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HYDROPHOBIC ION PROBE STUDIES OF MEMBRANE DIPOLE POTENTIALS

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Hydrophobic anions of dipicrylamine and of sodium tetraphenylborate have been employed as probes of interfacial dipole potential variations in lipid bilayer membranes. Systematic variation of dipole potentials has been achieved by introduction of compounds incorporating N^+ and B^- charge centers. Distribution of hydrophilic and and hydrophobic groups relative to these charge centers has been shown to control the orientation in the membrane/solution interface of the electric dipole moment formed by these centers. Thus triphenyl-[4-trimethylphenylammonium] borate orients with the B^- center, surrounded by phenyl groups, embedded in the membrane, while the smaller methylated N^+ center is directed toward the aqueous phases. This orientation has been confirmed using dipicrylamine probe ions. Results obtained in this system have been interpreted quantitatively using a previously developed model incorporating discrete charge effects. A second class of compounds, tri-*n*-alkylamine borane (T_nAB) complexes having the generic formula $(C_nH_{2n+1})_3N^+B^-H_3$, have also been synthesized for this study, using even-carbon alkyls ranging from ethyl to decyl. Molecular orientation of the complex is with the N^+ center and its associated alkyl groups directed into the membranes, while the protonated B^- center is directed toward the aqueous phases, as confirmed by use of tetraphenylborate ions as probes.

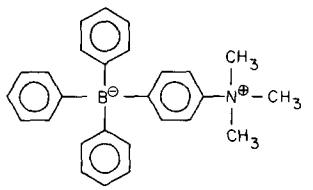
Introduction

The oriented adsorption of dipolar molecules at the surface of a bilayer membrane would be expected to modify its transverse electrostatic potential profile. This effect has been observed for uncharged dipolar molecules such as cholesterol [1,2], phloretin [3,4] and other compounds [5,6]. Cholesterol raises the positive interior potential of membranes, while phloretin has the opposite effect. It appears likely that the orientation of the dipoles contributed by such interfacially adsorbed molecules could be determined by the distribution of hydrophobic and hydrophilic groups relative to the charge centers which comprise the molecular dipole. To test this hypothesis systematically, we have synthesized amine borane compounds with a variable distribution of hydrophobic and hydrophilic groups relative to the N^+ and B^- charge centers on the molecules. Thus, triphenyl-[4-trimethylphenylammonium] borate (TTB) has a B^- charge center surrounded by phenyl groups, connected by a bridging phenyl group to a much smaller methylated N^+ center. Thus, orientation of this molecule with the B^- center directed toward the membrane interior is expected. In addition, we have synthesized tri-*n*-alkylamine borane complexes having the generic formula $(C_nH_{2n+1})_3N^+B^-H_3$ and designated by the abbreviation T_nAB. Even-*n* alkyl groups ranging from ethyl to decyl have been employed. In this case the expectation is that the hydrophobic alkyl chains

Abbreviations: TTB, triphenyl-[4-trimethylphenylammonium] borate; DMSO dimethyl sulfoxide.

attached to the N^+ center would be more favorably accommodated in the membrane than would the protonated B^- center.

The initial observation by Ketterer et al. [7] of current transients accompanying voltage pulses applied to bilayer membranes in the presence of hydrophobic ions has opened the possibility of using such a system to probe changes of the electrostatic potential profile encountered by a charge crossing the membrane, in particular, changes of the profile accompanying oriented adsorption of dipolar molecules at the membrane/solution interfaces. In favorable cases the relaxation time of the current transient is long compared with the membrane charging time. Thus the membrane is effectively voltage-clamped throughout the duration of the hydrophobic ion current transient. The relaxation time, the initial conductance, and the total charge translocated during the transient provide information about the energy barrier experienced by the hydrophobic ion. Thus, for example, adsorption of dipoles at the membrane/solution interfaces with their positive ends directed into the membrane would be expected to raise its positive interior potential and thereby facilitate the translocation of hydrophobic anions, reducing the relaxation time of the corresponding transient. Reversal of the sense of dipole orientation would increase the relaxation time. Hydrophobic ions used to probe the electrostatic potential of bilayer membranes include the anions, tetraphenylboron [3–5,7,8], dipicrylamine [6,7,9,10], and the cations tetraphenylarsonium, [3,5] and tetraphenylphosphonium [1,2].



triphenyl-[4-trimethylphenylammonium]borate

(TTB)

$(C_nH_{2n+1})_3\text{-}N^{\oplus} : B^{\ominus}H_3$

Tri-n-alkylamineborane complex

(T_nAB)

Fig. 1. Structures of the dipolar boron-nitrogen compounds used are illustrated. Locations of positive and negative charge centers are indicated.

