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Apparent Activation Volumes of Hydrophobic Ions and Carriers in Planar Lipid Bilayers

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Summary. A gas-free high-pressure cell has been developed to measure planar bilayer conductances induced by hydrophobic ions and ionophores as a function of hydrostatic pressure. Plots of log conductance versus pressure for valinomycin and nonactin-mediated potassium transport in egg phosphatidyl choline-decane membranes are essentially linear over a pressure range of 1 to 818 atm. Calculated activation volumes give similar results for both nonactin and valinomycin yielding values of + 48 and + 42 cc/mole, respectively. The valinomycin activation volume agrees reasonably well with the results obtained by Johnson and Miller (*Biochim. Biophys. Acta* **375**:286–291, 1975) for K^+-valinomycin transport in liposomes. In contrast to the activation volumes for nonactin and valinomycin, relaxation measurements of tetraphenyl boron (TPB) and dipicrylamine (DPA) give very small values of < 5 cc/mole for the translocation rate constant, k_t. Similarly, steady-state conductance measurements on tetraphenyl arsonium (TPA) and carbonylcyanide *m*-chlorophenylhydrazone (CCCP), give small values of 6 and 7 cc/mole, respectively. These low figures do not support transport theories based on the formation of bilayer holes or kinks (H. Träuble, *J. Membrane Biol.* **4**:193–208, 1971). The low values for TPB and TPA are especially interesting because their cross-sectional areas are not much different than those of valinomycin and nonactin. Pressure-induced changes in membrane dielectric constant and thickness which lower the bilayer electrostatic barrier could explain the low values for the hydrophobic ions. Additionally, larger activation volumes might be expected for carriers such as nonactin and valinomycin that undergo significant rearrangement and change in hydration during surface complexation of cations.

Key Words activation volume · planar bilayers · hydrophobic ions · ionophores · pressure · electrostriction

Introduction

The influence of large hydrostatic pressures is an important subject in the investigation of the mechanical properties of biological systems. Productive studies have been performed on the pressure dependencies of enzyme, membrane, and whole animal functions with significant effects found for pressures ranging from 1 to 1000 atm [10, 13, 21, 22]. However, little is known about the specific changes which occur in cell membrane organization and structure on exposure to hyperbaric environments. In this paper, we examine the pressure dependence of membrane conductances produced in planar lipid bilayer membranes in a gas-free high-pressure cell. Two classes of membrane permeants are considered: hydrophobic ions and carriers. For hydrophobic ions, we examine dipicrylamine (DPA), tetraphenyl boron (TPB), and its positively charged analog tetraphenyl arsonium (TPA); for carriers we use valinomycin, nonactin, and the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). From an examination of the pressure-induced variations in the steady-state and relaxation currents of these relatively simple molecules we draw conclusions about the general effects of hydrostatic pressure.

Although pressure is one of the few experimental variables capable of providing information about the mechanical aspects of membrane transport properties, hydrostatic pressure experiments have been largely limited to measurements of pressure variations of phase transition temperatures in phospholipid vesicles [16, 19, 20, 29]. The first evaluation of a model transport system was performed by Johnson and Miller [14]. They measured the inhibition of the exchange rate of radioactive Na^+, K^+, K^+ complexed to valinomycin, and the nonelectrolyte glucose as a function of pressure in lipid vesicles. Recently, Bruner and Hall [7] reported the first measurement of pressure effects on the conductance induced by the voltage-dependent pore former alamethecin in a planar Montal-Mueller type membrane. In each type of experiment, significant pressure dependencies were found with large positive activation volumes for both the carrier- and pore-mediated conductance mechanisms.

The component pressure sensitivities of a transport system can be interpreted thermodynamically as either an activation volume ($\Delta V^{\neq}$) for rate pro-

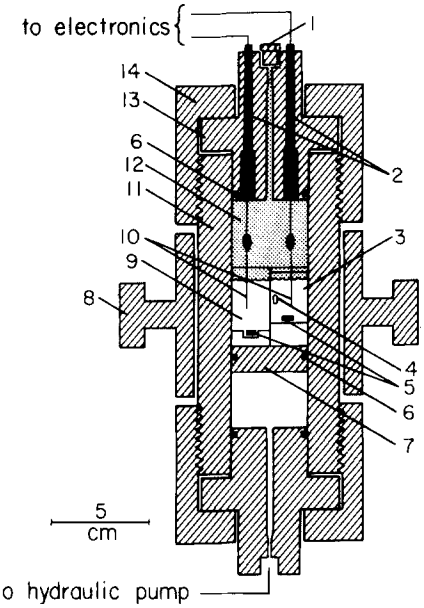

Fig. 1. Cross-section of the high pressure cell used for bilayer experiments. (1) plug, (2) acrylic electrode insulators, (3) 4-ml delrin cup, (4) 1-mm aperture, (5) magnetic fleas, (6) Buna "O" rings, (7) piston, (8) stirrer electromagnets, (9) acrylic membrane chamber, (10) silver/silver chloride electrodes, (11) cylinder body, (12) tetradecane, (13) cap, (14) collar

