

Ionic Permeation of Lipid Bilayer Membranes Mediated by a Neutral, Noncyclic Li^+ -selective Carrier Having Imide and Ether Ligands. I. Selectivity Among Monovalent Cations

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Summary. We have found that Simon's neutral, noncyclic, Li^+ -selective complexone, which has imide and ether ligands, renders lipid bilayer membranes selectively permeable to certain cations and anions. The present paper characterizes the ability of this molecule to carry monovalent cations; and we show it to be most selective for Li^+ among the alkali cations, the first reconstitution of Li^+ -selective permeation in lipid bilayer membranes. This complexone acts as an "equilibrium-domain" carrier for $\text{Ag}^+ > \text{Li}^+ > \text{Tl}^+ > \text{Na}^+ > \text{NH}_4^+ > \text{Rb}^+ > \text{Cs}^+$ over a wide range of experimental conditions. The major type of membrane-permeating species formed is a 2:1 carrier/cation complex dominant except at the lowest salt and carrier concentrations where a 1:1 carrier/cation, with a similar selectivity sequence, can be detected. Among the group *Ia* cations the selectivity sequence in bilayers, $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$, is similar to that previously found for this molecule in thick solvent-polymer membrane electrodes. We find this carrier to be more selective to Ag^+ than to any other monovalent cation yet studied. This high Ag^+ selectivity is used, together with the dependence of the selectivity on the nature of the N-amide substituents, to argue that the imide oxygens play a major role as ligands.

Key words: Lithium, carrier, selectivity, bilayers, membrane electrodes, Li selectivity, Ag selectivity, amide ligands

Those neutral, cyclic complexones such as valinomycin [6, 39], the macrotetralide actins [14, 43] and cyclic polyethers [14, 38] which selectively bind the *larger* alkali cations (K^+ , Rb^+ , Cs^+), have also been found

to produce selective permeation to these cations in lipid bilayers. They have, moreover, been shown to operate as carriers whose selectivities in the bilayer reflect their equilibrium complexation abilities [15, 26, 43]. In marked contrast, similar complexones (e.g., the cryptates [32] and perhydroantamanide [46]), which are selective to the *smaller* alkali cations (Li^+ and Na^+), have failed to yield selective permeation to these cations in either artificial or natural membranes [40]. Instead, they induce selective permeation to the larger cations, presumably because of kinetic limitations on the complexation reactions for cyclic molecules with the smaller cations.

In view of the biological importance of selective membrane permeation to the smaller alkali cations [1, 9, 10, 23, 41], and the previous absence of any carriers selective to these cations in bilayers, we decided to test whether Simon's small noncyclic Li^+ and Na^+ selective complexones [2, 3, 22], which might be expected to have less kinetic limitations on complexation than cyclic molecules, would produce selective permeation to the smaller cations in bilayers. This was indeed found to be the case for the Na^+ -selective complexone by Eisenman and Kuo [13, 28, 29], and subsequently by us for his Li^+ -selective molecules, as reported briefly elsewhere [33].

In this paper we will characterize one of Simon's Li^+ -selective complexones, N,N'-diheptyl-N,N',5,5-tetramethyl-3,7-dioxanonan diamide (Fig. 1), hereafter called ETH149, following Simon's terminology [25], which we will show to act as a carrier in bilayers, selective to Li^+ among the group *Ia* cations. We will, further, establish that this molecule forms a major membrane-permeating species with a clearly defined 2:1 carrier/ion stoichiometry and a minor permeant species with a 1:1 carrier/ion stoichiometry. For both species the ion loading and unloading reactions are sufficiently rapid compared to translocation that they are at equilibrium (i.e., in the "equilibrium-

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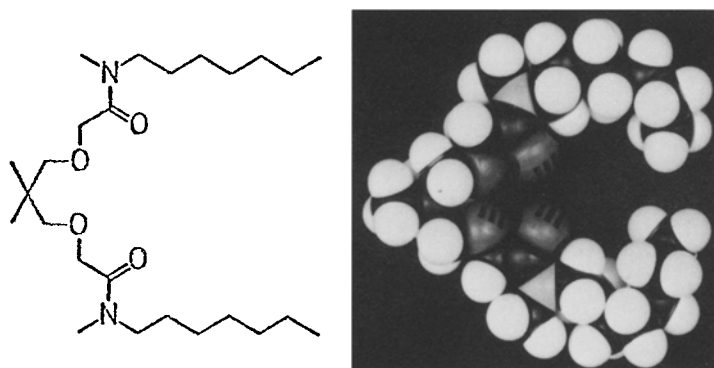


Fig. 1. The chemical formula and CPK model of Simon's Li^+ -selective complexone: N,N'-diheptyl-N,N',5,5-tetramethyl-3,7-dioxanonandiamide (ETH149)

domain" (20, 30). We will also show that this molecule is even more selective to Ag^+ than to Li^+ .

In the course of studying this complexone in bilayers we have found that in addition to its anticipated ability to carry monovalent cations this molecule could, surprisingly, also carry monovalent anions [33, 34, 36] and even act as a "symport" carrier of charged mixed cation-anion complexes [35]. We will report on these properties in subsequent papers of this series¹.

Materials and Methods

The complexone ETH149 used in this study was a gift from W. Simon. Membranes were formed by the air bubble technique [43] from GMO/decane or PE/decane solutions (25 mg/ml) on the aperture (usually 1 mm diameter) of a Teflon chamber, and the usual procedures for steady-state characterization of electrical properties of bilayer membranes [26] were followed, except when noted otherwise.

Because of the abundance of different types and molecularities of membrane-permeating species encountered with this complexone, the experiments had to be carefully designed to make the desired species dominant in a given situation. Thus, before carrying out any selectivity studies, it was generally necessary to establish the stoichiometry and charge of the principle membrane-permeating species by combining quantitative studies of concentration dependence of membrane conductance together with parallel studies of the sign and magnitude of the slope of the zero-current dilution potentials. Through careful selection of the salt employed (e.g., using impermeant divalent anions when characterizing monovalent cations and *vice versa*) and through control of salt and complexone concentrations we have been able to characterize the selectivity of complexes of a variety of stoichiometries.

