studied using an NMR method. Binding is appreciable and is affected by cholesterol in the membrane and counterions in solution. The passage of the organic anions through the membrane has also been followed. These measurements indicate that it is naive to use organic anions to measure membrane potentials in a simple manner.

Introduction

The interaction of ions with phospholipids accounts in part for the ion binding capacity of natural membranes. The interaction of simple inorganic ions, especially cations, with the phospholipid polar groups is now fairly well understood [1—5]. Relatively fewer studies of the interaction of bilayer membranes with hydrophobic ions, and the trans-bilayer transport of such lipid-soluble ions, have been undertaken [6—8]. Details of such interactions should lead to a description of the factors which modulate membrane binding and permeability. These quantities have become very important as hydrophobic anions and cations are used increasingly to assess membrane energy states.

For example, in efforts to follow the membrane potentials of energised biological vesicles and organelles it is common practice to use a variety of techniques based upon the addition of either anions such as thiocyanate or tetraphenylboride, ϕ_4B^-, and of series of organic cations ranging from methylamine to positively charged dyestuffs. It is recognised that this chemical approach to the study of potentials may not be simple in so far that these ions may well bind to the surface of the membrane as well as permeating it. In this way the method of studying the uptake the ions on energisation would not measure the potential across the membrane (one aqueous phase to another) but it would measure a sum of this potential and that due to the bound charges of the mem-

brane. It is also the case that ions of the one sign which can carry charge across a membrane may also carry counterions with them. The total transport of the ion is then not related to a potential difference. With these points of view in mind we decided to conduct an investigation into the binding of various ions to the surface of a very simple vesicle membrane, that prepared by sonication of phosphatidylcholine, egg lecithin.

We have undertaken, therefore, a NMR study of the interaction between phospholipid bilayer vesicles and the lipid-soluble ions, tetraphenylboride (TPB^-) and tetraphenylarsonium (TPA^+). The binding of these ions at the bilayer surface may readily be detected and quantified in terms of the induced ring current shifts of the phospholipid resonances. These secondary shifts depend on the spatial disposition of the aromatic rings of the bound ions relative to the groups which give rise to the shifted resonances. The transfer of the lipid-soluble ions across the vesicle bilayer can be monitored through the interaction of the ions at the internal bilayer surface, for this surface can be seen in the NMR spectra independently from the external surface. The dependence of the interaction on both phospholipid composition and ionic strength was investigated and enables a description of the extent to which membrane structural variables and solution conditions influence each of the conductance parameters, binding and transport.

Methods and Materials

Single shell bilayer vesicles were prepared by sonication under N_2 of aqueous phospholipid dispersions of varying composition (see text). Sonication was also carried out in the presence of different salt concentrations and some of these preparations were dialysed subsequently against O_2—free distilled water in order to study vesicles with an asymmetric salt distribution, the salt being trapped inside.

The binding titrations were carried out by addition of aliquots of the required tetraphenyl ion solution to a known volume containing sonicated vesicles (typically 15 mg/ml phospholipid). Proton magnetic resonance spectra were recorded at 90 and 270 MHz on Bruker instruments operating in the Fourier Transform mode. The kinetic experiments to monitor the equilibration across the vesicle bilayer were carried out at 270 MHz with a spectral accumulation time of about 25 s per titration point. ^{31}P spectra were obtained using a WH-90 spectrometer operating at 36.43 MHz. The temperature was controlled at 27°C unless otherwise stated.

All non-synthetic lipids and glycerophosphorylcholine ($CdCl_2$ salt) were obtained from Lipid Products (Grade I) and were used without further purification as was the dipalmitoyl phosphatidylcholine obtained from Koch-Light. Phosphatidylserine was obtained as the sodium salt. Tetraphenylboride (sodium salt) was purchased from the Sigma Chemical Co. and tetraphenylarsonium chloride from Cambrian Chemicals. All other reagents used were Analar grade.

Results

In the description of this work the concentration of reagent added to a micelle or vesicle system is given as the analytically added amount in the

aqueous phase. The reagents are all salts, e.g. sodium tetraphenylboride and their solubilities in the lipid phases are relatively small. Thus although the abscissae in Fig. 1—8 are the analytically added concentrations of reagents they differ from the true free ion concentrations in the aqueous phase by less that 10%. This was directly confirmed by analysis of the partition between vesicles and an aqueous phase using conventional equilibrium dialysis procedures.