cesses where $\Delta V^{\neq}$ is the difference in volume of the activated state and the volumes of the reactants, or as a reaction volume (ΔV_e) which is the difference between the volumes of reactants and products at equilibrium. From thermodynamics and transition state theory, we have for the reaction,

$$\text{REACTANTS} \overset{K^{\neq}}{\rightleftharpoons} \text{ACTIVATED STATE} \rightarrow \text{PRODUCTS}$$

$$k = \frac{RT}{Nh} K^{\neq}$$

$$= \frac{RT}{Nh} \exp(-\Delta G^{\neq}/RT)$$

where k is the specific rate coefficient, $K^{\neq}$ is the equilibrium constant between reactants and activated state, R, N and h are the universal gas constant, Avogadro's number, and Planck's constant, respectively, and $\Delta G^{\neq}$ is the free energy of activation. Taking logs and differentiating with respect to pressure P at constant temperature gives

$$\frac{\partial(\ln k)}{\partial P} = \frac{-\partial}{\partial P}\left(\frac{\Delta G^{\neq}}{RT}\right) = \frac{-\Delta V^{\neq}}{RT}. \quad (1)$$

For the equilibrium case, we have

$$\frac{\partial(\ln K_e)}{\partial P} = \frac{-\partial}{\partial P}\left(\frac{\Delta G}{RT}\right) = \frac{-\Delta V_e}{RT}. \quad (2)$$

From these last two equations, it can be seen that mechanisms requiring a volume expansion will be inhibited with increases in pressure. Conversely, when a volume reduction takes place, reactions are promoted by pressure.

The ability to measure steady-state and transient currents through a planar membrane make them an excellent experimental system for studying pressure effects on transport. In the case of the hydrophobic anions TPB and DPA the translocation step through the membrane interior can be evaluated separately from the surface binding [3, 17]. Thus, it is possible to evaluate different pressure responses of the component bilayer structures. Vesicle experiments such as those of Johnson and Miller do not permit the separation of pressure effects on the binding and translocation steps, and are thus limited to an evaluation of the overall permeation process.

Through characterization of the transport steps, it may be possible to determine the appropriateness of certain permeation mechanisms such as the Traüble kink model [28]. Johnson and Miller suggested from their results (with valinomycin, glucose and the ions K^+ and Na^+) that activation volumes may be quantized according to the size of the kinks necessary to accommodate a transported molecule. This concept assumes that the important volume changes affecting membrane transport are determined by particular bilayer hydrocarbon structures rather than through an associative interaction between permeant and membrane. On the other hand, diffusion models such as that of Hildebrand [11] for bulk hydrocarbons or nonassociated liquids in which pressure increases liquid viscosity by reducing the free volume, suggest that different permeants would experience the same pressure dependence in the hydrocarbon phase thus requiring pressure-dependent membrane surface properties to explain differences in activation volumes among various transported molecules.

Important factors in considering pressure-dependent effects include the sites or steps of major volume expansions or contractions, and the accompanying changes in fluid and mechanical properties. In addition we also calculate that changes in membrane electrical parameters that are expected upon application of pressure, can produce large effects on transport and, in fact, may be necessary to explain results obtained with TPB, DPA, TPA, and CCCP. A preliminary report of this work was presented in abstract form [23]. At that time we became aware of similar work being undertaken by B.E. Aldridge and L.J. Bruner [1]. We would like to thank Professor Bruner for providing us with results of their work which substantially agrees with our own.

Materials and Methods

Figure 1 shows a cross-section of the high pressure cell constructed for the work presented here. The inner and outer diameters of the cell are 3.88 and 7.38 cm, respectively. The walls and end caps are made from 304 stainless steel. The caps are held in place by threaded collars made of 41 leaded-42 alloy steel. Electrical leads pass through acrylic insulators inserted in the top cap and terminate in silver/silver chloride electrodes. Pressure is applied to the cell using a hand-operated ENERPAC hydraulic pump capable of sustaining 20,000 psi. Pressures as high as 1,000 atm can be generated in the cell within 5 sec.

