Biochimica et Biophysica Acta, 649 (1981) 441–448
Elsevier/North-Holland Biomedical Press

BBA 79485

A STUDY OF Li^+-SELECTIVE PERMEATION THROUGH LIPID BILAYER MEMBRANES MEDIATED BY A NEW IONOPHORE (AS701)

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(Received May 19th, 1981)

Key words. Li^+-selective transport, Ionophore, Lipid bilayer

The neutral, noncyclic, imide and ether containing ionophore AS701, has been developed as Li^+-selective molecule, to be used potentially as an aid in the Li^+-therapy of manic-depressive illness. The present report is a characterization of this molecule in neutral lipid bilayer membranes. This ionophore was found to render the bilayers Li^+-selective, acting as a selective carrier of monovalent cations. In addition, this molecule was found to be capable of acting as a selective carrier of monovalent anions. For both types of ions, the rate-limitting step in the process of permeation was found to be the diffusion of the carrier-ion complex through the membrane. The membrane-permeating species were found to be 2 : 1 carrier-ion complexes, carrying either a monovalent cation or a monovalent anion. The selectivity sequence among the ions studied being: $Li^+(1) > ClO_4^-(0.7) > Na^+(0.07) > K^+(0.016) > Rb^+(0.0095) > Cs^+(0.0083) > Cl^-(0.001)$. Mg^{2+} and SO_4^{2-} were found to be impermeant (under present experimental conditions). This sequence shows that the AS701 molecule has low selectivity for ions present in biological media, among those studied (i.e. Na^+, K^+, Mg^{2+}, Cl^- and SO_4^{2-}). This indicates that these ions will not interfere in the Li^+ permeability induced by this carrier in vivo, and that the carrier will not interfere in the normal transport processes of these ions.

Introduction

The lithium ion is an effective therapeutic agent in the treatment of manic-depressive illness [1]. To be effective, fairly large daily doses of lithium, administered as a lithium salt, are required [2], often leading to undesirable side-effects [3,4]. It would, therefore, be of interest to find a means of reducing the daily doses, while maintaining the therapeutically-effective level and rate of uptake of lithium into various body tissues, especially the brain [5].

One possible way of achieving this could be by the use of ionophores selective to lithium. To this end, Shanzer and his colleagues (Shanzer, A., Samuel, D. and Korenfeld, R., in preparation) have developed a neutral, noncyclic molecule (Fig. 1) code-named AS701. They have found this molecule to be capable of extracting Li^+ from aqueous to bulk organic phases. Furthermore, when Li^+ complexed to the ionophore was injected into rats (interventriculary, interperitonally or into the tail vein), Li^+ uptake into several tissues, including brain, was found to increase up to a factor of 4, compared to lithium as a salt.

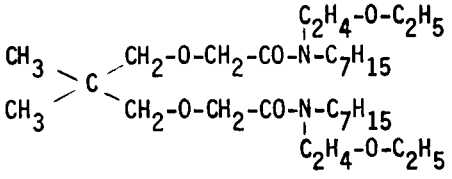

Fig. 1. Structure formula of Shanzer's Li^+-selective ionophore AS701. (Simon's Li^+-selective ionophore ETH149, has methyl groups instead of the diethyl ethers as *N*-amide substituents).

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Although this molecule has the desired potential, it is of considerable importance to characterize the behavior of this ionophore and related molecules in the media where it is expected to act, i.e. in biological membranes.

In the present communication we report studies of the activity of this molecule in planar lipid bilayer membranes, which is a first step in this direction. Such membranes are well established as model systems in which molecular aspects of ionophore-mediated selective ionic permeation can be studied, under clearly-defined and well-controlled conditions [6,7]. We have found these model membranes to be suitable for studying several issues, pertinent to the activity of AS701 ionophore in biological systems.

(1) The ionic selectivity of this ionophore system. Does this molecule act as an Li^+-selective ionophore in ultrathin lipid bilayer membranes? To be therapeutically-effective, this molecule should not only have high selectivity for Li^+, but should, also, have poor selectivity for all other ions present in the biological media. This includes not only the monovalent and divalent cations, i.e. H^+, K^+, Na^+, Ca^{2+} and Mg^{2+}, but also monovalent anions such as Cl^-, I^- and various organic anions. Anion carrying ability has been found by Margalit and Eisenman [8–10] for a structurally-related molecule. Simon's Li^+-selective carrier ETH149 [11] (see legend to Fig. 1 for details of structure). Therefore, the possibility of reactivity towards anions was explored with the present AS701 ionophore.

(2) The mechanism by which the AS701 ionophore mediates selective ionic permeation of membranes. Does this molecule act in the membrane as a carrier or as a channel-former? What and where are the rate-limiting steps in the process of permeation? How many types of ion-ionophore permeant species are formed and what are the ion and ionophore stoichiometries of each permeant species?

(3) The effects of the membrane lipid composition on the two issues discussed above.

In this report we will present mechanistic and selectivity studies, establishing that this ionophore acts in lipid bilayers as an Li^+-selective carrier.