To study the monovalent cations with the least complication from anions, we used sulphate salts since we found that this divalent anion was not detectably carried across the bilayer by the present complexone, whereas monovalent anions were carried to a significant degree. For the highly permeant Ag^+ ion, owing to limitations in the solubility of its sulphate salt, we used AgNO_3 . Also, occasionally, LiCl was used instead of Li_2SO_4 . Anion effects in these

cases were negligible, since the permeabilities for Cl^- and NO_3^- were found to be only 2 and 3%, respectively, of those for Li^+ and Ag^+ .

When characterizing the selectivity of the 2:1 species by measuring zero-current membrane potentials the following experimental conditions were employed to ensure that all permeant species were in that desired stoichiometry: Complexone concentration exceeded 10^{-7} M in the symmetrical aqueous phases which were also symmetrical in the concentration of the sulphate salt of the major monovalent cation, whose concentration ranged from 0.1–1.0 N. Li^+ , the minor ion, was then added to one side only as Li_2SO_4 to concentrations exceeding 0.1 N.

Results

Membrane Conductance at Zero-Current

Thick vs. thin membranes. From Simon's measurements on thick solvent-polymer membranes whose dielectric constants range from 8–25 [21], it was conceivable that there might be some detectable conductance induced in interference-colored lipid membranes despite the lower dielectric constant of our solvent (decane or hexadecane). We therefore designed an experiment to measure the steady-state conductance of bilayer (black) *vs.* interference-colored membranes, utilizing the tendency of GMO/hexadecane membranes to be more stable (compared to GMO/decane membranes) in a reasonably thick interference-colored state and their ability to be forced to blackness by an applied voltage of several hundred millivolts.

A typical experiment is illustrated in Fig. 2, which compares for interference-colored *vs.* black GMO/hexadecane membranes the dependence of the steady-state Li^+ -conductance on complexone concentration. For each complexone concentration an interference-colored membrane was formed and its zero-current conductance was measured. Then a potential of several hundred millivolts was applied momentarily to force the membrane to go black, and the conductance of the new state was measured. It can be seen in Fig. 2 that complexone additions up to 10^{-6} M in

¹ Margalit, R., Eisenman, G. 1981. Ionic permeation of lipid bilayer membranes mediated by a neutral noncyclic Li^+ -selective carrier having imide and ether ligands. II. Selectivity among monovalent anions. III. "Symport" membrane permeation by multivalent complexes of cations together with anions. (*in preparation*).

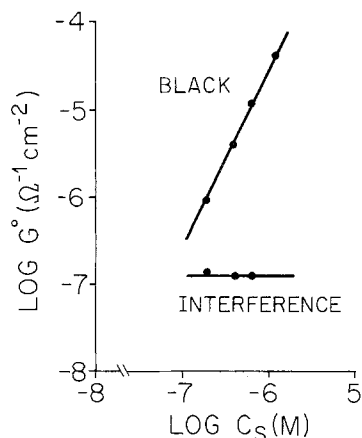


Fig. 2. Dependence of zero-current conductance of black and interference-colored GMO/hexadecane membranes on complexone concentrations, in the presence of 1 N LiCl. Ordinate: Logarithm of membrane conductance. Abscissa: Logarithm of aqueous complexone concentration. The points are experimental, the solid lines drawn to slopes of 0 and 2, for interference-colored and black membranes, respectively

the aqueous phase have no effect on the conductance of the thick membrane, even in the presence of 1 M LiCl. In contrast, when the membrane is thinned to the bilayer (black) state, a striking increase of conductance is observed and there is now a clear 2nd power dependence of membrane conductance on complexone concentration.

These results serve as an important control that the conductance properties studied throughout this work were uniquely those of the bilayer. They also indicate that there is no detectable conductance of the type seen in Simon's bulk membranes [2]. Consistent with this, in our usual GMO/decane bilayers we have invariably found the membrane conductance to be proportional to the black area, the conductance

of the torus and interference-colored areas always being negligible. Conductances throughout this paper are expressed per unit area of the black film (measured optically).

Dependence on complexone concentration. As the first step in characterizing the effects of this complexone on ion permeation through lipid bilayer membranes, we have measured the increase in zero-current conductance of GMO-membranes with increasing complexone concentrations. Over the complexone concentration range of 10^{-8} – 10^{-5} M the membrane conductance increases in a simple and regular manner, as illustrated in Fig. 3 for 0.01, 0.1, and 1.0 N solutions of the sulphate salts of the indicated monovalent cations. The different membrane conductances seen among the various salts sharing a common divalent anion clearly indicate the existence of selectivity among the monovalent cations, with the sequence $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$ among the alkali cations. However, conductance data such as these cannot, by themselves, exclude some participation of the divalent anion in the permeating complexes, so that additional data of other electrical properties of the membrane must be acquired before attributing the selectivity to monovalent cation permeability solely.

Putting this question aside for the moment, the regularity of the increase in membrane conductance makes it possible to deduce the complexone stoichiometry from data of the type illustrated in Fig. 3. From the data presented for 0.1 and 1.0 N salts it is clear that all points fall onto lines of slope=2 (i.e., a second power dependence) at complexone concentrations exceeding 10^{-7} M, implying that the membrane-permeating species involves two complexone molecules (i.e., a complexone stoichiometry of 2).