The membrane bilayer chamber consists of a delrin cup with a 1-mm aperture inserted into an acylic holder producing two compartments of 4 and 6 ml. Magnetic fleas are used to stir the aqueous compartments and are driven by four electromagnets attached to the outer wall of the high pressure cell. A variable oscillator is used to power the magnets.

All experiments reported here used membranes made from 1% (wt/vol) Type IX egg phosphatidylcholine from Sigma Chemical Co., St. Louis, Mo. Membranes were made in either unbuffered 150 mM NaCl at pH 6 or 150 mM NaCl plus 10 mM Tris at pH 7.2. For the carrier experiments, 10 mM KCl was also added to the electrolyte. Nonactin, valinomycin and CCCP were obtained from Sigma. Sodium tetraphenylboron, and tetraphenyl arsonium chloride were obtained from Aldrich. Experiments were carried out by placing the membrane chamber filled with electrolyte and dissolved ionophore or hydrophobic ion in the high pressure cell and filling the cell with tetradecane. The tetradecane insures proper electrical insulation of the membrane chamber and electrodes, and acts as the pressure-transmitting medium. Bilayers are produced on the delrin cup aperture by inserting a Pasteur pipet wetted with the decane-phospholipid mixture through a hole in the top cap. The membrane is then formed by brushing a bubble across the aperture.

Membrane formation and steady-state conductance are monitored using a standard voltage-current clamp circuit. Membrane capacitance is measured by voltage-jumping the membrane and measuring the charging time. For steady-state measurements under voltage clamp, the membrane current was monitored using an *x-y* plotter. Relaxation measurements are performed using a voltage-jump circuit similar to that of Ketterer et al. [17]. The membrane relaxation current, observed as a voltage drop across a series resistor is digitized using a Neurolog 8-bit signal averager or a 10-bit Biomation 1010 waveform recorder.

Results

Results of a typical high-pressure experiment on the steady-state conductance of valinomycin are illustrated in Fig. 2. In this case, the K^+-valinomycin conductance in an egg phosphatidyl choline membrane is monitored continuously as the hydrostatic pressure is varied from 1 to 750 atm. A fourfold decrease in conductance is noted at the highest pressure. When the membrane is returned to one atmosphere, the conductance returns close to its original value. Pressurization and depressurization can be repeated many times without the membrane breaking. Capacitance checks on membranes at different pressures showed small changes ($< +10\%$),

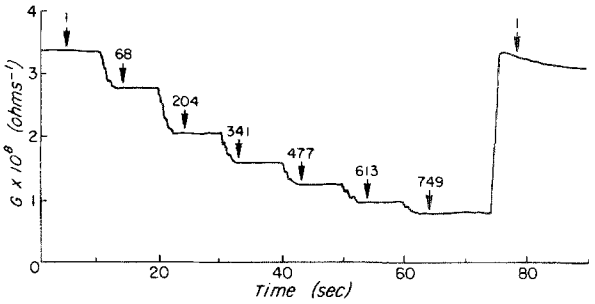

Fig. 2. Steady-state conductance *vs.* pressure for a valinomycin-treated phosphatidyl choline membrane. Unbuffered electrolyte consisted of 150 mM NaCl plus 10 mM KCl at pH 6. Valinomycin added to membrane-forming solution (0.4 mg/ml) and aqueous phase (10^{-7} M). V_c = 50 mV and T = 25°C

ruling out the possibility that decreases in membrane area account for the drops in conductance.

Not shown here is a slower and less reproducible variation of the valinomycin conductance following the application of pressure. When a steady pressure of 500 atm or higher is applied for more than five minutes, the initial conductance decrease is followed by an increase which eventually stabilizes a factor of 2 or more above the initially inhibited level. This proceeds with a half-time of approximately 5 to 15 min. However, the change in conductance with change in pressure is independent of this drift. If, for example, the pressure is relieved, the conductance increases rapidly attaining a new level with the percentage increase equaling the original decrease when pressure was first applied. Subsequently, the conductance decreases with a half-time of 5 to 15 min to the original baseline. Capacitance measurements again rule out membrane area changes accounting for the observed results. These relatively slow changes in conductance, also, occur for nonactin. The effect may be the result of changes in partitioning of carrier between the membrane and aqueous phases, or between thin areas of the bilayer and the thicker solvent domains such as the torus and interspersed microlenses. Solvent repartitioning between torus and bilayer is also possible. If pressurization-depressurization cycles are completed within a few minutes, the long time variations in conductance are not observed which supports the possibility that slow diffusion processes such as bilayer-solvent repartitioning are important. It should be noted that all the data reported here were obtained with the faster cycles where drift is insignificant.

