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SPECIFICITY OF Na^+ BINDING TO PHOSPHATIDYLSERINE VESICLES FROM A ^{23}Na NMR RELAXATION RATE STUDY

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Summary

^{23}Na NMR relaxation rate measurements show that Na^+ binds specifically to phosphatidylserine vesicles and is displaced partially from the binding site by K^+ and Ca^{2+} but to a considerably less extent by tetraethylammonium ion. The data indicate that tetraethylammonium ion affects the binding of Na^+ only slightly, by affecting the surface potential through its presence in the double layer, without competing for a phosphatidylserine binding site. Values for the intrinsic binding constant for the Na^+-phosphatidylserine complex that would be consistent with the competition experiments (and the dependence of the relaxation rate on concentration of free Na^+) fall in the range 0.4—1.2 M^{-1} with a better fit towards the higher values. We conclude that in the absence of competing cations in solution an appreciable fraction of the phosphatidylserine sites could be associated with bound Na^+ at 0.1 M Na^+ concentration.

Introduction

Early studies of the interaction of Na^+ with negatively charged phospholipids showed that Na^+ at high concentrations was able to displace Ca^{2+} from acidic lipid monolayers [1,2]. This interaction between monovalent ions and acidic phospholipids has been attributed to screening of the negative charges rather than to specific binding of the ion to the lipid [3,4]. However, differences have been observed in the interaction of two monovalent ions, Na^+ and tetraethylammonium ion (Et_4N^+) with phosphatidylserine. Aggregation and precipitation

Abbreviations: Et_4N^+, tetraethylammonium ion; TNS, toluidinylnaphthalene sulfonate.

of phosphatidylserine vesicles occurs in the presence of NaCl concentrations greater than 0.5 M [5—7] but not in the presence of several molar Et_4N^+ [6,7]. Furthermore, the effects of Na^+ on electrophoretic mobility and ^2H NMR spectra are less than those of Ca^{2+}, but greater than those of Et_4N^+ [7]. Hauser and his coworkers attributed these differences to the varying degree of hydration of the ion-lipid complex [7]. In this paper we emphasize the importance of specific binding of Na^+ to phosphatidylserine. Since phosphatidylserine is present in nerve membranes, the amount of Na^+ bound and the resultant degree of charge neutralization may be an important factor in their function.

In a previous study [8], the binding of Ca^{2+} and Mg^{2+} to phosphatidylserine vesicles could be explained only by assuming a moderately strong, specific binding of Na^+ to phosphatidylserine. The values of the intrinsic binding constants of Na^+, Mg^{2+} and Ca^{2+} to phosphatidylserine which best described the data in the above study were 0.8, 4 and 35 M^{-1}, respectively. In view of the relatively large concentration of Na^+ in physiological solutions, a significant fraction of the negatively charged headgroups of phosphatidylserine may be neutralized by Na^+. An independent confirmation for this charge neutralization involving 'tight' Na^+ binding was discussed by Nir and Bentz [9] whose calculations of rates of aggregation predicted fast aggregation of phosphatidylserine vesicles in 1 M Na^+ solution, as opposed to no aggregation in 1 M solution of another monovalent cation, such as tetramethylammonium, which was not expected to bind significantly to phosphatidylserine.

We present here the results of a study which uses ^{23}Na relaxation rates to probe the binding of Na^+ (and, via competition experiments, that of other ions) to phosphatidylserine vesicles. One aspect of the binding which is of particular interest is whether the sodium ion, partially or completely hydrated, binds, however weakly, to some specific group (e.g., carboxylate or phosphate) and is relatively fixed at the surface of a phosphatidylserine vesicle (i.e., in a Stern layer) or whether it is held in a layer near the surface (i.e., in a diffuse double layer or Gouy-Chapman layer) by nonspecific electrostatic interactions with the net negative charges at the surface. By nonspecific electrostatic interactions we mean those which would be essentially the same for all monovalent cations of a given size. The two situations cited above represent limits on what may well be a continuum of binding types; at one extreme is the strong specific binding displayed by divalent ions such as Ca^{2+}; at the other stands the presumably nonspecific binding of hydrophobic cations such as the tetraalkyl ammonium ions. Since the observed ^{23}Na relaxation rates will be a number average over free Na^+ (in bulk solution) and Na^+ bound to phosphatidylserine, the concentration dependence of the relaxation rates and the effect of competition with other ions may, in principle, be used to answer this question.