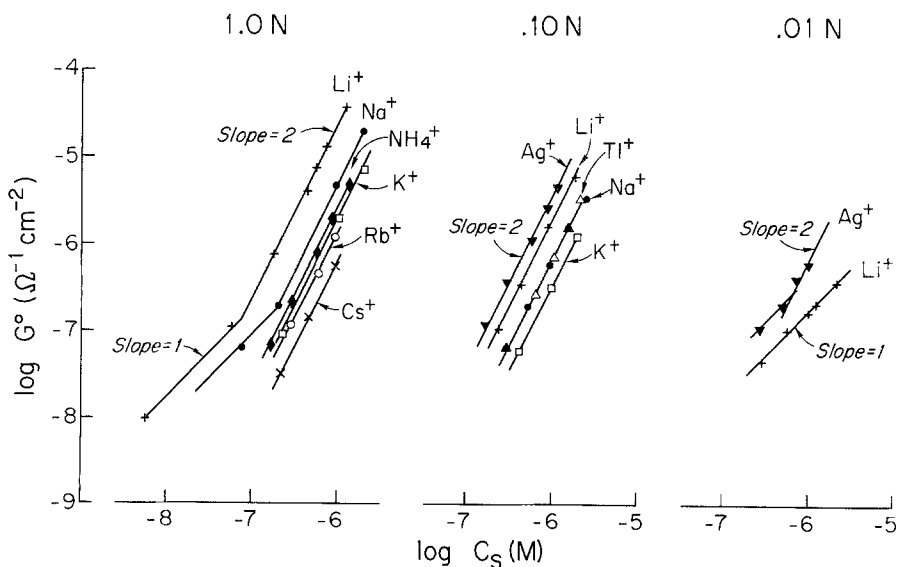


Fig. 3. First and second power dependences of zero-current conductance of GMO membranes on complexone concentrations, for 0.01, 0.10 and 1.0 N sulphate salts of the indicated cations (excepting nitrate salt for Ag^+). Ordinate: Logarithm of membrane conductance. Abscissa: Logarithm of aqueous complexone concentration. The points are experimental, the lines drawn to the indicated slopes

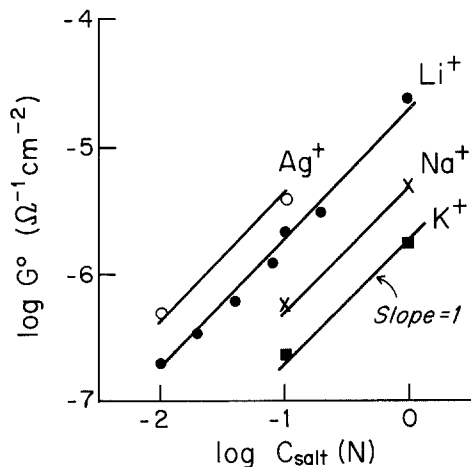


Fig. 4. First power dependence of zero-current conductance of GMO membranes on salt concentration, for symmetric aqueous complexone concentration of 10^{-6} M, and sulphate salts of the indicated cations (excepting nitrate salt for Ag^+). Ordinate: Logarithm of membrane conductance. Abscissa: Logarithm of salt concentration. The points are experimental the lines drawn to a slope of 1

Under more specialized circumstances, lines of slope = 1 (commonly encountered with the nonactin-like and valinomycin-like carriers) were also observed, implying a membrane-permeating complex with a complexone stoichiometry of 1. For example, this stoichiometry can be seen in Fig. 3 for 0.01 N Li^+ over the entire complexone concentration range. Although most of the conductances corresponding to a slope of 1 are of low magnitudes, they are still well above the background conductance of the unmodified membrane, the latter being less than 10^{-8} Ω^{-1} cm^{-2} . Furthermore, the data for 0.01 N Li^+ , which follow a slope of 1, are within the range of conductances observed for less-conducting ions (*cf.* the data for 1.0 N Cs^+) which follow a slope of 2. We therefore believe that the first power dependance of the conductance on complexone concentration, observed under specialized conditions, truly indicates a complexone stoichiometry of 1. Despite the two types of complexone stoichiometries, we invariably found a first power dependance of the membrane conductance on the ion activity, as typified by the slope of 1 for all the data points illustrated in Fig. 4 at a constant, bilateral aqueous complexone concentration of 10^{-6} M. This finding implies an ion stoichiometry of 1 for both types of complexone stoichiometries.

To see clearly that both types of complexone stoichiometries are encompassed in these data, one has to compare the data illustrated in Fig. 4 with those illustrated in Fig. 3 at the complexone concentration of 10^{-6} M. Doing this, one can see that for Na^+ , K^+ and Ag^+ all the conductances illustrated in Fig. 4 correspond to a complexone stoichiometry of 2 in

Fig. 3. With Li^+ , the data points illustrated in Fig. 4 at the region of low salt concentration correspond to a region in Fig. 3 where the complexone stoichiometry appears to be 1, while the data points illustrated in Fig. 4 at the region of high salt concentrations correspond to the region in Fig. 3 where the complexone stoichiometry is 2.

The conductance data of Figs. 3 and 4, taken together, imply that the two permeant species detected have 1:1 and 2:1 complexone/ion stoichiometries, respectively. The carried ion, in both species, is *presumably* the monovalent cation; but as we have already indicated, identification of the sign and magnitude of the charge carried by each type of permeant complex requires a membrane-potential measurement, which we will present in a following section.

As expected from simple equilibrium chemistry, the transition from the 1:1 to the 2:1 complex is observed in Fig. 3 to be dependent on complexone concentration. However, as observed from these same data, the transition also appears to be dependent on salt concentration, which is unexpected, especially since, for all cases where one of the two possible species is dominant, we have never observed an ion stoichiometry other than 1.²

The process involved in the peculiarly sharp transition from the 1:1 to the 2:1 species has not yet been elucidated. Therefore, in the following we have specifically avoided basing conclusions on data measured at concentrations where this transition might be taking place or where the system would contain significant concentrations of both species. In particular, we have designed the zero-current membrane potential measurements of selectivities so that only one type of species, usually the 2:1, is present.