Figure 3 shows a plot of the log of conductance *vs.* pressure for the carriers valinomycin and nonactin, the hydrophobic ion tetraphenyl arsonium, and the protonophore carbonylcyanide chlorophenylhydrazone. Analogous to Eq. (1) the activation vol-

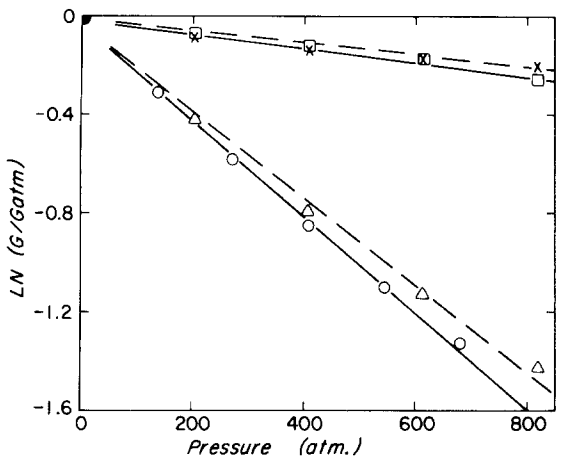

Fig. 3. Normalized plot of log conductance *vs.* pressure for valinomycin (○ – ○), nonactin (△ – △), CCCP (□ – □), and TPA (× – ×). Nonactin and valinomycin conditions same as Fig. 2. $[CCCP]_{aq}$ = 10 μM at pH 7.2 and $[TPA]_{aq}$ = 1 mM at pH 6. Fitted lines give ΔV's of 48, 42, 7 and 6 cc/mole for valinomycin, nonactin, CCCP and TPA, respectively. Each point is the average of four separate membranes. Standard errors of the mean were about the size of a symbol

ume for the steady-state conductances are given by

$$\log (G_p/G_o) = -P\,\Delta V^{\neq}/RT \qquad (3)$$

where G_o and G_p are the conductances at zero atmospheres and at pressure P. Individual points are averages of data taken from four separate bilayers for each of the permeants analyzed. The activation volumes are calculated from the slopes of the fitted lines which are essentially linear over the pressure range of our experiments.

Values of $\Delta V^{\neq}$ for valinomycin and nonactin are quite similar with magnitude of 48 and 42 cc/mole, respectively. The valinomycin value is in reasonably good agreement with the 40 cc/mole results of Johnson and Miller [14] for K^+-valinomycin exchange permeability in liposomes. These values are consistent with the expected activation volumes of viscosity for a relatively well-ordered hydrocarbon structure such as bilayer interior [12]. In sharp contrast, TPA and CCCP show very small pressure dependencies, giving activation volumes of 6 and 7 cc/mole. These small values are not reconcilable with simple diffusion through a bulk hydrocarbon phase and suggest that competing pressure-dependent electrostatic and other rate-affecting membrane properties are involved.

To resolve the pressure dependence of different rate steps in hydrophobic ion transport, tetraphenyl boron and dipicrylamine relaxation experiments were performed. Figure 4 shows the TPB relaxation current at atmospheric pressure and at 817 atm. Only a slight change in the relaxation time is observed corresponding to a $\Delta V^{\neq}$ of < 5 cc/mole for the translocation rate constant, k_i. (*See* Ketterer et al. [17] for their thorough description of this type of measurement.) For dipicrylamine, no change in relaxation time consistent with the application of pressure is ever observed, giving an apparent $\Delta V^{\neq}$ of zero for the translocation step. Also, for both TPB and DPA, the relaxation current which is a measure of the surface distribution coefficient [3, 6, 17] remains essentially constant regardless of pressure, thus the reaction volume ΔV_e for the surface absorption-desorption equilibrium is also apparently close to zero.

The lack of pressure response of TPB and DPA is not consistent with viscosity variations expected with pressure for a hydrocarbon bilayer. At first sight, the lack of pressure dependence of the translocation step, which according to the established models for hydrophobic ion permeation of bilayers corresponds to diffusion through the hydrocarbon interior, is particularly puzzling [3, 6, 17]. Various explanations are possible, however, and in general necessitate the inclusion of other membrane pressure-dependent properties besides simple viscosity.