Binding to zwitterionic phospholipids

The interaction of tetraphenylboride (TPB^-) and tetraphenylarsonium (TPA^+) with zwitterionic phospholipids was characterized by monitoring the changes in chemical shift of various phospholipid group proton magnetic resonances upon binding of either the anion or cation to egg yolk phosphatidylcholine in bilayer vesicles and to lysophosphatidylcholine in monolayer micelles.

Sodium tetraphenylboride (TPB^-). Addition of increasing amounts of sodium TPB^- to a solution of phosphatidylcholine vesicles led to increasing upfield shifts on the resonances of the polar head group (Fig. 1), smaller shifts being observed for the resonances of several groups which lie deeper in the hydrocarbon phase (Table I). These titration curves (Fig. 1) reflect the increase in binding, which is here shown to be in fast exchange. Smaller shifts were observed at higher phospholipid concentrations. The magnitude of the relative shifts for the different resonances was independent of both phospholipid and TPB^- concentrations and was also found to be unaffected by ionic strength ($0.01 < \mu \leqslant 0.3$). Since the relative magnitudes of the induced shifts are determined by the orientation of bound TPB^- relative to the different groups of phosphatidylcholine, these results suggest that the interaction may be described in terms of a single set of phosphatidylcholine-TPB^- complex species.

Similar data were obtained upon TPB^- binding to lysophosphatidylcholine micelles (Table I) although in this case the magnitude of the absolute shift induced was more strongly dependent on ionic strength. The relative shifts are seen to be very similar with both lipids. The mode of binding of TPB^- to both bilayer vesicles and micelles is thus identical, the interaction site being localized at the phospholipid/water interface as indicated by the differential shifts

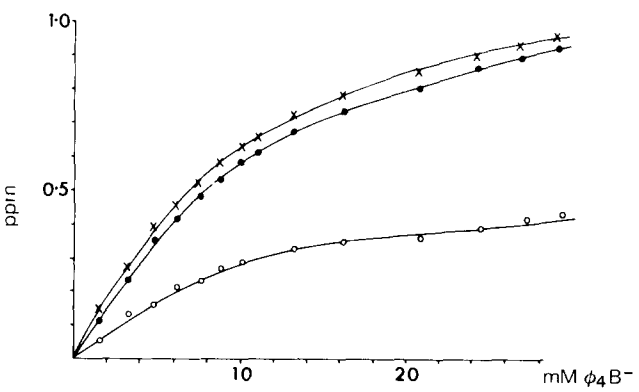

Fig. 1. Chemical shifts of the proton resonances of the polar group of phosphatidylcholine (20 mg/ml) induced upon addition of tetraphenylboride. ●———●, $N(C\underline{H}_3)_3$; X———X, $C\underline{H}_2N$; ○———○, $POC\underline{H}_2$.

TABLE I

TETRAPHENYLBORIDE-INDUCED SHIFTS FOR VARIOUS PHOSPHOLIPID RESONANCES

	Phosphatidylcholine *		Lysophosphatidylcholine **	
	Observed shift ***	Relative shift	Observed shift ***	Relative shift
$N(C\underline{H}_3)_3$	+0.67	93	+0.85	95
$C\underline{H}_2N$	+0.72	100	+0.89	100
$POC\underline{H}_2$	+0.33	46	+0.42	47
^{31}P	+0.06	8	+0.06	7
$C\underline{H}_2OP$ / $OCOC\underline{H}$	no shift			
$OCOC\underline{H}_2$	+0.09	12		
$C\underline{H}_2CO$	+0.25	35		
$C\underline{H}_2$-C-CO / $-C\underline{H}_2$-C=C	no shift			
$C\underline{H}_2$-(C=C)$_2$	+0.08	11		
$C\underline{H}$=CH / $(C\underline{H}_2)_2$ / $-C\underline{H}_3$	no shift			

* Phosphatidylcholine concentration = 26 mM (20 mg/ml).

** Lysophosphatidylcholine concentration = 28.8 mM (15 mg/ml).

*** TPB^- concentration = 13.3 mM; shifts are given in parts per million.

observed. No interaction was however detected between TPB^- and glycerophosphorylcholine, the monomeric form of the phosphatidylcholine polar group, in free aqueous solution. The lipid layers are essential for the interaction.