Materials and Methods

The ionophore used in this study was synthesized by Dr. A. Shanzer. Glyceryl-monooleate (monoolein) was purchased from Sigma Chemical Co., and bacterial phosphatidyl ethanolamine (PE) from Supelco. Membranes were formed on the aperture (usually 1 mm diameter) of a teflon chamber, from monoolein/decane or PE/decane solutions (25 mg/ml). Steady-state electrical properties of the bilayer membranes were measured, following previously described procedures [13,14].

Results

I. Membrane conductance

IA. Membrane zero-current conductance. Dependence on ionophore concentration. We have found zero-current conductance of monoolein membranes to increase in a regular manner with increasing ionophore concentration. Typical results are illustrated in Fig. 2, for 1.0 N chloride salts of the cations indicated.

Several features of the dependence of membrane conductance on ionophore concentration are immedi-

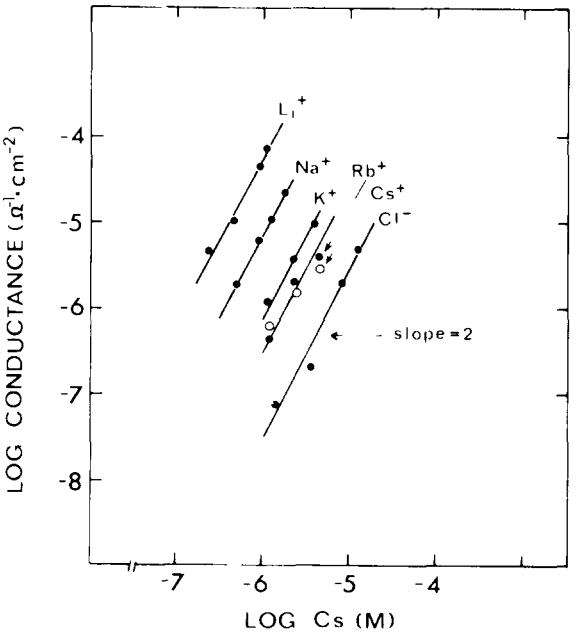

Fig 2 Dependence of the zero-current conductance of monoolein membranes on ionophore concentration, in presence of 1.0 N chloride salts of the indicated alkali cations and Mg^{2+} Ordinate. Logarithm of the membrane conductance. Abscissa Logarithm of aqueous ionophore concentration. Points are experimental, lines drawn to slopes of 2.

ately obvious: First, the levels to which the membrane conductances increase depend on the salt species present. The order of enhancing conductance among the alkali-cation salts being: LiCl > NaCl > KCl > RbCl > CsCl. Since these salts share a common anion, this indicates there is some selectivity among the monovalent cations, with Li^+ favored over the other cations. Secondly, in presence of magnesium chloride, the membrane conductance increases in a pattern similar to that observed in presence of the alkali cation salts, although to lower conductance levels (an order of magnitude lower than in presence of RbCl or CsCl, three orders of magnitude lower than in presence of LiCl).

Presumably, in presence of the alkali cation salts, the cation is complexed and transported, at least with the salt species showing the higher conductance levels. In presence of the magnesium chloride salt, one would expect the cation also, although divalent, to be the transported ion. However, based on our previous experience [8–10] with Simon's structurally-related Li^+-selective carrier (ETH 149) [11] we expect the chloride to be the ion complexed and transported across the membrane. Evidence for the sign and magnitude of the charge carried by the permeant complexes, which can be obtained from zero-current membrane potentials, will be introduced in a following section.

A third feature of the dependence of membrane zero-current conductance on ionophore concentration is illustrated by the regular manner of the conductance increase. All data points fall onto lines with a slope of 2, which implies an ionophore stoichiometry of two for all ion species tested [14]. We have found this ionophore stoichiometry to be the only one detected, over the concentration ranges studied (0.01–1.0 M salt and 10^{-7}–10^{-5} M ionophore).

IB. Membrane zero-current conductance. Dependence on salt concentration.

We have found the zero-current conductance of monoolein membranes to increase linearly with increasing salt concentration, for all salt species studied. Typical results for selected species: LiCl, NaCl and $Mg(ClO_4)_2$ are illustrated in Fig. 3. Clearly all data points fall onto slopes of 1, indicating an ion stoichiometry of 1. It is also seen that the conductances induced in the membrane in presence of $Mg(ClO_4)_2$ are on a level similar to those induced in

