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CHAOTROPIC ANIONS AND THE SURFACE POTENTIAL OF BILAYER MEMBRANES

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SUMMARY

The chaotropic anions perchlorate and thiocyanate adsorb to artificial phospholipid membranes. The negative electrostatic potential they produce at the surface of the membranes was measured by two independent techniques. The conductance produced by neutral carriers of cations and anions was measured to estimate changes in the surface potentials of planar black lipid films and the electrophoretic mobility of phospholipid vesicles was used to monitor changes in the zeta potentials of spherical bilayer membranes. Qualitatively similar results were obtained with the two techniques. The results, moreover, agreed with the change in surface potential produced by these anions at an air water interface, as measured directly with an ionizing electrode (Randles, J. E. B. (1957) Discuss. Faraday Soc. 24, 194–199). The results obtained with artificial bilayers may explain the observation (Wieth, J. O. (1970) J. Physiol. 207, 581–609) that thiocyanate increases the sodium or potassium and decreases the sulfate permeability of erythrocyte membranes.

INTRODUCTION

When the chloride salts of an alkali metal cation are added to water, the surface tension increases [1]. This demonstrates, via the Gibbs equation [2], that the concentration of electrolyte at the airwater interface is less than in the bulk aqueous phase. This depletion of electrolyte concentration at the interface can be understood in terms of coulombic forces alone, at least in very dilute solutions [1]. If the airwater interface is considered as merely two juxtaposed dielectrics, an ion within a few Ångstroms of the interface will be subject to ion-dipole or "image" forces driving it into the medium of higher dielectric constant [1, 3–6].

In contrast to chloride, some large "chaotropic"* or "structure breaking"

Abbreviation: DTFB, 5, 6-dichloro-2-trifluoromethylbenzimidazole.

* The term chaotropic (tending to disorder) was originally applied to the ability of anions such as ClO_4^- and CNS^- to denature DNA [7]. Hatefi and Hanstein [8] argue convincingly, however, that this term should have wider connotations. They use the word chaotropic to refer to the ability of ClO_4^- and CNS^- to disorder water structure and therefore facilitate the transfer of apolar groups into water. Such apolar groups tend to be excluded from pure water mainly by a "hydrophobic" or entropy effect [8, 9].

anions [10, 11] are subject to adsorptive forces at the air/water interface, examples being perchlorate and thiocyanate. These adsorptive forces are in some cases strong enough to overcome the repulsive "image" forces and $HClO_4$ actually decreases the surface tension of water. This demonstrates, via the Gibbs equation, that the electrolyte is accumulating at the surface.

It must be admitted that the forces which cause chaotropic anions to adsorb to an air water interface are not well understood. The reader interested in a reasonable speculation is referred to Hatefi and Hanstein [8]. Even in a bulk aqueous phase Eisenstadt and Friedman [10] note that "the picture of the structure breaking ions is not clear because there is little consistency among the results of the various quantitative measures of this effect". The structure breaking property is apparently important, because perchlorate and thiocyanate are less strongly adsorbed from the unstructured solvent dimethylformamide than from water [11].

Surface potential measurements provide an independent technique for observing an excess of chaotropic anions at an air/water interface (e.g. Randles [1]). These potentials can be measured with an ionizing or vibrating electrode in the air above the interface. The addition of an alkali metal chloride to pure water produces little change in the surface potential; 1 M KCl, for example, produces an increment of only +2 mV. The addition of 1 M $NaClO_4$ or KCNS, however, produces surface potential increments of +48 and +53 mV, respectively [1]. This indicates a preferential adsorption (or decreased desorption) of the chaotropic anion, and these surface potential changes correlate qualitatively with the observation that the surface tension increment is less for KCNS than for KCl.

The change in surface potential at the air/water interface could arise from two causes: (1) the adsorption of the chaotropic anion and the concomitant production of an ionic double layer or, (2) a change in the dipole potential due to an orientation of water molecules at the surface. As stated by Randles [1] "there is no certain way of deciding the relative extent of the two contributions, but the most plausible interpretation of the experimental results is obtained on the assumption that the potential difference due to the ionic double layer is predominant". We will proceed on this assumption, which can be justified experimentally for the phospholipid bilayers.