Discussion

In general, transport of an ion across a membrane can be categorized into steps which include diffusion through the adjacent aqueous phase, binding to a carrier or membrane surface site, translocation through the low dielectric interior, association with the opposite membrane-aqueous boundary, dissociation from carrier or membrane site, and diffusion into the bulk. Each step may have a corresponding volume change and contribute to the overall pressure dependence of the transport process. In the pressure range of 1 to 1000 atm, bulk aqueous electrolyte properties such as conductivity or viscosity change negligibly [12], and consequently need not be considered. In contrast, the pressure sensitivity of the surface reactions and the diffusion step through the hydrocarbon, which are generally rate limiting, are likely to be significant. Modifications of membrane transport properties that may help explain our results include: (1) changes in membrane hydrocarbon viscosity; (2) alteration of membrane electrostatic parameters, including dielectric constant, thickness, dipole potential, and surface charge; and (3) equilibrium shifts of specific surface reactions with membrane sites or carriers. Also, it should be noted that with the exception of surface charge, conditions (1) and (2) apply to the same transport step so that the volume change associated

with diffusion through the hydrocarbon would be the sum of the two.

Condition (1) can explain in a simple way the apparent activation volumes for nonactin and valinomycin. As pressure on the membrane is increased, carrier molecules encounter fewer and fewer appropriate membrane vacancies because of the increased energy required to produce them. Viscosity increases in bulk hydrocarbons when subjected to hydrostatic pressure are well known [12], and could account for the major part of the conductance decreases observed in our experiments. The activation volume for viscosity-controlled transport would be given by

$$\frac{\Delta V^{\neq}}{RT} = \frac{\partial(\ln \eta)}{\partial P} \tag{4}$$

where η is the viscosity of the hydrocarbon interior.

This simple view suggests that all permeants would be subjected to the same viscosity variations and should therefore give the same pressure dependence and activation volume. Although nonactin and valinomycin give approximately the same values for $\Delta V^{\neq}$, TPB, TPA, CCCP and DPA have very small apparent $\Delta V^{\neq}$'s ($\leq$ 7 cc/mole). The relaxation measurements on TPB and DPA, which presumably permit a determination of the rate through the central barrier, seem to indicate that applied pressure produces little or no viscosity change in the membrane interior. In addition, Johnson and Miller obtained a $\Delta V^{\neq}$ of 20 cc/mole for Na^+ and K^+ ion exchange in liposomes, further arguing against a simple change in viscosity.

The Träuble or kink model [28] of membrane permeation overcomes the main objection to the viscosity argument, namely the inability to account for dissimilar activation volumes of permeants, by assuming that the hydrocarbon tails produce different size holes in the membrane by the summing of trans-gauche chain conformation. Johnson and Miller attributed the activation volumes they obtained for valinomycin-K^+ and for K^+ and Na^+ to 2g2 kink-mediated diffusion for the former, and 2g1 kink for the latter. However, the Träuble model does not explain the much smaller volumes found for TPB, TPA, DPA and CCCP. Therefore, other factors affecting membrane permeability besides changes in kink formation and viscosity must be operating.

Calculations show that condition (2) could be important in characterizing apparent activation volumes for the charged permeants studied in this paper. It has been shown, for example, that thickness is a significant factor in determining the effective dielectric barrier of bilayers for the hydrophobic

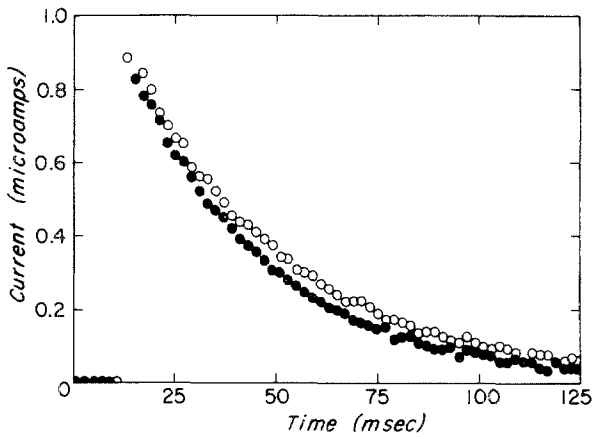

Fig. 4. Superimposed digitized traces of TPB relaxation currents following 50-mV voltage step at 1 atm (●) and at 818 atm (○). Relaxation times are 21.2 and 24.5 msec. [TPB] = 10^{-7} M (initial capacitive spike off scale)