An early ^{23}Na NMR relaxation rate study of Na^+ binding to phosphatidylserine vesicles [10] was interpreted in terms of weak binding. Other investigations [11—13] of multilamellar model membranes and of liquid crystal systems have shown very strong effects on the continuous wave NMR spectra of ^{23}Na. Moreover, Gustavsson and Lindman [14] have shown that for aqueous surfactant systems in which micellar aggregation occurs, alkali metal ion NMR relaxation rate data can be interpreted to show specific binding of the fully hydrated ion to alkyl carboxylate end-groups, but not to alkyl sulfates. In the

former case, hydrogen bond interactions between the carboxylate and water coordinated to Na^+ were inferred to be present, whereas they were thought to be less important for the more acidic sulfate groups. In the latter case, electrostatic interactions, which varied with alkali metal ion type, were invoked to account for the enhanced concentration of cations at the micelle-solution interface, i.e., a Stern layer type model was implicitly used.

Experimental

Phosphatidylserine was isolated from beef brain by chromatography through DEAE-cellulose and finally through silicic acid, and washed extensively with EDTA to remove divalent cations [15,16]. No impurity was detected in thin-layer chromatography on silica gel H (Applied Science Labs., State College, PA) in a solvent of chloroform/methanol/7 M ammonia (230 : 90 : 15, v/v). The phosphatidylserine was stored in chloroform (10 μmol/ml) under nitrogen in sealed ampoules at —50° C. A freshly opened ampoule was used for each experiment. All chemicals and solvents were reagent grade. Water was twice distilled, the second time in all-glass apparatus.

Sonicated unilamellar vesicles were prepared [17] in a buffer comprising 100 mM NaCl, 2 mM L-Histidine, 2 mM *N*-Tris(hydroxymethyl)methyl-2-amino-ethanesulfonic acid (TES) and 0.1 mM EDTA adjusted to pH 7.4. The preparation was centrifuged at $1 \cdot 10^5 \times g$ for 30 min in a Beckman model L3-50 ultracentrifuge (Beckman, Spinco division, Palo Alto, CA) to remove large vesicles. The supernatant (20—24 μmol phosphatidylserine/ml) was dialyzed for 2—3 h at room temperature against buffer containing the appropriate concentration of cations, so that the specified concentration of ions in bulk solution ('free') was that of the dialysate solution. No correction for binding to phosphatidylserine was required since the volume of buffer solution was at least 100-fold greater than that of the sample solution; moreover, for the Ca^{2+} competition experiments, the buffer solution against which the sample was being dialyzed was changed several times during the course of the dialysis. The samples were kept under a nitrogen or argon atmosphere at all times to prevent oxidation, and NMR experiments, which for the most dilute samples lasted no longer than 3 h, were carried out immediately after dialysis was completed.

Spin-lattice relaxation rate measurements were carried out at 15.870 MHz by means of a Bruker B-KR 321s pulsed NMR spectrometer modified for external field frequency control and FT NMR with signal averaging. 15—24 points for a given T1 Inversion Recovery pulse sequence [18] were computer fitted, by a non-linear least squares method, to the magnetization recovery curve, $M_z(t) = M_0(1 - 2\,e^{-t/T_1})$. The Carr-Purcell, Meiboom-Gill pulse sequence [19] or linewidths were used to determine values of the spin-spin relaxation rate, $1/T_2$. All measurements were carried out at ambient temperature, 28 ± 2° C.