Dilution of the surface density of polar groups upon introduction of other neutral lipids into the bilayer vesicles enabled investigation of the effect of the distribution of binding sites upon the absorption of TPB^- at the phospholipid/aqueous interface. These experiments were carried out using a constant phospholipid concentration. The observed shifts (and hence the extent of adsorption at the phospholipid/bulk solution interface) were unaffected by the introduction of dipalmitoyl lecithin. Mixed lipid vesicles were also formed at various mol ratios of mono-olein to phosphatidylcholine. Over the concentration range of added TPB^- ($\leqslant$2.8 mol ratio, anion to total phospholipid) slightly smaller shifts were observed with increasing proportions of mono-olein to phosphatidylcholine (Fig. 2). Experiments using sonicated mono-olein dispersion showed no detectable binding of TPB^- to the glycerol backbone or the hydrocarbon phase. The relative shift of different resonances were unaffected in all these experiments.

The extent of binding was more markedly affected in vesicles of the two component system, cholesterol-phosphatidylcholine (Fig. 2). Although the total concentration of phospholipid was fixed, increasing proportions of cholesterol resulted in a reduction of the amount of TPB^- bound to the external phospholipid surface in these mixed lipid bilayers. The differential shifts for the various phospholipid resonances were invariant with increasing TPB^- concentration in each of the two component systems investigated. This result precludes the possibility of a different mode of interaction (and spatial disposition of TPB^- at the bilayer/water interface) in the different bilayers.

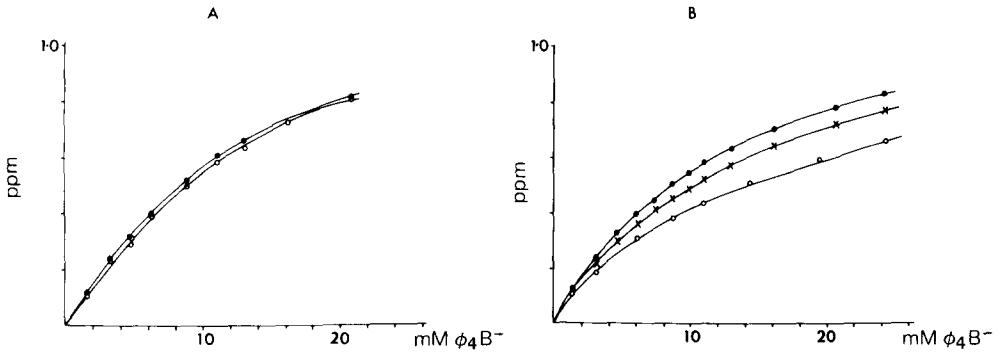

Fig. 2. Influence of neutral lipids on the chemical shift of the $N(C\underline{H}_3)_3$ resonance of phosphatidylcholine (20 mg/ml) induced by tetraphenylboride. (A) ●———●, 3 : 1 mol ratio phosphatidylcholine:mono-olein; ○———○, 1 : 1 mol ratio phosphatidylcholine:mono-olein. (B) ●———●, 3 : 1 mol ratio phosphatidylcholine:cholesterol; X———X, 3 : 2 mol ratio phosphatidylcholine:cholesterol; ○———○, 1 : 1 mol ratio phosphatidylcholine:cholesterol.

The study of the effect of ionic strength on the TPB^--phosphatidylcholine interactions was restricted to the use of sodium and lithium salts since the TPB^- forms insoluble complexes with K^+, Ca^{2+} and Mg^{2+}. It was however possible to examine the influence of the trivalent lanthanide cations at concentrations $\lesssim 10^{-3}$ M. Experiments were carried out using homogeneous phosphatidyl choline bilayers with both an asymmetric (extravesicular) and symmetric distribution of the different salts.