ions but not for the carrier valinomycin [4, 5]. Also, very small changes in dielectric constant can produce very significant conductance changes [8]. Both of these factors are described by the following equation given by Parsegian [24] for the dielectric barrier at the center of a bilayer:

$$\frac{\Delta G(\varepsilon, l)}{RT} = Q\frac{1}{r}\left(\frac{1}{\varepsilon_{HC}} - \frac{1}{\varepsilon_{H_2O}}\right) - \frac{2Q}{\varepsilon_{HC} l}\ln\frac{2\varepsilon_{H_2O}}{(\varepsilon_{HC} + \varepsilon_{H_2O})} \tag{5}$$

where $\Delta G(\varepsilon, l)/RT$ is the barrier height in dimensionless units, Q is equal to 280 Å, r is the ion radius, ε_{HC} and ε_{H_2O} are the hydrocarbon and aqueous dielectric constants, and l is the membrane thickness in Ångstroms.

Since the dielectric barrier is simply the free energy change for an ion transferring from one dielectric medium to another, differentiation with respect to pressure will yield the volume change (ΔV_{EL}) associated with this process. Assuming that the dielectric constant of water varies negligibly with pressure, and setting $\ln 2\ \varepsilon_{H_2O}/(\varepsilon_{HC} + \varepsilon_{H_2O})$ equal to $\ln 2$, we obtain the following from Eq. (5):

$$\frac{\Delta V_{EL}}{RT} = \frac{Q}{\varepsilon_{HC}^2}\left(\frac{2\ln 2}{l} - \frac{1}{r}\right)\frac{\partial \varepsilon_{HC}}{\partial P} + \frac{2Q\ln 2}{\varepsilon_{HC} l^2}\left(\frac{\partial l}{\partial P}\right). \tag{6}$$

The above equation gives the difference in volumes of electrostriction produced by an ion in two different dielectric media, and may be regarded as

the Drude-Nernst Equation [12] corrected for the finite membrane thickness.

To assess the importance of electrostriction, we need to obtain values for $1/\varepsilon_{HC}^2$ $(\partial\varepsilon_{HC}/\partial P)$ and $1/l^2$ $(\partial l/\partial P)$. Estimates of the dielectric term can be obtained from the following modification of the Clausius-Mossotti equation

$$\varepsilon_{HC} = \frac{V(P) + 2A}{V(P) - A} \tag{7}$$

where ε_{HC} is the dielectric constant, $V(P)$ is the molar volume as a function of pressure, and A is the molar polarizability which is independent of density. Letting V_o = the molar volume at $P = 0$, and β = the membrane compressibility then $V(P)$ can be approximated by $V(P) = V_o \exp(-\beta P)$. Inserting this approximation into Eq. (7) and differentiating, we have

$$\frac{\partial\varepsilon}{\partial P} = \frac{3A\ V_o e^{-\beta P}}{(V_o e^{-\beta P} - A)^2}. \tag{8}$$

With $\varepsilon_{HC} = 2$, $V_o = 800$ cc/mole, and $\beta = 3 \times 10^{-5}$ atm^{-1} [15] and by using Eqs. (7) and (8), we obtain a value of 1×10^{-5} for $1/\varepsilon_{HC}^2$ $(\partial\varepsilon_{HC}/\partial P)$.

Assuming membrane compression is isotropic and an initial membrane thickness of 50 Å, we estimate $1/l^2$ $(\partial l/\partial P)$ to be approximately -2×10^{-7} $Å^{-1}atm^{-1}$. From Eq. (6), we then calculate ΔV_{EL} to be -15 cc/mole for TPB ($r = 4.2$ Å) [9] and -10 cc/mole for nonactin ($r = 6.2$ Å) [18]. In these two examples, dielectric changes account for 90% or more of the electrostrictive volumes. Of course, these values are estimates and the actual electrostrictive volume changes might be quite different. Higher values of the membrane compressibility will give larger negative volumes. In addition, anisotropy of the membrane compressibility parameters will alter the comparative importance of the thickness and dielectric terms. It should be noted that Eq. (6) defines a relationship between the activation volume and the size of an ion. In general, the larger the ion, the smaller the importance of electrostriction and the greater the apparent effect of other volume changes.

Dipole potential changes appear to be ruled out by our experiments with TPB and TPA. A change in dipole potential should increase the conductance of one species but inhibit the other [2]. However, we observed pressure to slightly inhibit both. Additionally, since we used phosphatidyl choline, changes in membrane surface charge with pressure need not be considered.