Dependence on Lipid Composition

The magnitude of conductance increase produced by the present complexone in presence of salts of monovalent cations depends upon the lipid composition of the membrane in a way quite similar to that characteristic of the usual cyclic carriers of monovalent cations. This is apparent in Fig. 5 where conductances of PE and GMO membranes in the presence of a Li^+ salt are illustrated. The conductances measured

² This nonideal dependence of the stoichiometric transition on salt concentration should have been reflected in deviations from a slope of 1 in the Li^+ data presented in Fig. 4, since these data go from the range of the 1:1 to that of the 2:1 stoichiometries; but, as clearly seen from Fig. 4, we detected no deviations. However, this discrepancy seems to occur only at the specific complexone concentration employed in the experiment illustrated in Fig. 4 (i.e., 10^{-6} M), as can be easily ascertained upon examining the data presented for 0.01, 0.1 and 1.0 N Li_2SO_4 in Fig. 3 point by point over the entire complexone concentration range.

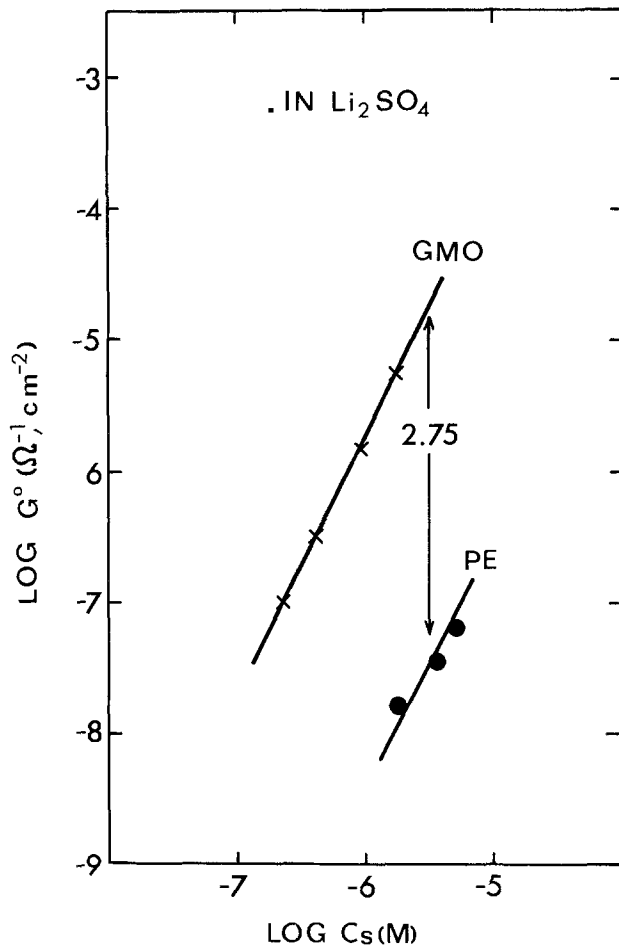


Fig. 5. Dependence of membrane zero-current conductance on complexone concentration for GMO and PE membranes, in the presence of $0.1 \text{ N Li}_2\text{SO}_4$. Ordinate: Logarithm of membrane conductance. Abscissa: Logarithm of aqueous complexone concentration. Points are experimental, solid lines drawn to slopes of 2

for PE membranes are seen to be three orders of magnitude lower than those for the GMO membranes, an effect closely similar to that observed for the nonactin/ K^+ and trinactin/ K^+ complexes [4, 44]. In contrast to such high sensitivity of typical carriers to the dipole potential difference between GMO and PE [4, 15, 24, 26, 42] the corresponding difference in conductance between these two lipids for a typical channel such as gramicidin A is quite small [4, 5, 45]. This supports the other evidence that we will present that the Li^+ selective complexone functions in the bilayer membrane as a carrier for cations and not as a channel.

Conductance-Voltage Behavior

The steady-state conductance-voltage behavior has been found to be useful for assessing the location and voltage-dependence of the several possible rate determining steps in the permeation process [16, 26, 30, 31]. We have, therefore, characterized the conductance-voltage behavior for both the 1:1 and the 2:1 complexes. Since we will later give evidence that the present molecule functions via a carrier mechanism, we will interpret the data using the theory which has been well developed [7] for carriers, with which our results will be seen to be in accord.

Typical data are illustrated in Fig. 6, presenting the dependence on voltage of the chord-conductances, measured with triangular waves and normalized to the zero current conductance in the usual way [16, 26, 30]. The conductance-voltage characteristic was found to be completely frequency independent over the range of $0.01\text{--}0.20 \text{ Hz}$ and thus represents the true

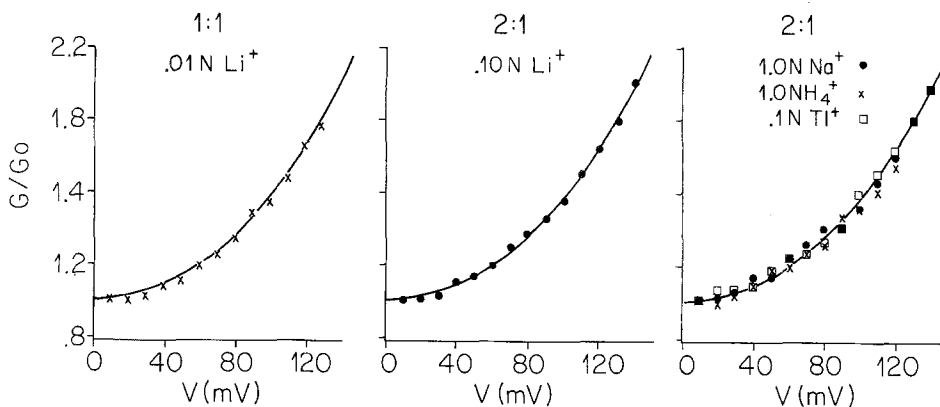


Fig. 6. Conductance-voltage behavior of GMO membranes in the presence of 10^{-6} M symmetric aqueous complexone concentrations and sulphate salts of the indicated cations, under experimental conditions forming 1:1 complexone/ion complexes with $0.01 \text{ N Li}_2\text{SO}_4$ solutions and 2:1 complexone/ion complexes with 0.1 N sulphates of Li^+ or Tl^+ and 1.0 N sulphates of Na^+ or NH_4^+ . Ordinate: The membrane conductance G , normalized to G_0 , the membrane conductance at the limit of zero-current. Abscissa: The applied potential. Points are experimental, curves drawn to the "equilibrium-domain" equation given in the text, with a value of $P_2 = 0.26$ for the "barrier-shape" parameter

steady-state behavior. For comparison with the experimental data points, theoretical curves on all the figures were drawn according to the following "equilibrium-domain" equation [7, 16, 26] with a value of 0.26 for P_2 , the "barrier half-width".