Above a 0.2 mol ratio (TPB^-:phosphatidylcholine) enhancement of the degree of binding of TPB^- was observed with increasing NaCl concentration in the external bulk solution (Fig. 3), the effect being more marked in the case of vesicle preparations sonicated in the presence of NaCl (Fig. 4). At NaCl concen-

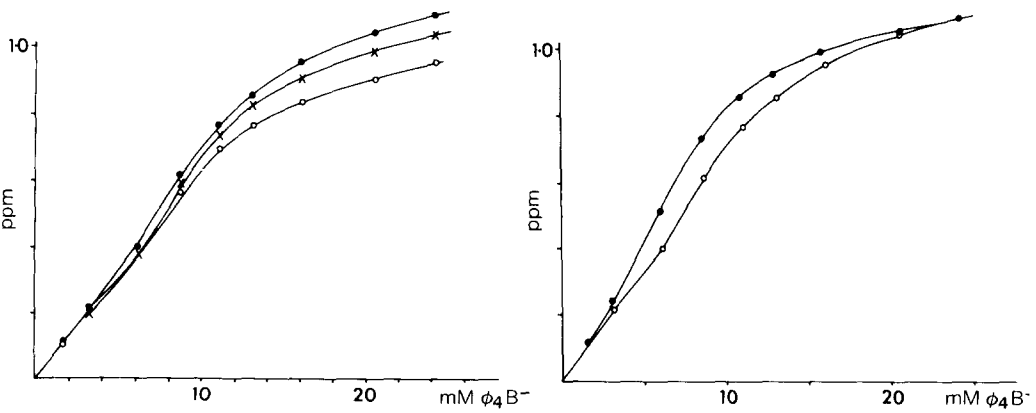

Fig. 3. Effect of NaCl in the bulk solution on the induced shift of the $N(C\underline{H}_3)_3$ resonance of phosphatidylcholine (20 mg/ml) upon addition of tetraphenylboride. ●———●, 200 mM NaCl; X———X, 100 mM NaCl; ○———○, 50 mM NaCl.

Fig. 4. Chemical shift of the $N(C\underline{H}_3)_3$ resonance of phosphatidylcholine (20 mg/ml) induced by tetraphenylboride in the presence of different NaCl distributions ●———●, 200 mM NaCl in internal and external aqueous solutions; ○———○, 200 mM NaCl in external solution only.

trations >0.25 M addition of TPB^- resulted in severe linebroadening of the spectral resonances, the vesicle solution turning rapidly opaque.

No specific cation dependence of the binding of TPB^- could be detected based on a comparison of the influence of NaCl and LiCl. It was, however, observed that low concentrations of $LaCl_3$ ($<10^{-3}$ M) resulted in an ehancement of the degree of TPB^- binding greater than that found at 0.2 M concentrations of the monovalent cation solutions. In the presence of 10^{-3} M $LaCl_3$ the aggregation/fusion of the phosphatidylcholine vesicles occurred above a mol ratio of 0.15 (TPB^-:phospholipid).

Similar results were obtained using lysophosphatidylcholine micelles though the enhancement in the amount of bound TPB^- induced by the presence of cations was found ot occur at all mol ratios of TPB^-:lyosophosphatidylcholine. In both micellar and vesicular systems there was no influence on TPB^- binding upon variation of the couteranion in solution: Cl^-, NO_3^-, SCN^- or ClO_4^-. This suggests that these anions are bound more weakly. That they are bound will be shown in subsequent publications. The relative induced shifts for the various resonances were invariant in all the experiments described.

Tetraphenylarsonium chloride. The interaction of tetraphenylarsonium (TPA^+) with phosphatidylcholine in both homogeneous and mixed (neutral) lipid bilayers was also studied. No shifts were observed for any of the phospholipid resonances up to the highest (solubility limited) concentration of TPA^+ used ($\sim 35 \cdot 10^{-3}$ M). This contrasts with the relatively high affinity of TPB^- for phosphatidylcholine, and unlike the enhancement in binding induced by increasing cation concentration observed in the case of the lipid-soluble anion, addition of various anions (Cl^-, NO_3^-, SCN^-, ClO_4^- and F^-, sodium salts) did not result in cooperative binding of the lipophilic cation.

Effect of negatively charged phospholipids on binding of TPB^- and TPA^+

Mixed lipid bilayers. Addition of TPB^- to a solution of phosphatidylcholine/phosphatidic acid vesicles resulted in shifts on resonances of several groups of phosphatidylcholine, the magnitude of these shifts being strongly dependent on the relative concentration of phosphatidic acid (Fig. 5). The same total phosphatidylcholine concentration was used in each case. The derived relative shifts were the same as those obtained in the case of the homogeneous phosphatidylcholine vesicles. These results indicate that while the affinity of TPB^- for phosphatidylcholine in negatively doped vesicles falls with increasing overall charge, the mode of interaction between the phospholipid and the cation is unchanged. A decrease in the degree of binding was also observed upon introduction of phosphatidylserine (Fig. 6), the effect being larger than had been observed for phosphatidic acid. Increasing mol ratio of phosphatidylserine however led to a decrease of the inhibition of TPB^- binding to phosphatidylcholine in this mixed lipid system, though the effect of phosphatidylserine was still inhibitory. The possibility that this reversal of inhibition with increasing mol fraction of phosphatidylserine was a consequence of the presence of Na^+ as counterion to phosphatidylserine (see Methods and Materials) was investigated by observing the influence of NaCl in the external aqueous medium upon the TPB^--induced spectral shifts (Fig. 6). These are seen to be significantly larger than in the absence of NaCl for TPB^- concentrations greater than a mol frac-