The changes in surface potential at the bilayer/water interface were measured by two independent techniques. The conductance, G^+, produced by a neutral carrier of cations, nonactin, has been shown [12–15] to be proportional to $\exp -(F\psi/RT)$, where ψ is the change in the electrostatic potential at the surface of the phospholipid bilayer.

$$G^+ \propto \exp -(F\psi/RT) \tag{1}$$

The conductance, G^-, produced by a neutral carrier of anions, DTFB, is proportional [16] to $\exp +(F\psi/RT)$.

$$G^- \propto \exp +(F\psi/RT) \tag{2}$$

The conductance responds to changes in surface potential due to either ionic double layers or dipoles. We also measured the electrophoretic mobility of phospholipid vesicles in the presence of chaotropic anions. The electrophoretic mobility is pro-

portional to the zeta potential, ξ, the potential at the hydrodynamic plane of shear, (e.g. Shaw [17]). The available evidence [14] suggests the mobility responds only to changes in the ionic double layer potential.

MATERIALS AND METHODS

The experimental apparatus and methods used for making conductance measurements on planar black lipid membranes of the Mueller-Rudin type have been previously described [18]. In brief, the experimental chamber was milled from a single piece of Teflon and had two compartments of 25 ml capacity separated by a thin wall having an aperture of 1.4 mm diameter. The phospholipids for these experiments were obtained from Supelco, (Bellefonte Pa.) and the glycerol monooleate was obtained from Sigma (St. Louis). All lipids migrated as single spots in the thin-layer chromatography control experiments. A small volume of lipid (1–2 μl) dissolved in *n*-decane to a concentration of 25 mg/ml was deposited on the aperture with a Pasteur pipette, which was flamed immediately prior to the experiment to remove organic impurities. Membranes were formed by passing a bubble over the aperture and observed via a Wilde M5 stereoscopic microscope equipped with a calibrated eye-piece reticule. Solutions were stirred with Teflon-coated magnetic stirrers and additions of microliter quantities of nonactin or DTFB dissolved in ethanol were made in the immediate vicinity of the stirrers. The total concentration of ethanol in the chamber never exceeded 0.2 %, whereas an ethanol concentration of 1 % had a negligible effect on the carrier induced conductance of the membrane in control experiments. The conductance was determined by measuring the current with a Keithley 602 electrometer when ± 10 mV were applied to the system, the membrane being in series with the high resistance of the electrometer. The torus of the membrane was sufficiently small (membrane area > 1.5 mm^2) in these experiments that the phenomenon discussed by Hladky (19), Hladky and Haydon [20] and Benz et al. [21] was of no consequence.

The nonactin was a generous gift of Miss Barbara Stearns of Squibb and the 5, 6-dichloro-2-trifluoromethylbenzimidazole (DTFB) was synthesized in the Department of Chemistry, S.U.N.Y., Stony Brook by Ron Liotta following a modification of the procedure of Acheson et al. [22]. The aqueous solutions were prepared with 18 MΩ "super Q" (Millipore Corp.)water and reagent grade inorganic salts. The temperature was 22 °C for the experiments with the planar membranes.

The vesicles for the microelectrophoresis experiments were prepared by diluting 0.25 ml of phosphatidylethanolamine, (25 mg/ml in chloroform/methanol) with 2 ml of ethanol, evaporating to dryness in a rotary evaporator, then adding the aqueous solution and 3 glass beads. Shaking the solution for a few minutes produced multilaminar vesicles of the appropriate size for microelectrophoresis measurements. The measurements of the electrophoretic mobility were made on a commercially available (Rank Bros., Bottisham, Cambridge, U.K.) cylindrical microelectrophoresis apparatus based on a design by Bangham et al. [23]. All measurements were made at the theoretical stationary layer, the experimental justification for this being given by Bangham et al. [23]. Control experiments produced results identical with Seaman and Heard [24] on human erythrocytes, and with the values quoted by Shaw [17] for rabbit and dog erythrocytes. Experiments were performed alternatively on vesicles in

the control (0.25 M NaCl) and in the experimental solution (0.25 M NaCNS or $NaClO_4$), the solutions being buffered to pH 7.4 with 0.005 M Tris. A few experiments were done with KCl and KCNS to test the effect of the cation on the change in electrophoretic mobility induced by chaotropic anions and a few experiments were also done with different buffers, (citrate, phosphate). Neither the cation nor the buffer affected the results within experimental error.