Theoretical

The principal relaxation mechanism for ^{23}Na nuclei (spin quantum number, $I = 3/2$) comes from interaction of the nuclear quadrupole moment with fluctuating electric field gradients at the ^{23}Na nucleus. When the motional

narrowing condition * holds, the spin-lattice and spin-spin relaxation rates ($1/T_1$ and $1/T_2$, respectively) are equal and are given [20] by Eqn. 1

$$R = \frac{1}{T_1} = \frac{1}{T_2} = \frac{2\pi^2}{5}\left(\frac{e^2qQ}{h}\right)^2 \tau_c \tag{1}$$

where eQ is the nuclear electric quadrupole moment, eq is the principal component of the electric field gradient tensor (in a principal axis system), τ_c is the correlation time for the random motion giving rise to a fluctuating field gradient and h is Planck's constant. If the ^{23}Na nuclei exchange rapidly (compared to the relaxation rates) between several sites with different relaxation rates, the observed relaxation rate is just a number average over the relaxation rates at the various sites:

$$R_{obs} = \sum_i p_i R_i \tag{2}$$

where p_i is the probability of a nucleus being at site i and R_i is the relaxation rate at the site.

For our case we will initially consider a two-site model: 'free' (Na^+ in bulk solution) and 'bound' (to the phosphatidylserine vesicle) with spin-lattice relaxation rates R_f and R_b, respectively. At this point we do not distinguish between the two limiting types of binding mentioned in the Introduction. Since the vesicle membrane is essentially impermeable to Na^+ over the time required for an NMR experiment [21] and since the volume of solution contained within the vesicles is only a small fraction of the total, the contribution of Na^+ in the interior of the vesicles can be neglected. That is to say, the NMR signal from Na^+ in the interior of the vesicle (bound and free) is much less intense than that from Na^+ (bound and free) outside the vesicle. Then the observed spin-lattice relaxation rate is given by Eqn. 3:

$$R_{obs} = p_b R_b + (1 - p_b)R_f \tag{3}$$

where p_b is the fraction of Na^+ bound to the external surface of phosphatidylserine vesicles.

If the motional narrowing condition is not satisfied for the bound site, then the situation becomes more complicated [22]. Even though exchange between free and bound Na^+ is fast, the magnetization decay curves for both $1/T_1$ and $1/T_2$ relaxation will be biexponential. The spin-lattice magnetization decay curve would be the superposition of a slow and fast decaying component, with relative weights, respectively, of 80 and 20%. The spin-spin magnetization decay would also be the superposition of a slow and fast decaying component with, however, relative weights of 60 and 40%, respectively. Such behavior has been observed [23] for Na^+ ions in a multilamellar lipid dispersion in water, a cephalin fraction consisting of approximately 33% each of phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol. Expressions for the relaxation rates of the fast and slow decaying components have been given by Bull [22] in terms of the parameters in Eqn. 1. Since no biexponential character to the magnetization decay curves was observed in our study and

* $\omega_0\tau_c << 1$, where ω_0 is the ^{23}Na Larmor frequency at the magnetic field employed.

since the measured spin-lattice and spin-spin relaxation rates were the same, to within experimental error, we will assume the motional narrowing condition is satisfied and use Eqns. 1—3 to interpret our results.

The fraction of bound Na^+, p_b, is given by Eqn. 4:

$$p_b = \frac{0.66\, f\, C_p}{0.66\, f\, C_p + C_m} \tag{4}$$

where f is the fraction of phosphatidylserine to which the Na^+ is bound, C_p is the molar concentration of phosphatidylserine and C_m is the molar concentration of free Na^+; the factor 0.66 reflects the fraction of phosphatidylserine in the external monolayer of the sonicated unilamellar vesicles. (As pointed out above, only the Na^+ bound to the external surface is detected in the observed relaxation rates.) *