Hydrophobic ions at low aqueous phase concentrations (no more than 10^{-7} kmol/l) do not significantly perturb the electrostatic potential profile existing prior to their addition. The relaxation time of the observed transient currents is independent of the probe ion concentration, while the initial conductance increases linearly with this concentration, indicating ideal adsorption of these ions. As the concentration of hydrophobic ions is further increased, however, the relaxation time increases, while the initial conductance goes through a maximum and decreases [9,11,12]. These effects may be attributed to non-ideal adsorption of hydrophobic ions, i.e., to interfacial saturation, and to perturbation of the membrane potential profile by the adsorbed ions themselves. The latter phenomenon can best be explained by considering discrete charge effects. Hydrophobic ions adsorbed in the membrane will induce an interior electric field which can be viewed as due to a dipole consisting of the ion itself and an image charge of opposite sign located on the opposite side of the interface [12,13].

Similar considerations of imaging permit an adsorbed dipole to be regarded as two dipoles of equal strength and similar orientation. Since the exact location of these dipoles along the bilayer normal is not critical to the calculation of their effect on the electrostatic barrier [13], each dipole and its image may be replaced by a single dipole of twice the actual moment of the adsorbed species.

Thus, both adsorbed hydrophobic ions and dipolar molecules can be modelled as dipoles located at the membrane/solution interface. It is therefore natural to study simultaneously the effects of adsorbed ions and of dipolar molecules on the electrostatic energy barrier. Here we present data on (a) the modification of the membrane electrostatic barrier by adsorbed TTB, using dipicrylamine as a probe, and (b), the modification of the barrier by adsorbed T_nAB complexes, using tetraphenylborate as a probe. The data of (a) are compared quantitatively to the model of Kleijn and Bruner [13]. Additional data of Wang [14] are also used to test this model.

Theory

We summarize here the main features of the model of Kleijn and Bruner [13], which will be used below in the analysis of data presented. We adopt the view that both adsorbed ions and dipoles, embedded in the membrane near its interface with the surrounding solutions, can modify the electrostatic potential profile [15]. Though diffuse layer potentials can be included, our summary here is based upon an image charge analysis [16] in which the aqueous phase is treated as a perfectly conducting medium, while the membrane is modelled as a film of uniform and low dielectric constant. The mutual interaction between surface species, and between surface species and translocating charges, can be calculated on this basis, treating adsorbed ions and dipolar molecules as discrete entities.

The aqueous solutions are modelled as ideal, which is justified since aqueous concentrations of all adsorbed species are low (no greater than 10^{-5} kmol/l). The adsorbed phase is modelled as a non-ideal two-dimensional gas of two components (hydrophobic ions and dipolar molecules). Using subscripts d and q to label dipolar molecules and hydrophobic ions respectively, the model predicts the following relations between aqueous concentrations, $c_{d,q}$, and interfacial densities, $\rho_{d,q}$, of charged and polar species:

$$\rho_d \exp[-G_{dq}\rho_q - G_{dd}\rho_d] = S_d c_d \tag{1}$$

where

$$G_{dd} = -\pi\Gamma(1/3)\gamma_{dd}^{2/3};\ \gamma_{dd} = \frac{P_d^2}{4\pi\epsilon\epsilon_0 kT}$$

$$G_{dq} = -\pi\Gamma(1/3)\gamma_{dq}^{2/3};\ \gamma_{dq} = \frac{P_d P_q}{4\pi\epsilon\epsilon_0 kT}$$

Here S_d is a constant partition coefficient, Γ is the gamma function, and P_d and P_q are the effective dipole moment of the dipolar species (twice its intrinsic moment) and the dipole moment of the hydrophobic ion and its image charge, respectively. A similar expression for hydrophobic ions is obtained from Eqn. 1 by interchange of subscripts d and q. Eqn. 1 and its counterpart for adsorbed ions reduce to the ideal case for low $\rho_{q,d}$ and $c_{q,d}$ values. The exponential factor describes the interactions at the surface. The subscripted terms in G give the surface from which a molecule (ion) of the first subscript species is excluded due to interaction with a molecule (ion) of the second subscript species. Obviously $G_{dq} = G_{qd}$.