The zeta potential was calculated from the Helmholtz-Smoluchowski equation (e.g. Shaw [17])

$$\zeta = \frac{4\pi\eta u}{\varepsilon} \tag{3}$$

where ζ is the zeta potential, η the viscosity, ε the permittivity and u the mobility. We make the conventional assumption that the values of the viscosity and dielectric constant in the double layers are equal to their bulk values and Eqn 3 becomes at 25 °C.

$$\zeta = 12.85\, u \tag{4}$$

where ζ is in mV and u is in $\mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$.

RESULTS

The lower half of Fig. 1 illustrates that perchlorate increases the conductance of a cation selective membrane. The bilayers were formed from a zwitterionic lipid, phosphatidylethanolamine, and a neutral carrier of cations, nonactin, was added to

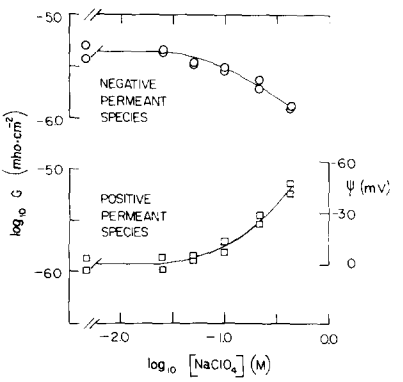

Fig. 1. The effect of perchlorate on the conductance of anion and cation selective membranes. The black lipid membranes were formed from a mixture of phosphatidylethanolamine and decane in a solution containing 10^{-1} M NaCl and 10^{-2} M KCl buffered to pH 7.0 with $5 \cdot 10^{-4}$ M Tris. One solution (lower, squares) also contained 10^{-6} M nonactin, a neutral carrier which selectively transports K^+ across the membrane and the other solution contained $1.2\ 10^{-6}$ M DTFB, a weak acid, the neutral form of which selectively transports its anion across the membrane. Note that perchlorate increases the conductance of the cation selective membrane and decreases the conductance of the anion selective membrane. The results of two experiments are shown to illustrate typical scatter in the data. Similar results were obtained with membranes formed from phosphatidylcholine. The changes in conductance are attributed to a change in the electrostatic potential at the surface of the membrane (right hand ordinate).

the aqueous phases. The conductance produced by nonactin can be used [12–15] to estimate the change in surface potential, ψ, of a bilayer membrane via Eqn (1). The changes in potential inferred from Eqn 1 are illustrated on the right hand ordinate. The highest concentration of perchlorate examined, about 0.5 M, produced a change in surface potential of −45mV.

Artifacts which could give rise to the increase in conductance are ruled out by control experiments. The increase in conductance could not, for example, be due to an increase in carrier mediated sodium conductance because this ion is weakly complexed by nonactin [25], and we observed experimentally that the addition of 0.5M NaCl instead of $NaClO_4$ produced no increase in conductance when nonactin was present. The increase in conductance is, furthermore, not due to the permeation of perchlorate. Perchlorate is more permeable than chloride, as might be expected from the lower Born energy [4] required to solubilize the larger ion in a bilayer. The conductance produced by this ion in the absence of nonactin (see the left point in Fig. 2) is, nevertheless, always an order of magnitude less than the conductance due to the nonactin-potassium complex. Finally, the conductance increased linearly with the concentration of nonactin whether perchlorate was present or not, as expected theoretically [25]. These experiments did not rule out the possibility that perchlorate was affecting some variable other than the electrostatic potential (e.g. viscosity, dielectric constant) and control experiments were done with a negative permeant species. The results are illustrated in the upper portion of Fig. 1.