$$\frac{G}{G_0} = \frac{2P_2 \sinh \phi/2}{\sinh P_2 \phi} \quad (1)$$

Clearly, at least for the selected cations studied (i.e., Li^+ , Na^+ , Tl^+ and NH_4^+), the conductance-voltage behavior of the present molecule is quite in accord with that theoretically expected for an "equilibrium-domain" carrier where the translocation of the complex is the rate-limiting step and the reactions of loading and unloading the ion at the membrane-solution interfaces are effectively at equilibrium [16, 26]. This behavior is consistent with our initial postulate that the complexation reactions for this noncyclic molecule are sufficiently rapid that they are never rate determining, which also has the important implication that the selectivities are directly related to ion-binding equilibria [8, 14, 20]. We shall present additional data in support of this conclusion, which will allow us to extend the inference of "equilibrium-domain" to all monovalent cations studied in this work.

Two additional points should be noted concerning the conductance-voltage behavior of the present molecule. First, the "barrier" is sharper than that characteristic of valinomycin and the nactin homologues (for which $P_2=0.35$ in GDO/decane [16, 26], and for which we find an identical value in GMO/decane membranes. It is of interest that for the related Na^+ -selective complexone a somewhat narrower barrier is seen ($P_2=0.22$ (G. Eisenman and K.H. Kuo, unpublished,)) and even a narrower one ($P_2=0.18$) (G. Eisenman and K.H. Kuo, unpublished) has been found for the cyclic polyether dicyclohexyl-30-crown-10. It seems that the sharpness of the barrier correlates with the thinness of the hydrophobic "skin" of the complex. Second, and a little disturbing, is the finding that the barrier shape of the 1:1 complex is indistinguishable from that of the 2:1 complex. Since these two complexes differ in their shielding, it would seem that conductance-voltage behavior is not sensitive enough to detect these differences.

Membrane Potentials at Zero-Current

"Dilution-potentials". The data presented so far already indicate that, of the two types of ions present in the medium, the monovalent cation and the divalent anion, the former is the ion translocated by this ionophore. To verify this we have measured membrane "dilution-potentials" generated under a concentration gradient of a single salt in order to deter-

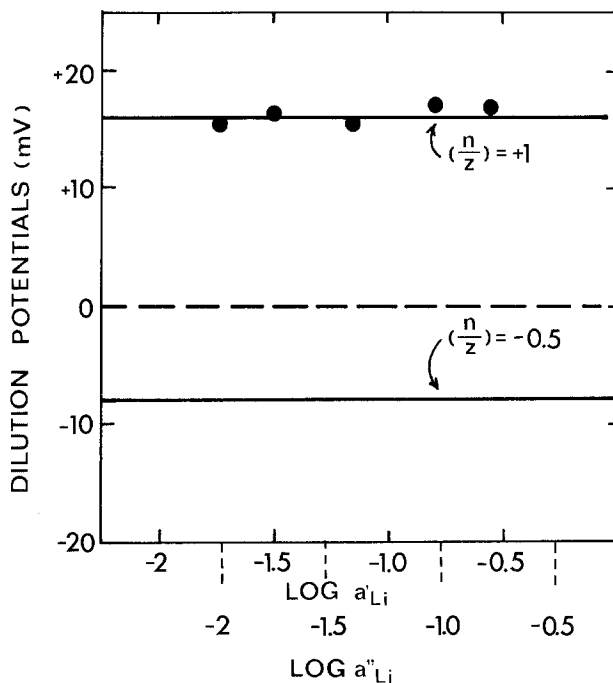


Fig. 7. Membrane zero-current "dilution-potentials" for Li_2SO_4 gradients and symmetrical aqueous complexone concentrations of 10^{-6} M. Ordinate: Observed membrane potentials. Dual abscissa: Logarithms of the activities of the Li^+ ion on the dilute and concentrated sides. The points are experimental, the lines drawn to the theoretical expression given in the text, for (n/z) values of +1 and -0.5, respectively

mine the net charge of the translocated species. Figure 7 illustrates how one can assess the sign and magnitude of the charge of a permeant complex. The observed potentials, plotted as the data points, were measured at a constant, symmetrical complexone concentration of 10^{-6} M, for a variety of Li_2SO_4 concentrations, over the 0.01–1.0 N range, while holding the ratio of Li^+ activities across the membrane constant at a value of 1.9. The dual abscissa gives the activities of both the dilute and concentrated sides. The lines are the expectations from the following "Nernstian" type theoretical expression (see Appendix), for the cases of permeant complexes for which the ratio of ion stoichiometry to the charge, n/z , equals either +1 or -0.5:

$$V_o = \frac{RT}{F} \left(\frac{n}{z} \right) \ln \frac{a_i'}{a_i} \quad (2)$$

a_i' and a_i are the ion activities on both sides of the membrane, n is the ion stoichiometry (i.e., the number of ions per complex), and z is the net charge of the complex. It is important to note that the dilution-potential data can only yield the value of n/z which will give the sign of the charge z but not its absolute magnitude; but by using the value of n measured

from conductance it is possible to calculate the *magnitude* of the charge.