Changes in maximum height of the electrostatic potential barrier, as sensed by a charge crossing the membrane, can be separated into contributions due to adsorbed ions and to dipolar molecules. The former is:

$$W_q = \frac{\rho_q}{6\epsilon\epsilon_0}\left[2qP_q \exp(\gamma_{qq}/x^3)\Gamma(1/3)\Gamma(2/3,\gamma_{qq}/x^3) - qP_q\Gamma(1/3)\Gamma(2/3) - \frac{P_q^2}{2}\gamma_{qq}^{-1/3}\Gamma(1/3)\right] \tag{2}$$

where q is the electronic charge and x is the half-thickness of the membrane. The second gamma function on the right is the incomplete gamma function. Eqn. 2 includes a correction for the presence of adsorbed ions on both sides of the membrane. The change in potential energy sensed by a translocating charge due to adsorbed dipoles is:

$$W_d = \frac{\rho_d}{6\epsilon\epsilon_0}\left[4qP_d \exp(\gamma_{dq}/x^3)\Gamma(1/3)\Gamma(2/3,\gamma_{dq}/x^3) - 2qP_d\Gamma(1/3)\Gamma(2/3) - P_d P_q \gamma_{dq}^{-1/3}\Gamma(1/3)\right] \tag{3}$$

Adsorption and desorption rates for hydrophobic ions moving between the aqueous phases and membrane surfaces are slow compared with their membrane translocation rates [7]. In this case it can be shown that the relaxation time of current transients is inversely proportional to the translocation rate. Application of the Arrhenius equation to the rate constant leads to the expression

$$\tau = \tau_0 \exp[(W_q + W_d)/kT] \tag{4}$$

for the relaxation time, τ, of the transient current in the presence of adsorbed ions and dipoles. The relaxation time reduces to τ_0 when discrete effects are negligible, i.e., when $W_q, W_d \ll kT$.

The dipole moments $P_{q,d}$ and partition coefficients $S_{q,d}$ cannot be established independently.

They are determined by best fit to the experimental data.

Materials and Methods

Membrane formation and measurements

A mixture of 0.02 mg diphytanoylphosphatidylcholine (Avanti, Birmingham, AL, U.S.A.) per μl decane was used to form lipid bilayer membranes by the brush technique. Unbuffered solutions of KCl (1 kmol/l) were used. The conductance cell used has been described elsewhere [17].

Stock solutions of dipicrylamine and of sodium tetraphenylborate (Aldrich Chemical, Milwaukee, WI, U.S.A.) were prepared in DMSO. The dipolar compound, TTB, was also dissolved in DMSO, but the complexes, T_nAB, were diluted in *n*-butanol. Aqueous concentrations of DMSO and of *n*-butanol never exceeded 0.5% by volume.

The output of a pulse generator (Model DM-4, Continental Specialties, New Haven, CT, U.S.A.) was applied, through a voltage divider and series resistor, to Ag/AgCl electrodes immersed in the aqueous phases bathing the membranes. A storage oscilloscope (Model 7313, Tektronix, Inc., Beaverton, OR., U.S.A.) equipped with two differential amplifier inputs (Model 7422) was used to monitor membrane voltage and current. One differential input monitored the voltage drop across the electrodes, while the second monitored the voltage across the series resistor. The value of this current-sensing resistor was always made as large as possible, consistent with the requirement that the membrane charging time be kept short in comparison to the relaxation time of the hydrophobic ion current transient. Current sensing resistors in the range 0.5–200 kΩ were used. All current transients were photographed from the oscilloscope screen for subsequent analysis.

Data analysis

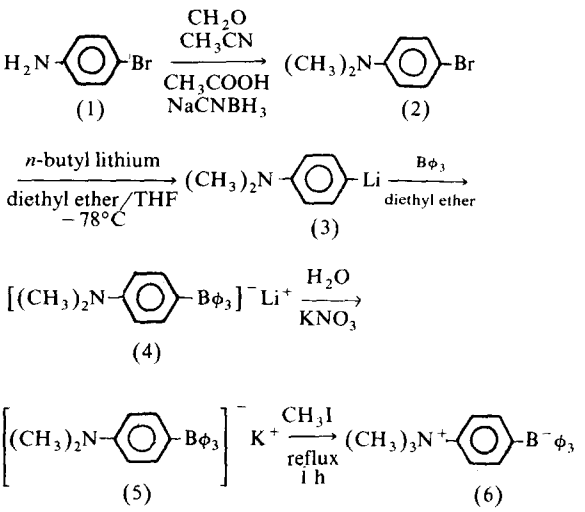
To fit the experimental data to the theory, Eqns. 1–4 were used to develop a computer algorithm, in Fortran describing the model. Tabulated values of the complete gamma function were used [18]. To evaluate the incomplete gamma function, it was written as:

$$\Gamma(2/3, z) = \Gamma(2/3) - \int_0^z e^{-t} t^{1/3} dt \qquad (5)$$

The integral was expanded in Taylor series. Integration term by term resulted in convergent series for the integral. Eqns. 1 were solved using a two-dimensional Newton-Raphson procedure [19]. To fit the empirical parameters to the model, a derivative-free nonlinear regression was used.

Synthesis of triphenyl-[4-trimethylphenylammonium] borate (TTB)

The following reaction scheme (I) was employed:



The starting material, *p*-bromoanaline (1) (Aldrich Chemical Co., Milwaukee, WI) was methylated using sodium cyanoborohydride (Aldrich) after the method of Borch and Hassid [20]. *N,N*-Dimethyl-*p*-bromoanaline (2), 2.2 g (10 mmol), were dissolved in 40 ml dry tetrahydrofuran (THF), and added dropwise over 30 min to a magnetically stirred cold (−78°C) solution containing 15 ml 1.6 M *n*-butyl lithium and 15 ml anhydrous diethyl ether. The mixture was stirred for an additional 3 h at −78°C to form (3). In another flask, 2.4 g (10 mmol) triphenylboron ($B\phi_3$) were dissolved in anhydrous diethyl ether, then added dropwise over 15 min to the cold solution containing (3). The solution was stirred at −78°C for an additional 1 h, then, under continuous stirring, was allowed to warm to room temperature overnight. The solvent was removed under nitrogen at reduced pressure, leaving the highly air-sensitive product, (4). The product was washed several times with

pentane, with excess solution being drawn off each time. Finally, residual pentane was removed under a reduced pressure of nitrogen. Then 50 ml water were added to the residue, followed by 3 g KNO_3, with heating to produce a beige precipitate, (5), which is slightly air-sensitive. 1 g (2.7 mmol) of (5) was dissolved in 25 ml acetone. Then 1 ml methyl iodide was added, and reacted under reflux for 1 h. The solvent was evaporated and the white powder residue was washed with diethyl ether and water to leave the white crystalline product, (6). The structure was confirmed by proton NMR in deuterated DMSO. Upon heating, the product slowly air-oxidized and partially sublimed at 330–340°C. This accords with the observations of Wittig and Herwig [21], who have given a brief prior report of the synthesis of (6).

Synthesis of tri-*n*-alkyl amine boranes

Complexes of the form $R_3N^+ \cdot B^-H_3$ were synthesized, where R included all even-carbon-*n*-alkyls from ethyl to decyl. This was accomplished in each case by direct reaction of the appropriate tertiary alkyl amine with borane-methyl sulfide complex, provided at a concentration of 10 M in excess methyl sulfide (Aldrich).

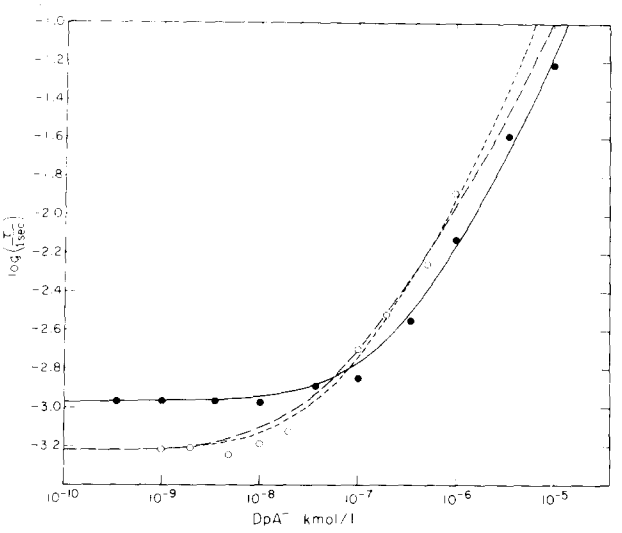
As an example we describe the synthesis, on a 20 mmol scale, of tri-*n*-octyl amine borane. Distilled hexane (14 ml) was added to a dry flask previously flushed with nitrogen. Then 8 ml (5.35 g or 18 nmol) of tri-*n*-octyl amine (Aldrich) were added, followed by dropwise addition over 5 min of 2.0 ml (20 mmol) boron-methyl sulfide. The exothermic reaction proceeded to completion under stirring for 1.5 h. Water (5 ml) was then added to the reaction flask and the mixture was further stirred for several hours, until all bubbling ceased. This signalled the removal of excess boron-methyl sulfide complex. Then solvents were removed under nitrogen, and the remaining clear substance was dissolved in diethyl ether. This solution was washed with saturated aqueous NaCl solution, then the diethyl ether layer was removed and dried over $MgSO_4$. The solvent was evaporated, leaving a clear white liquid product. The structure was confirmed by proton NMR of a specimen dissolved in deuterated chloroform. All complexes in this group were prepared in this way, at yields of 51–99%.