In the presence of the substituted benzimidazole DTFB the membrane is

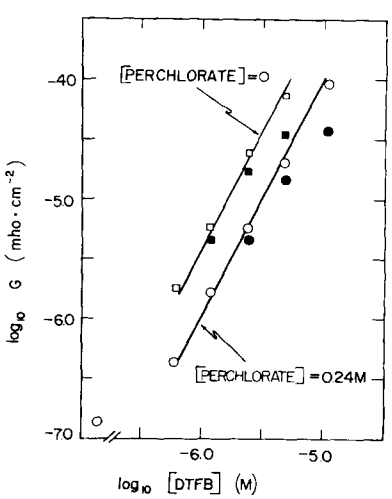

Fig. 2. The effect of DTFB on the conductance of a black lipid membrane formed from phosphatidylethanolamine. One membrane was formed in an aqueous solution containing 10^{-1} M NaCl, 10^{-2} M KCl buffered to pH = 7.0 with $5 \cdot 10^{-4}$ Tris (squares); the other solution contained, in addition, 2.4 10^{-1} M $NaClO_4$ (circles). The open circles and squares designate "instantaneous" measurements, the closed circles and squares steady state measurements when these differ from the "instantaneous" values. Note that the "instantaneous" conductance depends on the square of the DTFB concentration, the lines through the points being drawn with a slope of two. The difference between the steady state and "instantaneous" measurements is due to diffusion polarization in the aqueous unstirred layers and can be reduced by increasing the concentration of buffer in the solution.

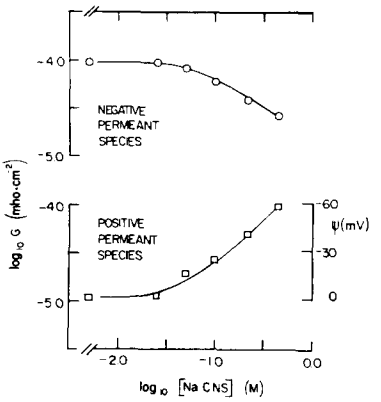

Fig. 3. The effect of thiocyanate on the conductances of anion and cation selective membranes. The membranes were formed from phosphatidylethanolamine in solutions containing 10^{-1} MKCl and $5\ 10^{-3}$ M Tris buffered to pH 7.3. In one case the solution also contained 10^{-6} M nonactin (lower, squares) in the other it contained $5 \cdot 10^{-6}$ M DTFB (upper circles). The points are the average of measurements from three different experiments in each solution. The right hand ordinate illustrates the change in surface potential inferred from the conductance measurements. Similar results were obtained with membranes formed from glycerol monooleate.

permeable to an HA_2^- complex [16, 26–28] formed between the neutral acid, HA, and its anion, A^-. The conductance [16] responds to the surface potential in the manner predicted by Eqn 2. To a first approximation, perchlorate produces a change in the conductance of an anion selective bilayer which is equal in magnitude but opposite in direction to that observed with a cation selective bilayer (Fig. 1). This strongly suggests that perchlorate is changing the electrostatic surface potential.

Fig. 2 is a control experiment which demonstrates that the anionic probe molecule behaves in a regular manner in the presence of perchlorate, an important consideration because some chaotropic anions (e.g. PF_6^-) appear to interact with these probes. The open squares in Fig. 2 illustrate that when DTFB is added to the bathing solution the "instantaneous" conductance increases quadratically with concentration. This observation is consistent with the permeant species being an HA_2^- complex [16]. The "instantaneous" conductance also increased quadratically with the concentration of DTFB when 0.24 M $NaClO_4$ was present, (open circles) but was lower, at any DTFB concentration, by about 0.5 log units because of the surface potential produced by perchlorate. The solid squares and circles illustrate the steady state values of the conductance when they differed from the instantaneous values because of diffusion polarization in the aqueous unstirred layers [29, 16]. The experiments illustrated in Figs 1 and 3 were done with a relatively low benzimidazole concentration and a high buffer concentration to minimize diffusion polarization and allow steady state conductance values to be used. (The steady state but not the instantaneous conductance depends on buffer concentration because it is the buffered hydrogen ions [30, 16] and not the HA_2^- complex which diffuse through the aqueous unstirred layers). We note, finally, that the decrease in conductance produced by perchlorate is not due to an increase in ionic strength because the addition of 0.5 M sodium chloride produced no decrease in the conductance due to DTFB. Both the

nonactin and DTFB probes thus behave in a regular manner in the presence of perchlorate. To the extent that the changes in conductance produced by this chaotropic anion (Fig. 1) are symmetrical in nature they can be interpreted in terms of a change in the electrostatic surface potential [15].