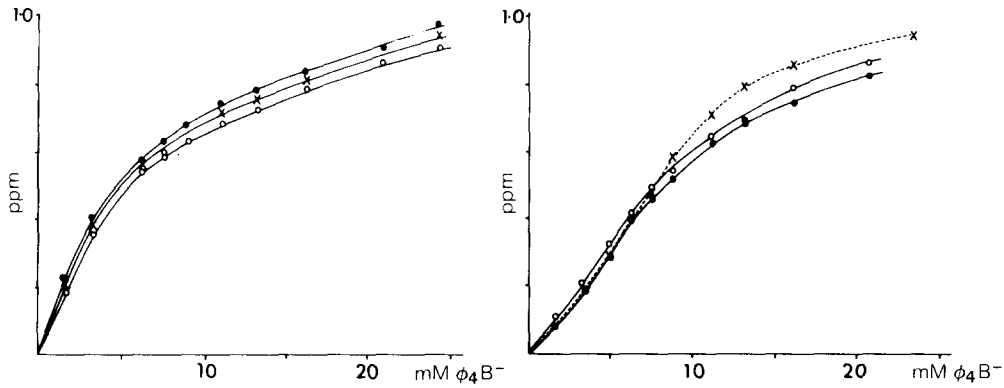

Fig. 6. Chemical shift of the $N(C\underline{H}_3)_3$ resonance of phosphatidylcholine (20 mg/ml) upon addition of tetraphenylboride to phosphatidylcholine/phosphatidylserine-cosonicated vesicles. ●———●, 23% mol ratio phosphatidylserine; ○———○, 37% mol ratio phosphatidylserine; +- - - - -+, 37% mol ratio phosphatidylserine in presence of 10 mM NaCl in the external aqueous solution.

Fig. 5. Tetraphenylboride-induced shifts of the $N(C\underline{H}_3)_3$ group resonance of phosphatidylcholine (20 mg/ml) in pbosphatidic acid/phosphatidylcholine-cosonicated vesicles. ●———●, phosphatidylcholine; X———X, 10% mol ratio phosphatidic acid; ○———○ 22% mol ratio phosphatidic acid.

tion equal to 0.4 (TPB^-:phosphatidylcholine). Though TPB^- binds preferentially to the zwitterionic phospholipid, addition of TPB^- to the mixed lipid vesicles also led to small shifts on resonances of groups deriving from phosphatidylserine molecules. These results were more readily observed in the case of homogeneous phosphatidylserine vesicles as will be described below.

Addition of TPA^+ to the two component, phosphatidylserine/phosphatidylcholine, bilayer vesicles led to shifts of several resonances attributable to phosphatidylserine, the largest shifts being induced for the glycerol $C\underline{H}_2CO$ and hydrocarbon chain $C\underline{H}_2CO$ proton resonances. Binding of TPA^+ to phosphatidylserine was reduced by the addition of NaCl to the external solution. No effects of TPA^+ on resonances of phosphatidylcholine was detected. Thus this lipid-soluble cation binds preferentially to phosphatidylserine, the interaction being localized deeper into the hydrocarbon phase than in the case of TPB^- binding to the polar group of lecithin.