The data in Fig. 7 clearly indicate that the complex is positively charged, with a value of $n/z = +1$. This already suggests that Li^+ is the translocated ion, and the argument can be made even more quantitative. Since we have already shown that $n=1$ (recall Fig. 4), the expected value of the term n/z can be $+1$ only if Li^+ is the translocated ion, whereas n/z must be -0.5 if sulphate is the translocated ion. Clearly the data points indicate that Li^+ (and not sulphate) is the sole transported ion, excluding any participation of the sulphate ion. The data for the lower end of salt concentrations correspond to the region of dominance of the 1:1 species while the data at the higher end correspond to the region of dominance of the 2:1 species (recall Fig. 3); and it is clear that the charged permeant species this complexone forms in the presence of sulphate salts of monovalent cations can be either simple 1:1 or "sandwich" 2:1 complexone/cation complexes. This is a particularly simple situation which holds in this complexone system for these sulphate salts, but is not generally true. For other types of salts, as will be shown in the second and third paper of this series³, permeation can involve cases similar to the present one where under given conditions the membrane contains only one type of permeant species but with a net charge other than $+1$, as well as cases where there is a mixture of permeating species with different charges and stoichiometries; in the latter cases identification of the net charge and stoichiometry of each of the permeant species becomes more complicated.

The Li^+ data presented in Fig. 7 are typical of dilution potentials of other monovalent cations studied in this work (under similar experimental conditions). They are also consistent with further results which we will present in the following section, where the potentials in mixtures of salts will be shown to conform to a theoretical expression suitable for permeation by monovalent cations only.

Potentials in mixtures of salts. To characterize the selectivity of this molecule further, we have measured the zero-current membrane-potentials produced in mixtures of salts, determining in detail the permeability ratios of the 2:1 complexes and in much less detail those of the 1:1 species. Several observations have directed us to emphasize the 2:1 species. From the data we have presented so far, this stoichiometry is seen to be dominant over sufficiently broad ranges of salt and complexone concentrations (e.g., sulphate salts at concentrations exceeding 0.1 N and complex-

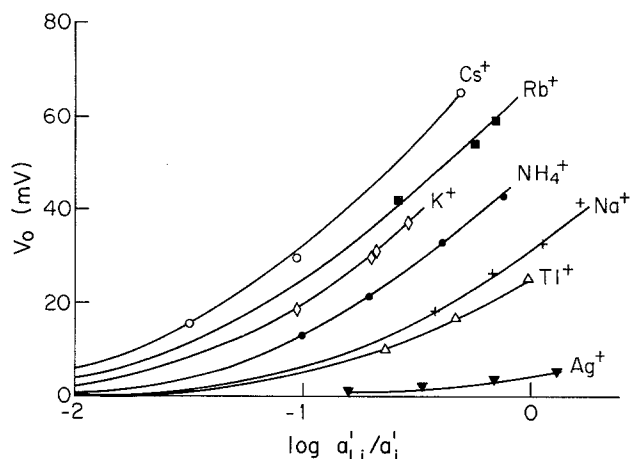


Fig. 8. Membrane zero-current potentials in salt mixtures of Li_2SO_4 and sulphate salts of the indicated cations (excepting nitrate for the Ag^+/Li^+ mixture), at symmetric aqueous complexone concentrations of 10^{-6} M. One-sided additions of Li^+ to symmetrical 1.0 N solutions of the major cation (excepting 0.1 N for Ag^+ and Ti^+), Li^+ final concentrations exceeding 0.10 N. *Ordinate:* Observed membrane potentials. *Abscissa:* Logarithm of the ratio of Li^+ activity to major cation activity. Points are experimental, curves are theoretical, drawn to Goldman-Hodgkin-Katz type equation for permeation of monovalent cations species only, for the permeability ratios given in Table 1

one concentrations greater than 10^{-7} M) that we could examine whether the permeability ratios are true constants or show dependence on salt concentration and/or on membrane potential. In contrast, the salt and complexone concentration ranges where the 1:1 complexes are the predominant species are rather narrow, being restricted to concentrations low enough to avoid the effects of transition to the 2:1 complexes but still high enough so that the electrical conductance of the bare membrane would be negligible.

Typical results for the 2:1 complexes are illustrated in Fig. 8, where the measured potentials are plotted as the data points and the solid curves are the theoretically expected potentials according the usual Goldman-Hodgkin-Katz type equation for membrane permeation by monovalent cations solely [8, 14]. The permeability ratios to which these curves were drawn are listed in the first column of Table 1. Most of the data presented in Figure 1 were measured at a major-ion⁴ concentration of 1.0 N. We have also measured membrane potentials in salt mixtures where the major ion concentrations were $10\times$ lower (not presented) and have obtained virtually identical permeability ratios, indicating that these ratios are independent of salt concentration.

Three features of the cationic selectivity of this system can be deduced from data of which those

³ See footnote 1, p. 212.

⁴ See Materials and Methods for definitions of major and minor ions.

Table 1. Comparison of permeability and conductance ratios for the 2:1 complexone/cation complexes

Ion	P_i/P_{Li}	G_i^o/G_{Li}^o
Li ⁺	1.00 (1.0)	1.00 (1.0)
Na ⁺	0.290	0.220 (0.35)
K ⁺	0.077 (0.095)	0.089
Rb ⁺	0.057	0.063
Cs ⁺	0.036	0.030
Ag ⁺	2.70	2.20 (3.0)
Tl ⁺	0.390	0.350
NH ₄ ⁺	0.134	0.110

(Numbers in parenthesis are the respective values for 1:1 complexone/cation complexes.)

presented in Fig. 8 are typical: First, selectivity among the monovalent cations does indeed exist in this system, illustrated by the clearly separated curves for each cation. Second, there is a good agreement between the observed potentials and the theoretical curves, the latter calculated assuming that only the monovalent cations participate in the membrane-permeating complex and with a stoichiometry of one cation per complex. This is consistent with, and is in support of, the “dilution-potential” data we have shown in the former section. Third, from the close agreement of the data and the theoretical curves it should be clear that the permeability ratios are voltage-independent. Such voltage-independence is characteristic of “equilibrium-domain” carriers and is not seen where kinetic limitations to complexation are present [15, 26, 30]. This is consistent with our conclusion from the conductance-voltage behavior (recall Fig. 6) that this carrier is in the “equilibrium-domain” for the species presented there and allows us to extend the conclusion to all species presented in Fig. 8.