Results and Discussion

In Fig. 2 we present plots of relaxation time versus aqueous-phase dipicrylamine concentration for two different membrane systems. In both cases dipicrylamine is the only adsorbing species present. Our data for diphytanoylcholine membranes (filled circles) are best fit to the model described above by the parameter values $P_q = 27.8$ D and $S_q = 0.245$ mm for the dipicrylamine ion/image charge dipole moment and for the dipicrylamine partition coefficient, respectively. The large value of P_q favors an accurate description of the system by our model.

Also shown in Fig. 2 are the experimental data of Wang [14] (open circles), who studied the translocation of dipicrylamine ions across dioleoyl phosphotidylcholine membranes. The best fit of our model to his data leads to $P_q = 28.3$ D and $S_q = 1.02$ mm. Wang also determined the adsorbed charge independently using high-amplitude pulse experiments, allowing a model-independent estimate of S_q. From his measured surface charge density at $1 \cdot 10^{-9}$ kmol/l aqueous concentration of dipicrylamine we determine $S_q = 1.77$ mm. In view of this discrepancy we have made a second fit to his data holding S_q fixed at 1.77 mm, and varying only P_q. An excellent fit is again obtained, this time with $P_q = 30.7$ D. This indicates that the variation of parameter values considered is within the error limits to be expected from our two-parameter fitting procedure.

The data of Wang [14] also permit a comparison of the observed and predicted values of interface charge density. Theoretical curves for both values of S_q are shown in Fig. 3. As expected, the curve for $S_q = 1.02$ mm underestimates the density of adsorbed surface charge at all aqueous phase concentrations of dipicrylamine, while the curve for $S_q = 1.77$ mm underestimates the surface charge density at higher aqueous phase concentrations of dipicrylamine, where non-ideal effects become important. This latter discrepancy can be explained by our assumption that the relative dielectric permittivity of the membrane is numerically equal to 2 throughout. While this value is accurate for the membrane interior, it is likely to be an underestimate near the more polar interfaces. This would cause an underestimate of the screening, and hence



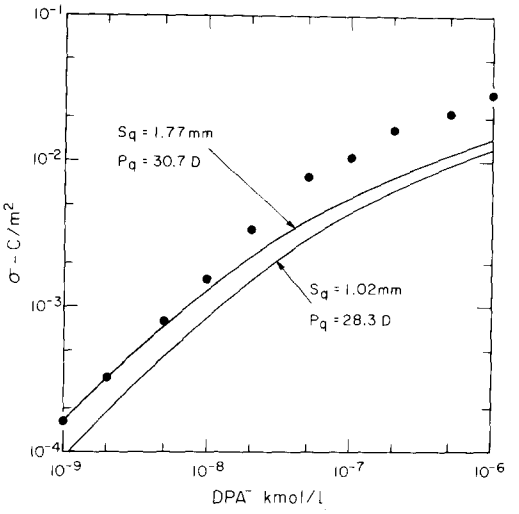


Fig. 2. (Left) Relaxation time of hydrophobic ion transient currents are plotted versus aqueous phase concentration of dipicrylamine, the only adsorbing species present. The data are for (●) diphytanoylphosphatidylcholine membranes used in the present study, and (○) dioleoylphosphatidylcholine membranes as measured by Wang [14]. Theoretical curves corresponding to two different pairs of parameter values have been fit to the latter data, as explained in the text. Parameters determined by best fit of theoretical curves to the data are S_q, the partition coefficient for ideal adsorption of dipicrylamine ions, and P_q, the dipole moment formed by an adsorbed dipicrylamine ion and its image charge.———, $P_q = 27.8$ D; $S_q = 0.254$ mm, ------, $P_q = 28.3$ D, $S_q = 1.02$ mm; — — —, $P_q = 30.7$ D, $S_q = 1.77$ mm.