The equations and procedure for calculating f, which have been given in more detail elsewhere [24], are summarized below:

$$\sigma = \frac{\sigma^0}{1 + \sum Y_0^{z_i} K_i C_i} \tag{5}$$

$$\sigma = \frac{1}{g}\left(\sum C_i [Y_0^{z_i} - 1]\right)^{1/2} \tag{6}$$

where C_i is the concentration of free cations i with charge z_i, σ and σ^0 are the surface charge densities, respectively, for the given cation concentrations and for very dilute solutions, and the K_i is an intrinsic binding constant for cation i. The quantity Y_0 is given by:

$$Y_0 = \exp[-e\Psi(0)/kT] \tag{7}$$

and reflects the enhancement in the concentrations of monovalent cations at a negatively charged surface the potential of which is $\Psi(0)$. In Eqn. 7, e is the magnitude of an electronic charge, k is Boltzmann's constant, and T is the absolute temperature. The quantity g in Eqn. 6 equals 272 at room temperature [3], and should be replaced by $272[78 \times 298/\epsilon T]^{1/2}$ at other temperatures. ϵ is the dielectric constant of the medium. The value of σ^0 is 1/70 in units of $e/Å^2$. The fraction f of phosphatidylserine molecules with bound Na^+ [24] is given by

$$f = Y_0 K_{Na} C_{Na} \sigma/\sigma^0 \tag{8}$$

and is thus readily calculated from the value of Y_0, which is obtained from a numerical solution for a given set of concentrations and binding constants.

Results

In Table I the values of R_{obs}, the measured values of the spin-lattice relaxation rate, are presented for several Na^+ concentrations (with 0.020 or 0.024 M

* Experiments in which samples of phosphatidylserine vesicles initially in a 0.1 M Na^+ solution were dialysed against 0.1 M K^+ solution (so that the concentration of Na^+ — bound and free — outside the vesicle was less than 0.01 M) verified this point: the intensity of the ^{23}Na signal after dialysis was reduced by more than a hundred-fold.

TABLE I

MEASURED AND CALCULATED RELAXATION RATES OF SODIUM IN A SUSPENSION OF SONICATED PHOSPHATIDYLSERINE VESICLES

Cation concentrations (M)	R (observed) (s^{-1})	R_{calc}	$R_{obs}-R_{calc}$	p_b * (fraction of Na bound)
0.05 Na^+	56.8	55.5	1.3	0.143
0.1 Na^+ **	41.3	42.1	−0.8	0.087
0.1 Na^+ + 0.1 Et_4N^+ **	39.25	40.35	−1.1	0.074
0.15 Na^+	33.32	34.0	−0.7	0.054
0.1 Na^+ + 0.0005 Ca^{2+} **	32.85	29.6	3.3	0.035
0.2 Na^+ **	29.55	32.5	−2.9	0.047
0.25 Na^+	30.12	29.2	0.9	0.033
0.5 Na^+ ***	(26.72)	(25.8)	0.9	0.017
0.5 Na^+ (vesicle-free medium)	19.45 ± 2.4			
0.25 Na^+ (vesicle-free medium)	20.56 ± 1.2			
0.1 Na^+ + 0.1 K^+	32.59 ± 1.3			

* Calculated from Eqns. 4—8 (K = 0.8 M^{-1}).

** In these cases the concentration of phosphatidylserine is 24 mM. In all other cases the concentration is 20 mM; $K_{Ca^{2+}}$ = 35 M^{-1} was assumed (ref. 8).

*** Results obtained from least squares calculations based on eight points.

phosphatidylserine) and for competition experiments with several metal ions (Ca^{2+} at 0.0005 M, K^+ at 0.1 M, Et_4N^+ at 0.1 M). Several qualitative features of these results should be noted. The addition of 0.1 M Et_4N^+ does not change appreciably the relaxation rate of a 0.1 M Na^+ solution, although 0.1 M K^+ and 0.0005 M Ca^{2+} do. The decrease in relaxation rate with added Ca^{2+} or K^+ corresponds to a decrease in p_b, the fraction of bound Na^+. Clearly the charge and concentration of the ions are not the only factors which affect competition with Na^+ for binding to the phosphatidylserine surface. From the relaxation rate changes, the binding of Na^+ to phosphatidylserine appears to be almost saturated at somewhat greater than 0.2 M Na^+.