Homogeneous phosphatidylserine bilayers. Addition of TPB^- to phosphatidylserine led to small upfield shifts on some of the phospholipid resonances (Fig. 7). Increasing NaCl concentration resulted in an enhancement of these shifts, most markedly in the case of the $C\underline{H}NH_3^+$ proton, and the derived differential shifts did not remain constant. Larger shifts were readily observed upon binding of TPA^+ to the external phospholipid/aqueous interface, the relative magnitude decreasing in the order $C\underline{H}OCO$ glycerol > $C\underline{H}_2CO$ > $C\underline{H}NH_3^+$ > $POC\underline{H}_2$ (Fig. 8). The extent of binding of TPA^+ to phosphatidylserine was not affect by the presence of Cl^- or F^- (both as sodium salt), and thus cooperativity of binding was not observed. The relative magnitude of the TPA^+-induced shifts, found to be independent of TPA^+ and salt concentrations, differs from that obtained upon the much less specific binding of TPB^- to the surface of phosphatidylserine bilayers. This result supports the observation from the mixed lipid system studies that the tetraphenylarsonium cation is

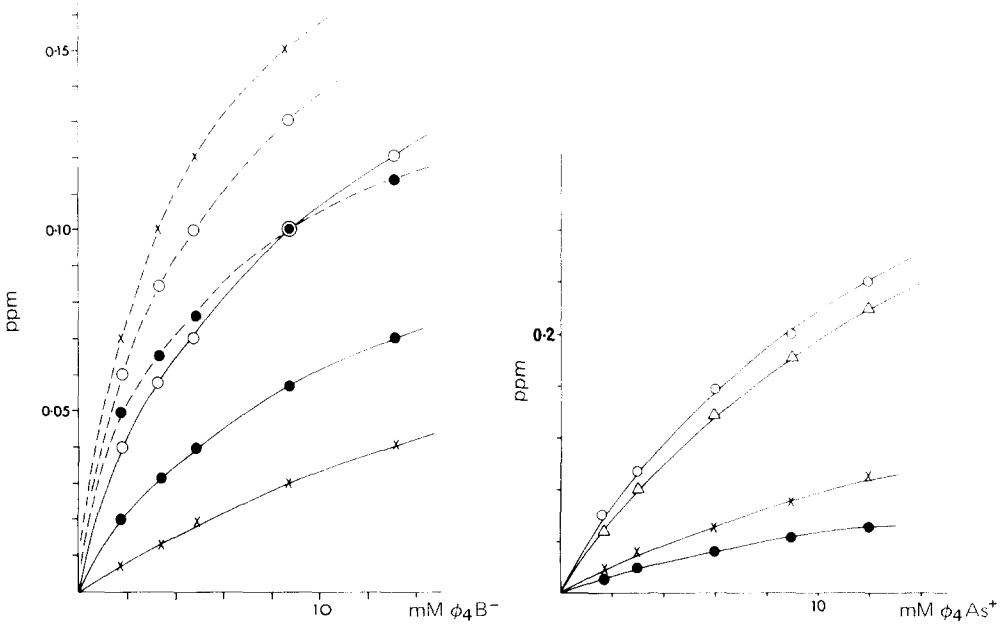

Fig. 7. Induced shifts of some of the proton resonances of phosphatidylserine (8 mg/ml) upon titration with tetraphenylboride in the absence and presence of NaCl (135 mM) in the extravesicular solution. ●———●, $POC\underline{H}_2$; ○- - - - -○, $POC\underline{H}_2$ in presence of NaCl; X———X, $C\underline{H}NH_3$; X- - - - -X $C\underline{H}NH_3$ in presence of NaCl; ○———○, $C\underline{H}_2CO$. ○- - - - -○, $C\underline{H}_2CO$ in presence of NaCl.

Fig. 8. Tetraphenylarsonium-induced chemical shifts of some of the proton resonances of phosphatidylserine (8 mg/ml). ●———●, $POC\underline{H}_2$; X———X, $C\underline{H}NH_3$; ○———○, $C\underline{H}OCO$, △———△, $C\underline{H}_2CO$.

disposed at the bilayer surface in a manner different to the position adopted by the tetraphenylboride anion nearer the aqueous medium.

Diffusion of TPB^- and TPA^+ across the bilayer

Addition of TPB^- to homogeneous phosphatidylcholine vesicles at anion concentrations below approx. 0.2 mol fraction (TPB^-:total phospholipid) led to the resolution of signals deriving from both inward facing and outward facing polar groups due to the interaction of TPB^- with the external bilayer surface only (Fig. 9). Coalescence of these signals occurred with time, indicative of the equilibration of the lipid-soluble anion across the bilayer with consequent binding to the phospholipid molecules at the inner membrane/water interface. The equilibration process was stongly dependent on the external TPB^- concentration and was observed to be sensitive to the presence of added cations (Fig. 9). The large enhancement in the rate of equilibration brought about by various lanthanide salts was not however accompanied by the diffusion of the trivalent cations across the bilayer. This conclusion is reached from the observation that the internal phospholipid resonances were unaffected by addition of either $LaCl_3$ or $PrCl_3$. The latter, paramagnetic cation when bound to the phospholipid polar group induces downfield shifts on the phospholipid resonances, i.e. in the opposite direction to the ring current shifts resulting from the binding of TPB^-. Such paramagnetic shifts observed for the external