Among the alkali cations the permeability ratios of the 2:1 species can be seen to fall into the sequence of: Li⁺ > Na⁺ > K⁺ > Rb⁺ > Cs⁺, with values quantitatively similar to the corresponding conductance ratios (for comparison, the latter are given in the second column of Table 1). The close agreement between the permeability ratios and the conductance ratios is another expected property of “equilibrium-domain” carriers.

We have included in Table 1 (in parenthesis) limited data for the selectivity of the 1:1 species. From these data it seems clear that the selectivity sequence of the 1:1 species (Ag⁺ > Li⁺ > Na⁺ > K⁺) is similar to that of the 2:1 complexes. However, the differences in the magnitudes of the observed ratios, while small, are probably significant.

Selectivity for the “Non-Noble Gas” Cations: Ag⁺, Tl⁺, NH₄⁺

Besides the alkali-metal cations which have “noble gas” type electronic shells, we have also measured membrane conductances and potentials for Ag⁺, Tl⁺ and NH₄⁺ to gain insight into the types of cationic ligands and their spatial array. Scanning Figs. 3, 4, 6 and 8, it is clear that Ag⁺, Tl⁺ and NH₄⁺ behave qualitatively like the alkali cations in regard to zero-current membrane conductance (Figs. 3 and 4), conductance-voltage behavior (Fig. 6), or zero-current membrane potentials (Fig. 8). The selectivity sequence is: Ag⁺ > Li⁺ > Tl⁺ > Na⁺ > NH₄⁺ > K⁺ > Rb⁺ > Cs⁺. Quantitatively, the conductance and permeability ratios listed in Table 1 show that these cations are more favorably selected than their like-size alkali-metal counterparts, being “supra 1a” in the terminology of Eisenman and Krasne [15]. The implications of this finding will be used in the following discussion to argue for ligand type and orientation in the complexes.

Discussion

Arguments favoring a carrier mechanism. The present molecule is one of the first of a new type of noncyclic complexones to be studied in bilayers, and, even though we have already interpreted the data in terms of a carrier mechanism, it is desirable to summarize the arguments that this complexone acts by a carrier mechanism in lipid bilayer membranes.

First, its small size and low stoichiometry (recall Figs. 1 and 3) make it unlikely that this complexone could form a channel long enough to span the bilayer. Second, the similar selectivity sequence observed in both the thin bilayers and in the thick solvent-polymer membranes [25] is expected for a carrier [12] but not for a channel [18, 19]. Third, the large difference in conductance observed for GMO *vs.* PE membranes (recall Fig. 5) due to surface dipole differences is exactly what is seen for typical carriers [4, 5, 42] and is in marked contrast to the very much smaller difference seen for the gramicidin A channel [4, 5, 45]. Fourth, the conductance and permeability ratios are independent of salt concentration over a wide range (recall Figs. 4 and 8), which is expected for “equilibrium-domain” carriers [20] but is not generally found for typical channels [18]. Fifth, the permeability ratios are in good agreement with the corresponding conductance ratios (*see* Table 1), which is expected for a carrier [8, 14] and contrast with the behavior of a channel where these ratios are the same only at the lowest salt concentration [18, 19].

These arguments favor a carrier mechanism so strongly that we felt it unnecessary to carry out further tests such as membrane freezing and melting [27].

Ligands and Structures of the Carrier/Cation Complexes

Each carrier molecule has four oxygen ligands with the potential to bind a cation (two imides and two ethers). One type of oxygen is likely to be the major (e.g., the closest) ligand for it is sterically impossible (recall Fig. 1) for a structure with a symmetrical array of these 4 ligands to be achieved in a 1:1 complex; and, even in a 2:1 complex, a symmetrical configuration in which both types of oxygens have equal participation also seems unlikely. One can argue for a major liganding role for the imide oxygens in both the 1:1 and the 2:1 complexes, at least for the smaller alkali cations, by noting the sensitivity of the selectivity sequence to the nature of the N-amide substituents. This sensitivity can be seen by comparing the selectivities of the present carrier with those of a structurally related molecule, Simon's Na^+ -selective complexone [2, 13, 28, 29], whose N-amide substituents differ in being aromatic (benzyl) instead of aliphatic. This difference between electron-donating (methyls and heptyls) and electron-withdrawing (benzyl) substituents should, by Eisenman's "field strength" theory [11, 12, 15], shift selectivity towards a lower field strength sequence in the Na^+ -complexone. This is indeed observed. The Na^+ -selective molecule has a lower field strength sequence VII ($\text{Na} > \text{K} > \text{Rb} > \text{Cs} > \text{Li}$) for both the 1:1 and the 2:1 complexes in contrast to the high field strength sequence XI ($\text{Li} > \text{Na} > \text{K} > \text{Rb} > \text{Cs}$) for both the 1:1 and the 2:1 complexes of the Li^+ -selective complexone.