The results of an analysis of the relaxation rate data, obtained by the procedure outlined in the Theoretical section, are also presented in Table I. The calculated values of R are given for a binding constant K = 0.8 M^{-1}. However, values for K of 0.1, 0.2, 0.4 and 1.2 M^{-1} were also used. For a given value of K, the values of p_b and f were calculated from Eqns. 4—8. Each K value then yields a set of linear equations (Eqn. 3) for which R_{obs} and p_b have specified values and R_b and R_f are unknown parameters to be determined from a linear least squares fit (see Table II). Two criteria were employed to test the goodness of fit: one was the agreement between calculated and observed R values; the second was the agreement between calculated and observed values of R_f. The latter was taken as the measured value, R_{obs}, for aqueous solutions not containing vesicles *.

The p_b values in Table I, calculated for K = 0.8 M^{-1}, are derived from seven data points. We have also included in this table the results for eight data points;

* Theoretical calculations and experimental data [25,26] demonstrate that the dependence of ^{23}Na NMR relaxation rates on concentration and the presence of other cations are essentially negligible for aqueous solutions at concentrations less than approx. 0.5 M in the absence of specific complexing agents such as phosphatidylserine.

TABLE II

SUMMARY OF LEAST SQUARES CALCULATIONS FOR DIFFERENT BINDING CONSTANTS OF Na^+ TO PHOSPHATIDYLSERINE

R.M., root mean square error.

Binding constant of Na^+ to phosphatidylserine M^{-1}	R_f (s^{-1})	R_b (s^{-1})	R.M.	Largest deviation between R_{obs} and R_{calc}	Fraction of phosphatidylserine bound to Na^+ for 0.1 M Na^+ in solution
1.2	20.2	255	1.9	−2.8	0.680
0.8	21.2	260	2.2	3.3	0.638
0.4	23.2	271	3.1	5.1	0.560
0.2 *	24.6	304	3.2	6.0	0.384
0.1 *	24.9	363	3.4	6.2	0.290

* Results obtained from least squares calculations based on eight points.

this set included R_{obs} for the 0.5 M Na^+ solution, for which aggregation of vesicle occurs. In these calculations we assumed that the binding constant for Et_4N^+ was zero, based on the results of the competition experiment between 0.1 M Na^+ and 0.1 M Et_4N^+ and also from observations indicating that in 1 M Et_4N^+ solution there is no aggregation of phosphatidylserine vesicles [6,7]. If the result for the Et_4N^+-Na^+ competition experiment is not included in the data set, the derived relaxation rate parameters are not appreciably changed. The result for the competition experiment between K^+ and Na^+ was not included in the data set, although it is evident that the binding constant for K^+ is roughly the same as that for Na^+. *

In Table II we summarize the results for other assumed values of the Na^+ binding constant. It appears that the fit becomes progressively worse as one goes from $K = 1.2\ M^{-1}$ to $K = 0.1\ M^{-1}$. Although the range of K values, 1.2—0.4 M^{-1}, gives a parameter fit within the experimental uncertainty, it is evident that the agreement is poor enough for $K = 0.2$ and 0.1 M^{-1} to justify exclusion of these two values.

Discussion

In the model outlined in the Theoretical section only two sites for Na^+ were considered, Na^+ bound to phosphatidylserine and Na^+ free in solution. In principle, three sites could have been considered: free, double-layer and bound to a specific phosphatidylserine site. Nir et al. [24] have presented expressions by which the amount of cation residing in the double layer at any distance away from the surface can be calculated. Such calculations show that under our experimental conditions a sufficient fraction of Na^+ is present in the double layer to contribute to the observed relaxation rate, if the relaxation rate of Na^+ at double-layer sites were appreciably different from that of free Na^+. For example, in a 0.1 M Na^+ sample, the amount of Na^+ contained in the double-layer region is about half that which is more tightly bound to the phosphatidyl-

* Preliminary results from similar competition experiments with Li^+ and Rb^+ indicate that this may also be the case for these ions.

serine vesicles if the binding constant K equals 0.8 M^{-1}.