Accordingly, an estimate of the net activation volume for the hydrocarbon phase of a bilayer reduces to the sum of the viscosity and electrostrictive contributions and is expressed as

$$\Delta V_{HC}^{\neq} = \Delta V_{\eta}^{\neq} + \Delta V_{El}^{\neq}.$$

For bulk linear hydrocarbons, $\Delta V_{\eta}^{\neq}$ of + 20 cc/mole have been determined [13]. The net result for the ions and carriers used in our study would put the estimated $\Delta V_{HC}^{\neq}$'s in the + 5 to + 10 cc/mole range which reasonably explains the low values we observe for the hydrophobic ions and the charged carrier CCCP. The electrostrictive volume component essentially cancels the positive viscosity term. In general, it appears that the translocation step through the membrane interior does not contribute appreciably to apparent membrane volume changes. The fact that nonactin and valinomycin activation have much larger apparent volumes suggests that interfacial contributions may be very important for these carriers.

Carriers and hydrophobic ions may differ significantly in the volume changes associated with their surface reactions because of the water of hydration associated with aqueous cations and membrane-adsorbed carriers. It is believed that uncomplexed carriers at the membrane adsorption plane have their polar moieties oriented towards the aqueous phase and are hydrogen bonded to a degree [26]. During complexation, water is released from both the carrier, which undergoes a conformational change, and the cation which is normally hydrated. This results in a considerable release of water producing a positive ΔV. The hydration of the carrier at the surface has been used to explain the fact that the measured rates of translocation for the complexed and uncomplexed forms are very close in value despite the high dielectric barrier to charge translocation [27]. Assuming that each potassium releases 4 molecules of H_2O and that the carrier releases 4 molecules for a total of 8 moles of H_2O per mole of product, a total $\Delta V^{\neq}$ of 25 cc/mole is expected [12]. Volume expansion of the membrane accompanying carrier diffusion across the dividing plane formed by the polar groups and the hydrocarbon tail may also contribute significantly to a positive $\Delta V^{\neq}$.

Another rationale to explain the low activation volumes for the smaller ions takes a very different view. Parsegian [25] has calculated that the passage of an ion across a bilayer produces a large local pressure jump due to coulombic forces between the ion and the surface. He estimates that a univalent ion 10 Å into the membrane will generate a local pressure of 200 atmospheres. Thus, ion penetration of a membrane may induce significant deformation of the surface permitting water molecules to penetrate the membrane, thereby lowering the electrostatic

barrier and reducing the local pressure sensitivity of the membrane to dielectric, and viscosity changes.

Although the preceding arguments are based on highly idealized models, they do show how the qualitative results we obtained could have occurred. Clearly, further experimentation is necessary to determine which combination of alternatives is the most important. We plan to examine the dependence of the valinomycin relaxation kinetics under pressure which will then allow us to determine the pressure dependence of the translocation steps for both the charged and uncharged forms. This would determine the contribution of surface dehydration effects and permit an evaluation of the electrostatic contributions as well. In addition, by doing experiments with folded monolayer membranes, we can largely eliminate uncertainties arising from solvent-bilayer repartitioning effects.