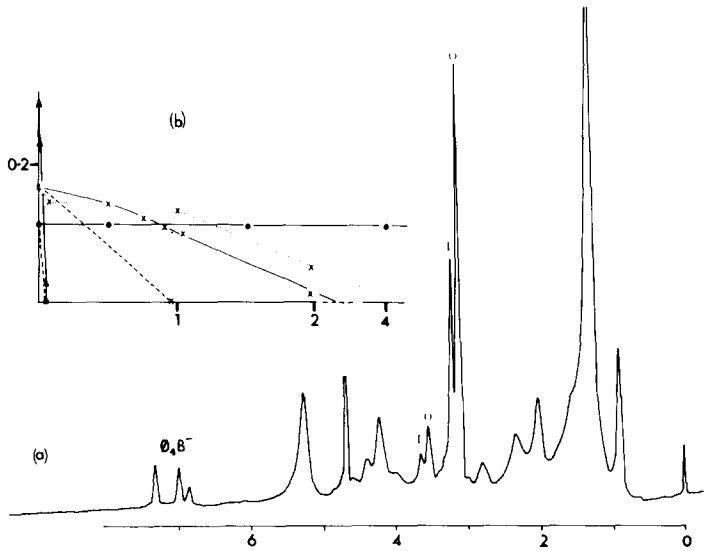

Fig. 9. (a) 270 MHz proton NMR spectrum of phosphatidylcholine (20 mg/ml) vesicles in presence of 1.74 mM tetraphenylboride. The resonances of groups on both inside and external facing surfaces are indicated. (b) Time dependence of equilibration under various solution conditions. ●———●, 1.74 mM tetraphenylboride; X———X, 3.37 mM tetraphenylboride; ▲———▲, 4.81 tetraphenylboride; X- - - - - X, 3.37 mM tetraphenylboride, 100 mM NaCl in both intra- and extravesicular aqueous solutions; ●- - - - - ●, 1.74 mM tetraphenylboride, 1 mM $LaCl_3$ in external solution; X· · · · · X, phosphatidylcholine vesicles prepared at a 33% mol ratio of cholesterol.

molecules were not detected for any of the internal phosphatidylcholine resonances (^1H or ^{31}P).

In the two component lipid system, cholesterol/phosphatidylcholine, increasing cholesterol content led to a notable decrease in the rate of equilibration of TPB^- across the bilayer (Fig. 9), though a certain amount of diffusion does occur at the early stages of the equilibration process. In these mixed lipid vesicle experiments it was noted that higher external TPB^- concentrations were required to induce virtually instantaneous equilibration. In the case of phosphatidylcholine, TPB^- mol ratios >0.2 resulted in superimposed signals from the phospholipid molecules on both sides of the bilayer. At such concentrations the rate of equilibration is faster than can be measured during the NMR experiment.

Experiments carried out to investigate the influence of H^+ concentration on the equilibration process showed that binding of TPB^- to phosphatidylcholine at the external surface and the bulk diffusion rate across the bilayer were independent of pH in the range 2—11.

Discussion

The binding of the tetraphenylboride anion to phosphatidylcholine molecules oriented at a bilayer (or micellar) surface shows a saturation similar to that seen for simple cations [2] but the data here presented do not lend themselves to simple analysis due to salt effects. The degree of loading is dependent upon ionic strength and the nature of the cation added. Inspection of the rela-

tive shifts for the various resonances of the polar group of phosphatidylcholine indicates that the bound anions lie at the membrane/water interface in a manner which permits the electrostatic interaction between the anion and $-N^+(CH_3)_3$ group while minimizing repulsion from the negatively charged phosphate group. Such binding may well result in some deformation of the packing of the polar group. The binding of the tetraphenylboride anion, Stokes radius 4.2 Å [10], will presumably be restricted by steric constraints and the resulting strain may well limit the number of anions that can be bound at the two-dimensional lattice of zwitterionic polar groups. This limitation on the number of binding states will be increasingly important at high loading.