We have assumed that the relaxation rate of Na^+ in the double-layer region is approximately that of free Na^+, on the basis of the following considerations. First, a good fit can be obtained with the assumption that differences between R_{calc} and R_{obs} are within experimental error and the derived value for R_f is close to that taken from vesicle-free solutions. Second, the addition of Et_4N^+ does not change the relaxation rate appreciably, although such a change would be expected if Na^+ in the double-layer gave an appreciable contribution to the relaxation rate and was displaced in approximate mole ratio by added Et_4N^+.

If Et_4N^+ does not enter the double-layer (possibly because of its size or the effects of a hydrophobically bound hydration shell), then of course it would not displace Na^+. However, this assumption requires considerable modification of a simple double-layer model, in which the concentration of ions in the bulk solution and their charge are the prime relevant variables in determining the concentration of ions in the double layer.

It may be noted that Eqns. 5 and 8 (see also ref. 24) do not give a linear relation between the fraction of Na^+ bound and its concentration (or binding constant). When Na^+ alone is present in solution, an increase in its concentration results in a reduction in the surface potential because of two effects. The first effect, screening, is expressed by the Gouy-Chapman treatment via Eqn. 6, which gives a reduction is surface potential for a constant surface charge with an increase in concentration of cations in solution. The second effect, charge neutralization, corresponds to a reduction in the surface charge density as given by Eqn. 5. An inspection of Table II illustrates the effect of charge neutralization. For instance, if the binding constant of Na^+ to phosphatidylserine (at 0.1 M Na^+) increases from 0.1 M^{-1} to 1.2 M^{-1} the fraction of charges neutralized does not increase by a similar factor of 12, but only by 2.3.

It would be expected that Et_4N^+ addition would decrease the fraction of Na^+ bound to phosphatidylserine, even if Et_4N^+ itself did not bind to phosphatidylserine, because of the altered potential near the phosphatidylserine surface due to increased cation concentration in the double layer. Such an effect is present, but is small, as can be seen from the data in Table I: the fraction of Na^+ bound to phosphatidylserine decreases by about 10% when 0.1 M Et_4N^+ is added to the 0.1 M Na^+ solution. This change was calculated from Eqns. 4 to 8 for K = 0.8 M^{-1}. For a smaller value of K, 0.4 M^{-1}, the calculated decreases in p_b are similar, somewhat greater than 10%.

Even if the two-site model we have presented gives a qualitatively consistent picture for the relaxation rate results, one may still ask how sensitive are our calculations to the choice of binding constants. As illustrated in Table II, the NMR results are better fitted by a binding constant of 1.2 M^{-1} than by 0.8 or 0.4 M^{-1}, but the differences are within the experimental uncertainty. Hence it might be argued that the NMR results are not sensitive enough to discriminate fully between this range of values. Nevertheless, inspection of the last column of Table II shows that over this range of K values (1.2—0.4 M^{-1}) a significant fraction of phosphatidylserine is calculated to be associated with bound Na^+. Even if smaller values for the binding constants are taken (0.2—0.1 M^{-1} — and these values, we emphasize, do not satisfactorily explain the NMR results), 30% of the phosphatidylserine is associated with Na^+ in a 0.1 M solution.

Despite possible charge neutralization by bound Na^+, divalent cations, such as Ca^{2+} at low bulk concentrations, compete effectively with Na^+ for phosphatidylserine binding sites. However, the values of $R_{obs} = 32.85\ s^{-1}$ for the 0.1 M Na^+, 0.0005 M Ca^{2+} sample rules out a complete displacement of Na^+. From the calculations outlined in the Theoretical section, one finds that 22% of the phosphatidylserine charges are associated with Na^+ and 53% are neutralized by Ca^{2+} for this case.