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References

1. Aldridge, B.E., Bruner, L.J. 1984. Hydrostatic pressure on transport of carrier-cation complexes and of hydrophobic anions. *Biophys. J.* **45:**39a (*Abstr.*)
2. Anderson, O.S., Finkelstein, A., Katz, I., Cass, A. 1976. Effect of phloretin on permeability of thin lipid membranes. *J. Gen. Physiol.* **67:**749–771
3. Anderson, O.S., Fuchs, M. 1975. Potential energy barriers to ion transport within lipid bilayers. *Biophys. J.* **15:**795–830
4. Benz, R., Frölich, O., Läuger, P. 1976. Influence of membrane structure on the kinetics of carrier-mediated ion transport through lipid bilayers. *Biochim. Biophys. Acta* **464:**465–481
5. Benz, R., Läuger, P. 1977. Transport kinetics of dipicrylamine through lipid bilayer membranes. *Biochim. Biophys. Acta* **468:**245–258
6. Bruner, L.J. 1975. The interaction of hydrophobic ions with lipid bilayer membranes. *J. Membrane Biol.* **22:**125–141
7. Bruner, L.J., Hall, J.E. 1983. Pressure effects on alamethecin conductance in bilayer membranes. *Biophys. J.* **44:**39–47
8. Dilger, J.P., McLaughlin, S.G.A., McIntosh, T.J., Simon, S. 1979. Dielectric constant of phospholipid bilayers and the permeability of membranes to ions. *Science* **206:**1196–1198
9. Grunwald, E., Baughman, G., Kohnstam, G. 1960. The solvation of electrolytes in dioxane-water mixtures, as deduced from the effect of solvent change on the standard partial molar free energy. *J. Am. Chem. Soc.* **82:**5801
10. Heremans, K. 1982. High pressure effects on proteins and other biomolecules. *Annu. Rev. Biophys. Bioeng.* **11:**1–21
11. Hildebrand, J.H., Lamoreaux, R.H. 1972. Fluidity: A general theory. *Proc. Natl. Acad. Sci. USA* **69:**3428–3431
12. Issacs, N.S. 1981. Liquid Phase High Pressure Chemistry. pp. 63–136. John Wiley, New York
13. Johnson, F.H., Eyring, H., Storer, B.J. 1974. Theory of Rate Processes in Biology and Medicine. pp. 273–369. Van Nostrand Reinhold, New York
14. Johnson, S.M., Miller, K.W. 1975. The effect of pressure and the volume of activation on the monovalent cation and glucose permeabilities of liposomes of varying composition. *Biochim. Biophys. Acta* **375:**286–291
15. Johnson, S.M., Miller, K.W., Bangham, A.D. 1973. The opposing effects of pressure and general anesthetics on the cation permeability of liposomes of varying lipid composition. *Biochim. Biophys. Acta* **307:**42–57
16. Kamaya, H., Ueda, I., Moore, P.S., Eyring, H. 1979. Antagonism between high pressure and anesthetics in the thermal phase-transition of dipalmitoyl phosphatidylcholine bilayer. *Biochim. Biophys. Acta* **550:**131–137
17. Ketterer, B., Neumcke, B., Läuger, P. 1971. Transport mechanism of hydrophobic ions through lipid bilayer membranes. *J. Membrane Biol.* **5:**225–245
18. Kilboun, B.T., Dunitz, J.D., Dioda, L.A.R., Simon, W. 1967. Structure of the K^+ complex with nonactin, a macrotetrolide antibiotic possessing highly specific K^+ transport. *J. Mol. Biol.* **30:**559
19. Liu, N., Kay, R.L. 1977. Redetermination of pressure dependence of lipid bilayer phase transition. *Biochemistry* **16:**3484–3486
20. MacDonald, A.G. 1978. A dilatometric investigation of the effects of general anesthetics, alcohols and hydrostatic pressure on the phase transition in smectic mesophases of dipalmitoyl phosphatidylcholine. *Biochim. Biophys. Acta* **507:**26–37
21. MacDonald, A.G., Miller, K.W. 1976. Biological membranes at high hydrostatic pressure. *In:* Biochemical and Biophysical Perspectives in Marine Biology. D.C. Malins and S.R. Sargent, editors. pp. 117–146. Academic, New York
22. Morild, E. 1981. The theory of pressure effects on enzymes. *In:* Advances in Protein Chemistry. C.B. Anfinsen, J.T. Edsall and F.M. Richards, editors. pp. 93–165. Academic, New York
23. Moronne, M.M., Macey, R.I. 1984. Hydrostatic pressure dependence of planar lipid bilayer conductances. *Biophys. J.* **45:**327a (*Abstr.*)
24. Parsegian, V.A. 1969. Energy of an ion crossing a low dielectric membrane: Solutions to four relevant electrostatic problems. *Nature (London)* **221:**844
25. Parsegian, V.A. 1975. Ion-membrane interactions as structural forces. *Ann. N.Y. Acad. Sci.* **264:**161–174
26. Shemyakin, M.M., Ovchinnikov, Yu.A., Ivanov, V.T., Antonov, V.K., Vinogradova, E.I., Shkrob, A.M., Malenkov, G.G., Evstratov, A.V., Laine, I.A., Melnik, E.I., Ryabova, I.D. 1969. Cyclodepsipetides as chemical tools for studying ionic transport through membranes. *J. Membrane Biol.* **1:**402
27. Stark, G., Ketterer, B., Benz, R., Läuger, P. 1971. The rate constants of valinomycin mediated ion transport through thin lipid membranes. *Biophys. J.* **11:**981–994
28. Träuble, H. 1971. The movement of molecules across lipid membranes: A molecular theory. *J. Membrane Biol.* **4:**193–208
29. Trudell, J.R. 1976. The effect of high pressure on phospholipid bilayer membranes. *In:* Extreme Environments: Mechanisms of Microbial Adaptation. M.R. Heinrich, editor. pp. 349–353. Academic, New York

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