Fig. 3. (Right.) Calculations of surface charge density based on the discrete charge model described in this paper are compared with the high-field experimental data of Wang [14]. Values for the parameter values S_q and P_q are shown. The model underestimates the surface charge density, as discussed in the text.

an overestimate of the mutual interaction between adsorbed species. This in turn would cause an underestimate of the surface density of dipicrylamine by our model in its present form.

The Gouy theory, by contrast, leads to an overestimate of the surface density of adsorbed charge [11,17]. This overestimate has been attributed to an underestimate by the Gouy theory of the magnitude of the electrostatic potential at the adsorption plane of hydrophobic ions. To raise the potential in the adsorption plane, Wang and Bruner [17] proposed that dielectric saturation occurs in the water near the surface. Andersen et al. [11] suggested that the plane of adsorption might move further into the membrane. They also suggested that a consideration of discrete charge effects might remove the discrepancy between Gouy theory and experimental observations. The present model indeed indicates that discrete charge effects in the form of dipole interactions between surface species can explain lowered adsorption at high concentrations of hydrophobic ions. The effect of the diffuse Gouy layer is small at high aqueous salt concentrations and has been neglected in our computations.

Fig. 4 shows experimental data for a system containing varying amounts of both dipicrylamine and the dipolar compound, TTB. Addition of TTB to the aqueous phases increases the relaxation time for translocating dipicrylamine, indicating that the interior potential of the membrane becomes more negative with increasing TTB adsorption. This result, which is also obtained using tetraphenyl borate as a probe (data not shown), confirms that the orientation of the TTB molecule in the interface is controlled by the hydrophobic properties of the aromatic groups which surround the B^- charge center (Fig. 1) and direct it into the membrane. Fitting the data of Fig. 4 to the model resulted in the dipole moments $P_q = 29.3$ D, $P_d = 26.8$ D, and