It could be argued that perchlorate produced a negative surface potential because it is preferentially adsorbed to the ethanolamine moiety of phosphatidylethanolamine. To examine this possibility we performed experiments similar to those illustrated in Fig. 1 on bilayers formed from phosphatidylcholine and from glycerol mono-oleate dissolved in decane. Perchlorate produced the same change in surface potential on bilayers formed from either phosphatidylethanolamine or phosphatidyl choline which makes it unlikely that the adsorption of perchlorate is due to a specific interaction with the primary or quaternary amines of the phospholipids. The changes produced by ClO_4^- in the permeability of glycerol mono-oleate membranes to anions and cations were, however, substantially less than the changes produced by ClO_4^- on membranes formed from the phospholipids. We do not understand why ClO_4^- has so little effect on the surface potential of membranes formed from this neutral lipid.

Fig. 3 illustrates that CNS^- enhances the conductance of a cation selective bilayer and depresses the conductance of an anion selective membrane. A comparison of Figs 3 and 1 illustrates that CNS^- and ClO_4^- produce virtually identical effects on the surface potential of a phosphatidylethanolamine bilayer. Similar effects were also observed with thiocyanate on bilayers formed from glycerol mono-oleate.

The changes in surface potentials illustrated on the right hand ordinates of Figs 1 and 3 could be due to either the production of a double layer or to the orientation of dipoles at the membrane solution interface. To distinguish between these two possibilities we also measured the effect of ClO_4^-, CNS^- and PF_6^- on the electrophoretic mobility of phospholipid vesicles formed from phosphatidyl ethanolamine. As Table I illustrates, all three of these chaotropic anions produce substantial changes in the electrophoretic mobility of the vesicles. Most of our experiments were done with perchlorate, and a concentration of 0.25M ClO_4^- produced a zeta potential of

TABLE I

THE ELECTROPHORETIC MOBILITY AND ZETA POTENTIAL OF PHOSPHATIDYLETHANOLAMINE VESICLES AT 25 °C

The temperature for all experiments was 25 °C. See the Appendix for a discussion of other measurements of the mobility of phosphatidylethanolamine vesicles.

Solution	Mobility* ($\mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$)	Zeta potential (mV)
0.25 M NaCl	$-0.08 \pm .12$ ($n = 4$)	− 1.0 mV
0.25 M $NaClO_4$	$-1.10 \pm .02$ ($n = 4$)	−14.0 mV
0.25 M NaCl	$-0.05 \pm .13$ ($n = 4$)	− 0.5 mV
0.25 M NaCNS	$-0.88 \pm .08$ ($n = 4$)	−11.0 mV
0.1 M KCl	−0.43 ($n = 2$)	− 5.5 mV
0.1 M KPF_6	−1.05 ($n = 2$)	−13.5 mV

* Mobility measurements ± standard deviation (n = number of sets of 10 individual measurements).

TABLE II

THE SURFACE POTENTIAL PRODUCED BY $NaClO_4$ AT MEMBRANE/SOLUTION AND AIR/WATER INTERFACES

Concentration of sodium perchlorate	Change in surface* potential at the membrane/solution interface (mV)	Change in surface** potential at the air/water interface (mV)	Change in *** zeta potential of membrane vesicles (mV)
0.39	−35	−30	—
0.25	−25	−20	−13
0.1	−10	−10	—
0.025	0	− 4	—

* Average of changes in potential predicted by the upper and lower portions of Fig. 1. Identical data were obtained on membranes formed from either phosphatidylethanolamine or phosphatidylcholine.