Further support for the imides as principle ligands, at least for the smaller cations, comes from the "supra 1A" selectivity observed for Ag^+ with the Li^+ carrier molecule. Although the ether ligands of this molecule might be considered as possible sources of this behavior, since the carrier cyclohexyl 30-crown-10, which has solely ether ligands, does exhibit a slightly "supra 1A" behavior for Ag^+ (G. Eisenman and K.H. Kuo, *unpublished*), it seems unlikely that the extremely strong Ag^+ preference of the present molecule could be due to the ethers since the ether ligands in nonactin have been found to produce a "sub 1A" behavior for Ag^+ [15] despite the fact that they were "supra 1A" behavior for Tl^+ [15]. In contrast, amide carbonyl ligands, such as are present in gramicidin A, do indeed show "supra 1A" selectivity for Ag^+ [19, 37]. Thus it seems reasonable

to postulate that the high Ag^+ selectivity in the present carrier is also indicative of imide ligands.

A third argument, implicating the imide oxygens as important ligands for the larger cations also comes from the "supra 1A" selectivity observed for NH_4^+ in the 2:1 complexes. A "supra 1A" selectivity for NH_4^+ indicates a tetrahedral array of oxygen ligands [15]. Therefore, a tetrahedral array of ligands is likely in the 2:1 complex; and CPK models indicate that the imide oxygens are better placed than are the ethers to enter into such an array.

We can now speculate as to the detailed structure of the 2:1 and 1:1 complexes. For the structure of the 2:1 complex, we suggest that the two participating carrier molecules fold to form an inner hydrophilic cavity, within which the cation is situated by an "induced fit," while the four heptyl chains are externalized as an hydrophobic shield. Within this cavity the imide oxygens are arrayed tetrahedrally around the cation with the ethers more distant. As to the 1:1 complexes, it seems likely that the cation interacts most closely with two imide oxygens while still being partially hydrated externally and interacting indirectly through interposed water molecules with the more distant ethers. Such a partially hydrated complex would fit with the observation that in ethanol solutions and in solvent-polymer membranes (where the dielectric constant is higher than in the bilayer) the dominant stoichiometry found for this carrier is 1:1 [22, 25] in contrast to the 2:1 stoichiometry characteristic of bilayers under similar experimental conditions.

We close by noting the above structures are consistent with our initial hypothesis that noncyclic complexones are flexible enough to avoid kinetic limitations of the type which apparently prevent the cyclic perhydroantamanide [46] and cryptate molecules [32] from acting as selective carriers for the smaller cations in bilayer membranes.

Conclusions

- 1) Simon's Li^+ selective complexone retains its selectivity for Li^+ in bilayer membranes, where it acts as an "equilibrium domain" carrier of monovalent cations.
- 2) Among the alkali cations it is Li^+ -selective, but it is even more selective for Ag^+ .
- 3) Under conditions where only monovalent cations are carried, the permeant species are well defined 1:1 and 2:1 carrier/cation complexes, with the latter usually dominant.
- 4) Both stoichiometries show an inverse lyotropic selectivity sequence $\text{Li} > \text{Na} > \text{K} > \text{Rb} > \text{Cs}$, similar to

that observed for complex formation in ethanol and in thick solvent-polymer membrane electrodes.

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Appendix

Our aim here is to extend certain expressions of zero-current membrane potentials for 1:1 carrier/cation complexes [8, 14] and for higher carrier stoichiometries [38] to include higher ion stoichiometries as well.

Following the general procedures and assumptions of Eisenman et al. [14] and Ciani et al. [8], but considering membrane permeating species with various valencies and expressing the ionic fluxes by the Nernst-Planck flux equations, zero-current conditions require:

$$-\frac{d\psi^*}{dx} = \frac{RT}{F} \frac{\sum_{j=1}^n z_j U_{R_{z_j}^*} \frac{dC_{R_{z_j}^*}}{dx}}{\sum_{j=1}^n z_j^2 U_{R_{z_j}^*} C_{R_{z_j}^*}} \quad (A1)$$

Where an asterisk (*) denotes parameters in the membrane phase, $R_{z_j}^*$ is the j th membrane-permeating species and carries a net charge of z_j ; $U_{R_{z_j}^*}$ and $C_{R_{z_j}^*}$ are its mobility and concentration in the membrane phase, respectively. This equation can be reduced to simpler expressions for certain specific cases. In the following we will present and integrate a reduced form of this equation which is relevant to the study presented in this report. Other reduced forms, relevant to the permeation modes we will report on in the 2nd and 3rd papers of this series⁵, will be discussed here.

When carrier and salt conditions in the medium are such that only one type of permeant species ($R_{z_j}^* = R^z$) of a particular stoichiometry is present in the system, Eq. (A1) can be reduced to the following "Nernstian" type expression:

$$-\frac{d\psi^*}{dx} = \frac{RT}{zF} \frac{d \ln C_{R^z}^*}{dx} \quad (A2)$$

where z is the valence of this species. Equation (A2) can be easily integrated across the membrane from 0 to d , to give

$$V_o = \frac{RT}{zF} \ln \frac{a'_{R^z}}{a''_{R^z}} \quad (A3)$$

Where a'_{R^z} and a''_{R^z} are the aqueous activities of the carrier/ion complexes on the (') and (") sides of the membrane, respectively.

The explicit form of R^z for the present case of the homogeneous complexation reaction between n ions of valence z_i and m molecules of the neutral carrier S , to form the complex $I_n S_m^z$ is



where

$$z = n z_i \quad (A5)$$

The aqueous activities of the complexes can be expressed in terms of the known salt and carrier concentrations:

$$a_{I_n S_m^z} = K_{I_n S_m^z} (a_i)^n (a_S)^m \quad (A6)$$

⁵ See footnote 1, p. 212.

Under conditions of symmetrical aqueous carrier concentrations on both sides of the membrane, approximating free ion and carrier concentrations to the total carrier and salt concentrations [8, 14], and inserting Eq. (A6) into (A3), the membrane potential V_o becomes

$$V_o = \frac{RT}{zF} \ln \frac{(a_i)^n}{(a_i')^n} = \frac{RT}{F} \left(\frac{n}{z} \right) \ln \frac{a_i}{a_i'} \quad (A7)$$

Since this potential is measured under a concentration gradient of a single salt, it is commonly referred to as the "dilution-potential."

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