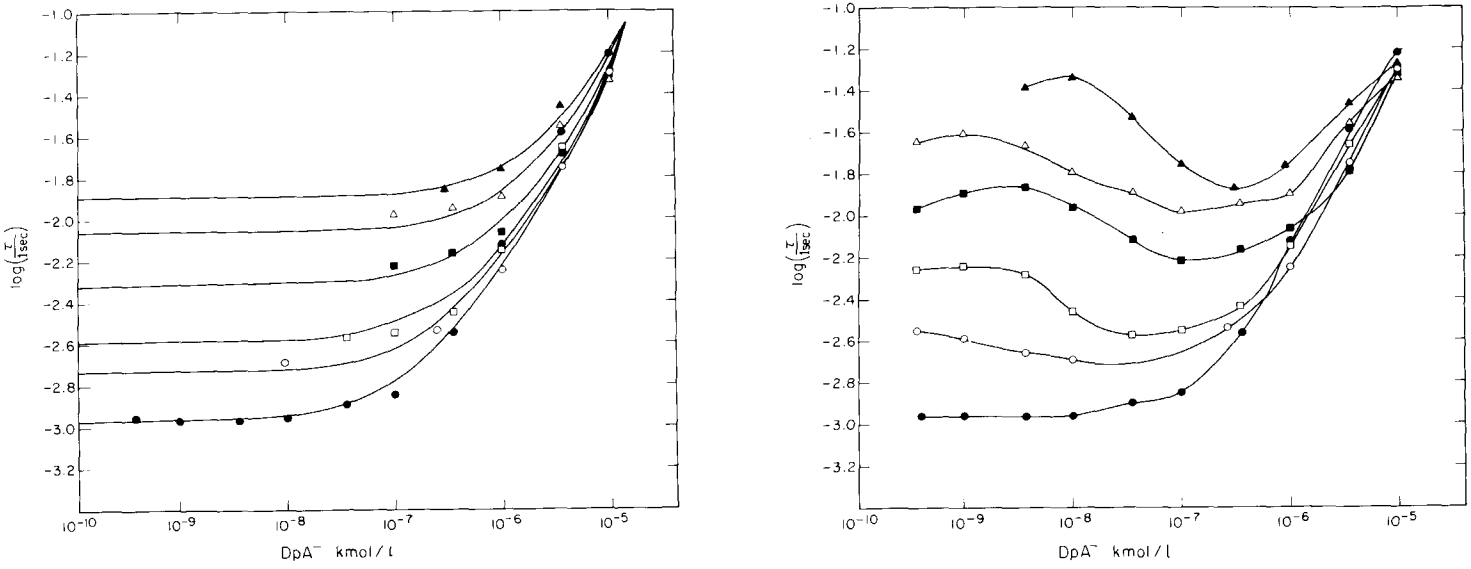


Fig. 4. Relaxation time of hydrophobic ion transient currents are plotted versus aqueous phase concentration of dipicrylamine, at various fixed aqueous phase concentrations of the dipolar compound, TTB: ●, zero; ○, $1 \cdot 10^{-6}$; □, $2 \cdot 10^{-6}$; ■, $5 \cdot 10^{-6}$; △, $1 \cdot 10^{-5}$; ▲, $1.5 \cdot 10^{-5}$ M TTB. All data shown are fit to the model described in the text using a single set of values for the four parameters: $S_q = 0.27$ mm; $S_d = 0.034$ mm; $P_q = 29.3$ D; $P_d = 26.8$ D.

Fig. 5. The data of Fig. 4 are repeated, but now with the complete data set shown. The increase of relaxation time with decreasing concentration of dipicrylamine, which is most evident at high TTB concentrations is interpreted in terms of complexation between the two species. Symbols as in Fig. 4.

the partition coefficients $S_q = 0.27$ mm and $S_d = 0.034$ mm. It should be recalled that P_d is twice the intrinsic dipole moment of the TTB molecule. Although all four parameters were fit simultaneously, the values for P_q and S_q are in excellent agreement with those obtained previously for diphytanoylphosphatidylcholine membranes in the presence of dipicrylamine ions alone (Fig. 2).

Our interpretation of the data of Fig. 4 is complicated, however, by an additional source of interaction between dipicrylamine ions and dipolar TTB molecules which becomes apparent when the full data set is considered (Fig. 5). Here it is seen that, as the dipicrylamine ion concentration is decreased from the highest values used, the observed relaxation time decreases toward a limiting value as described by the model presented (Fig. 4). Upon further reduction of the dipicrylamine (DPA^-) concentration, however, the relaxation time again increases in the presence of TTB. We attribute this latter increase to the formation of an interfacial complex $[TTB\text{-}DPA]^-$ between the adsorbed species. An attractive short range electrostatic interaction favoring such complexation could well occur, even though the dipole-dipole interaction between these species is expected to be repulsive at long range. The conditions of high interfacial concentration of TTB and low interfacial concentrations of dipicrylamine, under which the anomolous increase of relaxation time is observed, would certainly favor the preponderence of complexed over uncomplexed dipicrylamine. The relaxation time observed under this condition would reflect the requirement that the dipicrylamine ion have sufficient energy to dissociate from the complex as well as surmount the central barrier. As the aqueous phase concentration of dipicrylamine is increased, more of the uncomplexed anion would become available in the interfaces for translocation subject only to the interactions described by the model presented.

We next present results obtained with tri-*n*-alkylamineborane complexes (T_nAB). In each case these have been studied in situations where either the aqueous phase or both the aqueous phase and the membrane-forming solution were saturated with the complex under study. These procedures were followed because the membrane torus appears to act as a sink for the complex, preventing the attainment of stable equilibrium in non-saturated systems.

In general, the T_nAB complexes were found to shorten the relaxation time for translocation of hydrophobic anion probes. This is consistent with our expectation that the alkyl groups attached to the N^+ charge center would be directed toward the membrane interior, and would thereby orient the dipole moment of the complex in such a way as to increase the positive interior potential of the membrane. Since the relaxation time of the hydrophobic ion current is shortened, tetraphenylborate was used as a probe ion rather than dipicrylamine to allow better resolution. Initial relaxation times for these probes are approximately 100 ms and 1 ms, respectively. Qualitatively similar effects are observed with dipicrylamine ions, however (data not shown).