This contribution to the saturation effects is non-coulombic in origin, in contrast to the influence on binding due to the build up of charge upon anion adsorption at the interface. The affinity (or partition coefficient) for the anion to the phospholipid surface is thus a function of the degree of loading. This may be readily seen from the increase in anion binding induced by addition of different cations to the external solution, indicating cooperativity between the binding of anion and cation. Such cooperative effects have been previously observed for the binding of various inorganic cations to phosphatidylcholine [2]. The results described here indicate also that the ionic strength of the intravesicular aqueous solution influences the binding of TPB^- to the external surface. This suggests that it is possible to transfer information across the phospholipid bilayer either by electrostatic or conformational effects [1].

The partitition of TPB^- into the membrane/water interface is observed to be smaller upon incorporation of cholesterol into the hydrocarbon phase. The reduction in the degree of binding of tetraphenylboride was also found upon dilution of the adsorption sites in phosphatidylcholine/mono-olein mixture. In such two component systems there will be patches of free phosphatidylcholine available for interaction with TPB^-, the addition of other non-polar lipids preventing the disposition of the phosphatidylcholine molecules required for the binding of TPB^-, and thereby reducing the effective partition coefficient. Thus in such binary systems both non-coulombic and electrostatic effects are factors influencing the anion-phospholipid interaction at the aqueous interface.

The binding of TPB^- to phosphatidylserine is much weaker than the interaction observed with phosphatidylcholine bilayers, the effect of increasing ionic strength being a reorganization of the anion at the membrane surface indicative of the influence of surface charge on binding. As such, this lipophilic anion provides a useful probe of the membrane surface charge. Greater specificity of binding is found for the tetraphenylarsonium cation which interacts only with the negatively charged phospholipid molecules. The binding site of TPA^+ is localized deeper into the glycerol backbone region of the phospholipid polar group than in the case of TPB^-. The preferential binding of the lipophilic cation may provide an application for the molecule as a probe in surface recognition studies, or in studies on the clustering and organization of membrane constituents.

Permeation of TPB^- across the bilayer was observed to be ionic strength dependent, though the degree of loading was independent of ionic strength over the same concentration range of TPB^- (>0.2 mol ratio). This result suggests that the permeant species may well be the Na^+ complex (i.e. the neutral

molecule) rather than the anionic form of tetraphenylboride. The lack of pH dependence upon the rate of equilibration suggests that protons are not translocated together with the anion ($pK_a < 2$). It is not possible, however, based on the data obtained, to unequivocally identify the charge of the permeating species. The effect of small concentration of $LaCl_3$ markedly enhanced the rate of equilibration across the bilayer, even in the case of the cholesterol-phosphatidylcholine-mixed vesicles whwere the rate of equilibration decreased with increasing 'rigidity' of the hydrocarbon phase at higher cholesterol concentrations. Trivalent lanthanide cations readily displace Na^+ from the membrane surface, leaving fewer Na^+ available at the surface for TPB^- permeation as a neutral species. Whatever the charge of the permeating species, the data indicate that diffusion across the bilayer occurs only after a critical loading of the external surface has been achieved. The observation that the binding of cations at the external surface can cooperatively initiate such loading, and thereby trigger the flux of solute, is reminiscent of physiological gating effects. It is of interest in this respect to note that paramagnetic shifts were not induced on the resonances of tetraphenylboride upon addition of paramagnetic lanthanide salts. This indicates that the cations occupy different sites on the bilayer surface to the sites at which TPB^- is bound. The finding that such cation binding can influence transport is yet another example of 'action at a distance' resulting from cooperative effects at a phospholipid surface. Such effects are not restricted to the external surface alone as seen by the influence of the intravesicular ionic composition on the binding and equilibration of TPB^-.

Conclusions

This paper shows that the lipophilic anion, tetraphenylboride, binds to neutral phospholipid membranes, that binding is enhanced in the presence of Na^+, and that binding is altered by the incorporation of charged groups in the membrane. Thus extreme care must be taken in the interpretation of observed 'potentials' calculated from partition experiments using this anion. The rate of transport of the anion is markedly affected by the presence of gegen-ions.

The lipophilic cation tetraphenylarsonium, has similar properties although it binds more weakly and in a different fashion.

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