The fractional coverage of phosphatidylserine by bound Na^+ given by the above calculations (Table II) is in good agreement with that estimated for Na^+-octanoate micelles [14], although our calculated value for R_b, the relaxation rate of bound Na^+, is approximately three times greater than the value derived for the latter system. For the Na^+-octanoate system, Gustavsson and Lindman concluded [14] that fully hydrated Na^+ was bound to the micelle surface via hydrogen bonds between carboxylate groups and water in the first coordination sphere of Na^+. Our derived value for R_b is consistent with such a picture.

It could be argued also that the value of R_b derived here for bound Na^+ is too small for Na^+ complexed to a high molecular weight aggregate with a long rotational correlation time, such as a phospholipid vesicle. This argument can be answered as follows: first, the fluctuating electric field gradient may be small if the bound Na^+ ion is fully hydrated, i.e., if the symmetry at the ^{23}Na nucleus is perturbed only slightly; second, the correlation time for quadrupolar relaxation may correspond to some local motion (e.g. libration of the group binding Na^+, pseudorotation of the hydrated Na^+ ion, or exchange between double layer and the bound phosphatidylserine site) and thus be much smaller than a rotational correlation time for rotation of the phosphatidylserine vesicles.

The results and analysis presented here show that the binding of Na^+ to phosphatidylserine vesicles involves more than nonspecific competition with other ions for double-layer sites. A model in which an appreciable fraction of phosphatidylserine groups are associated with bound Na^+, in a complex which is more weakly bound than that of Ca^{2+}-phosphatidylserine, fits the results both qualitatively and quantitatively. An important assumption in our analysis has been that Et_4N^+ can compete and displace Na^+ from the double layer. However, if this were not the case, then an explanation would be needed as to why Et_4N^+ did not enter the double layer or displace Na^+ on essentially a statistical basis, and such a rationalization is not evident to us.

It may be of interest to add that the notion of some degree of tight binding of Na^+ to phosphatidylserine has been useful in explaining a variety of phenomena. Initially it was introduced [8,24] to explain the experimental results of binding of Ca^{2+} and Mg^{2+} to phosphatidylserine in the presence of Na^+. As we pointed out, the value of 0.8 M^{-1} [8,24] for the intrinsic binding of Na^+ to phosphatidylserine is in accord with our present NMR studies which provide direct evidence for the binding of Na^+. The degree of charge neutralization by sodium was also confirmed by calculations on rates of aggregation [9] and by experiments [5—7] which showed aggregation and precipitation of phosphatidylserine vesicles in the presence of Na^+ but not in the presence of Et_4N^+. On the other hand it might appear that our results for the surface potentials [24] were much lower than those of McLaughlin et al. [3] and

McLaughlin [27], which were obtained from conductivity measurements on black lipid membranes. We will omit here the discussion of this source of discrepancy (see discussion in ref. 24) because there are some new data (McLaughlin, S.G.A., personal communication) from electrophoretic measurements which confirm our previous values [24] for the surface potentials. The values of approx. −60 mV and −80 mV for the ζ potentials of phosphatidylserine liposomes in the presence of 0.1 M Na^+ and Et_4N^+, respectively, are in full agreement with our previously predicted values [9,24], giving the same differences (approx. 20 mV) between the surface potentials [9] and the ζ potentials measured previously in the presence of Na^+ and Ca^{2+} [28].

In addition, recent experiments with the fluorescent probe, TNS, (McLaughlin, S.G.A. and Eisenberg, S., personal communication) have given estimates of surface potential with phosphatidylserine vesicles in NaCl, which are substantially lower than those predicted by Gouy-Chapman equation. This anomaly could, of course, be explained adequately by 'tight' Na^+ binding which would reduce the potential of phosphatidylserine bilayers at the water interface.

The relatively 'tight' binding for Na^+ and K^+ reported here could be related to the specificity of alkali metal ions with phosphatidylserine membranes in terms of vesicle permeability [21] and bilayer transmembrane potentials [29] reported earlier. It would be of interest to examine whether such binding is also observed with other acidic phospholipids or whether it is related to the specific headgroup configuration of phosphatidylserine.

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