** From the data of Randles [31].

*** From Table I.

−14 mV. It is to be expected theoretically, and has been confirmed experimentally for certain molecules [14], that changes in the dipole potential do not produce changes in the electrophoretic mobility of phospholipid vesicles. We conclude, therefore, that the changes in zeta potential calculated in Table I are manifestations of an electrical double layer. The fact that the change in zeta potential (−13 mV) is less than the change in surface potential (−25 mV) does not, conversely, prove that the difference between these two measurements is due to a dipole potential. The zeta potential is measured at the hydrodynamic plane of shear and could be substantially less than the true surface potential at these relatively high salt concentrations, where the Debye length is < 10 Å. Note that CNS^- and KPF_6^- also produce a negative zeta potential.

DISCUSSION

Both the experiments with carriers conducted on the planar black lipid films (Figs 1–3) and the microelectrophoresis experiments conducted on the spherical bilayers (Table I) illustrate that the chaotropic ions perchlorate and thiocyanate adsorb to membranes and change the surface potential. The changes in potential produced at the surface of membranes formed from zwitterionic or neutral lipids correlate quite well with the changes in potential that the chaotropic ions produce at an air/water interface, the one exception being the effect of ClO_4^- on glycerol monooleate films. Perchlorate at a concentration of 0.25M, for example [31], produces a change in the surface potential of an air/water interface of about −20 mV as illustrated in Table II. This value agrees qualitatively with the value obtained for the change in surface potential of the black phospholipid films (−25 mV).

The above results might explain the observation [32] that 120 mM thiocyanate increases the permeability of the erythrocyte membrane to sodium and potassium some 2–3 times and decreases the permeability of the membrane to sulphate by a factor of about 2. These effects are similar in magnitude to the changes thiocyanate induces in the permeability of artificial black lipid membranes to cations and anions

(Fig. 3). Wieth [32] previously "concluded that the anion-induced changes of permeability are due to binding of anions to fixed cationic charges in the red cell membrane". The above results support his suggestion that the anion-induced changes could be electrostatic in nature but demonstrate that it is not necessary to invoke fixed cationic charges as the binding sites. On the red blood cell membrane, salicylate is even more effective than thiocyanate in enhancing the sodium and potassium and depressing the sulphate permeability [32]. It changes these variables by about an order of magnitude when present at 120 mM. This is consistent with the observation that salicylate enhances the cation and depresses the anion permeability of black lipid membranes by about an order of magnitude when present at decimolar concentrations [27]. Salicylate also affects the zeta potential of bilayer membranes [33]. Although both salicylate and thiocyanate adsorb to artificial black lipid membranes and produce an ionic diffuse double layer, the former anion probably adsorbs to the membranes by a hydrophobic [9, 27] mechanism whereas the mechanism by which the chaotropic anions adsorb is unknown. The effects of ANS and its analogs on the ionic permeability of erthrocytes have also recently been discussed in terms of an electrostatic mechanism [34].

APPENDIX

The zeta potential of phosphatidylethanolamine vesicles

Other investigators have also measured the electrophoretic mobility of phosphatidylethanolamine vesicles in NaCl solutions at a neutral pH. Papahadjopoulos and Weiss [35] observed a mobility of $-2.12 \pm 0.25\ \mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$, Barton and Jevons [36] a value of -1.4, Bonting and Bangham [37] -1.25 and we (Table I) a value of $-0.08 \pm 0.13\ \mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$ The reason for the large discrepancy in these results is unclear at the present time. We wish to stress, however, that the absolute value of the mobility, or zeta potential, in a NaCl solution is irrelevant to our major conclusion; which is that the chaotropic anions increase the magnitude of the negative zeta or surface potential of phospholipid bilayers. In a large series of preliminary experiments we also observed a negative electrophoretic mobility for phosphatidylethanolamine vesicles in NaCl solutions ($-0.32 \pm 0.14\ \mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$, 6 sets of 10 measurements), but perchlorate produced a change in the mobility (to a value of -1.3 ± 0.15) which agreed, within experimental error, with the values listed in Table I. We rejected these preliminary experiments because the phosphatidylethanolamine also appeared slightly negative when planar black lipid films were formed with the same lipid and investigated with the probes of surface potentials discussed in detail by Szabo et al. [15]. The difference between the measurements of the electrophoretic mobility of phosphatidylethanolamine vesicles in NaCl could thus be due to negative contaminants and we regard the value of zero as our most reliable estimate.

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