In Fig. 6, the approach to equilibrium as a function of time is shown for tri-*n*-decylamineborane ($T_{10}AB$) complex. The upper curve shows the time course of the observed tetraphenylborate relaxation time after saturating the aqueous phase with the complex (with a nominal addition of $1 \cdot 10^{-5}$ kmol/l). It is seen that about 2 h are required for equilibration. For a second experiment, a membrane-forming solution was prepared

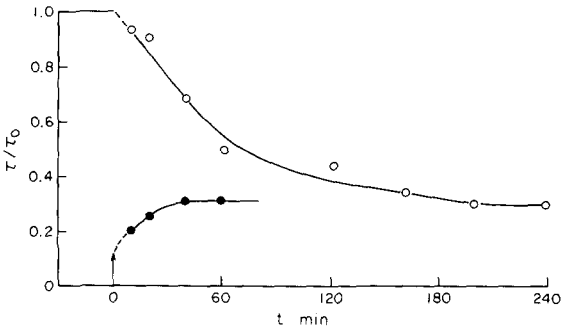


Fig. 6. The approach to equilibrium of the relaxation time of the hydrophobic ion current transient due to tetraphenylborate is illustrated. For the upper curve, the aqueous phases only are saturated with $T_{10}AB$ at the point in time indicated by the arrow. The membrane was fully black and equilibrated with $5 \cdot 10^{-8}$ kmol/l of tetraphenylborate in the aqueous phases prior to the addition of $T_{10}AB$. For the lower curve, obtained with tetraphenylborate and $T_{10}AB$ already in the aqueous phases, as above, a membrane was formed from a decane/diphytanoylphosphatidylcholine solution already saturated with $T_{10}AB$. Thinning time under these conditions was approx. 30 s. Both curves show the same endpoint.

using *n*-decane saturated with $T_{10}AB$ (nominal concentration of $1 \cdot 10^{-2}$ kmol/l). Prior to spreading of the membrane, the aqueous solutions were also saturated with the complex. The lower curve of Fig. 6 shows the time course of the tetraphenylborate relaxation time, with measurements commencing immediately after membrane thinning, which required about 30 s. While it appears that the membrane is initially supersaturated, a rapid increase of relaxation time to the same endpoint as for the upper curve is observed. Very similar results (data not shown) were obtained for the T_8AB and T_6AB complexes. All three complexes reduced the relaxation time of the probe ion by a factor of 2.5–3.0.

The shorter chain complexes, T_4AB, and T_2AB, reduced the probe ion relaxation time by larger factors, but, because of membrane instability introduced by the larger concentrations required for saturation, no stable endpoints could be observed.

Our finding that T_nAB complexes lower the barrier to translocation of hydrophobic anion probes raises the possibility that a uniform distribution of the complex throughout the membrane could elevate its average dielectric constant. This could then account for the observed effect, rather than the interfacial dipole fields produced by the adsorption of oriented polar molecules of T_nAB. This possibility can be excluded because we have been unable to detect any increase of membrane capacitance (under 10%) upon addition of T_nAB complexes alone in saturating amounts to the aqueous phases.

Conclusions

Our results indicate that the orientation of interfacially adsorbed dipolar molecules can be controlled by the distribution of groups with greater and lesser hydrophobicity relative to the orientation of the molecular dipole. Thus, TTB lowers the interior potential barrier of the membrane, which T_nAB complexes raise it. No clear trend in the modification of the barrier, as a function of alkyl chain length of the T_nAB complexes, could be detected. The limiting membrane solubilities of $T_{10}AB$, T_8AB, and T_6AB complexes appeared to be about equal. Complexes with shorter alkyl groups seemed to have higher limiting solubilities. A more complete dipole alignment normal to the membrane plane could not, however, be ruled out in the case of T_4AB and T_2AB complexes.

The fact that no stable equilibrium could be attained for non-saturated T_nAB containing systems suggests that lateral diffusion of adsorbed species on the membrane surface toward the torus is often imporant. One must be aware that observed adsorption 'equilibria' on membrane surfaces may in fact be steady states, reflecting a balance between diffusion through unstirred layers to the membrane surface, and lateral diffusion over the surface to the membrane torus [22].

The model developed by Kleijn and Bruner [13] has been found to give a good representation of the translocation of hydrophobic ions across a bilayer membrane. It provides an explanation, in terms of discrete charge effects, of the inability of the Gouy theory to describe adequately the adsorption of such ions. The model could be further improved by taking into account the variation of the dielectric constant of the membrane near its interfaces with the surrounding aqueous phases.

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