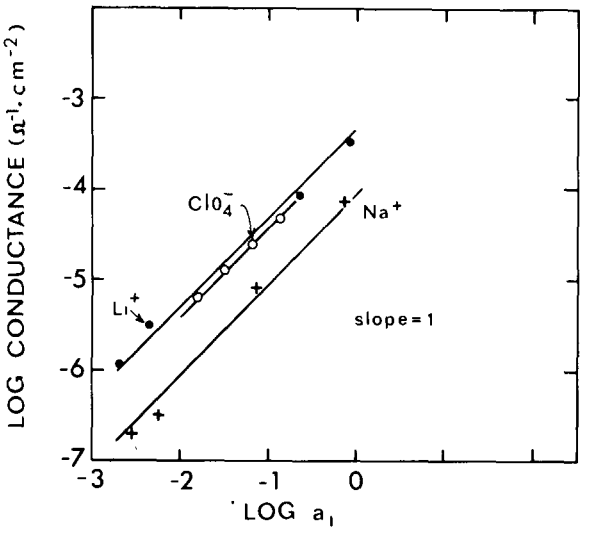

Fig. 3 Dependence of zero-current conductance of monoolein membranes on salt concentration, for Li_2SO_4, Na_2SO_4 and $Mg(ClO_4)_2$, at constant ionophore concentration of $3 \cdot 10^{-6}$ M. Ordinate: Logarithm of membrane conductance. Abscissa: Logarithm of the activity of the monovalent ion (for each case). Points are experimental, lines drawn to slopes of 1.

the membrane in presence of LiCl, under similar ionophore and salt concentrations. This is different from the chloride salt of magnesium, where the conductances are three orders of magnitude lower than those induced in presence of LiCl (recall Fig. 2). These different levels of activity of two magnesium salts are an indication of some participation (at least) of the monovalent anions in the membrane-permeating species.

The conductance data on this and the previous section (IA) indicate that the permeant species is a 2 : 1 ionophore-ion complex.

IC. Zero-current membrane conductance. Dependence on membrane lipid composition. A detailed study of the effects of the membrane lipid composition on the mechanism and selectivity of the present ionophore will be reported separately. Some preliminary data will, however, be reported here since, as will be discussed below, effects of lipid composition provide a criterion for distinguishing carriers from channel-formers.

It has been reported that typical carriers are highly sensitive to the dipole-potential difference between

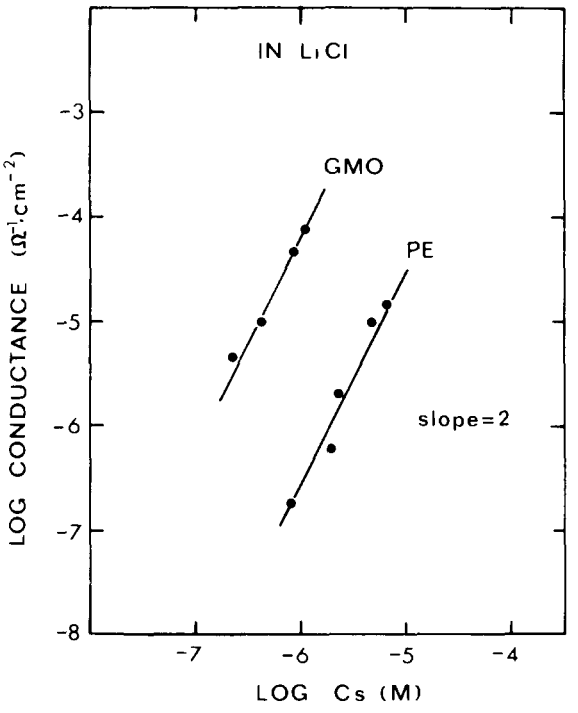

Fig 4. Increase of membrane zero-current conductance, with increasing ionophore concentration, in presence of 1.0 N LiCl, for monoolein (GMO) and PE membranes, showing the effects of the membrane lipid-composition on the levels of membrane conductance. Ordinate. Logarithm of membrane conductance. Abscissa Logarithm of aqueous ionophore concentration. Points are experimental, lines drawn to slopes of 2

monoolein and PE [15–18]. For example, in presence of nonactin-K^+ or trinactin-K^+ complexes, a three orders of magnitude drop in the magnitude of the zero-current conductance has been observed upon replacing monoolein by PE [15,16]. A response similar in magnitude and direction has been observed for the Li^+ complex of Simon's molecule ETH149 [19]. In contrast, for a typical channel such as gramicidin A, a rather small change in the magnitude of the zero-current conductance is observed upon replacing monoolein by PE, as the membrane lipid [20].

For the present AS701 ionophore, typical results of the effects of replacing monoolein by PE, on the membrane zero-current conductance, in presence of LiCl, are illustrated in Fig. 4. Clearly, the substantial decrease in membrane conductance on going from monoolein to PE membranes, is of the order typically found for carriers, rather than for channels. Additional experimental observations indicating carrier properties for this molecule will be considered in the discussion.

ID. Conductance-voltage behavior. Typical results of this type of experiment, for several salts, are illustrated in Fig. 5. The data points are the observed membrane conductance G, normalized to G_0, the membrane conductance at the limit of zero-current (G/G_0). The increase in membrane conductance with increasing applied potential is seen to be hyperbolic, in the presence of each of the salts tested. The conductances are independent of salt concentration over the 0.01–1.0 N range, and independent of sweep frequency over the 0.01–0.20 Hz range employed.

For carrier systems where the rate-limiting step in

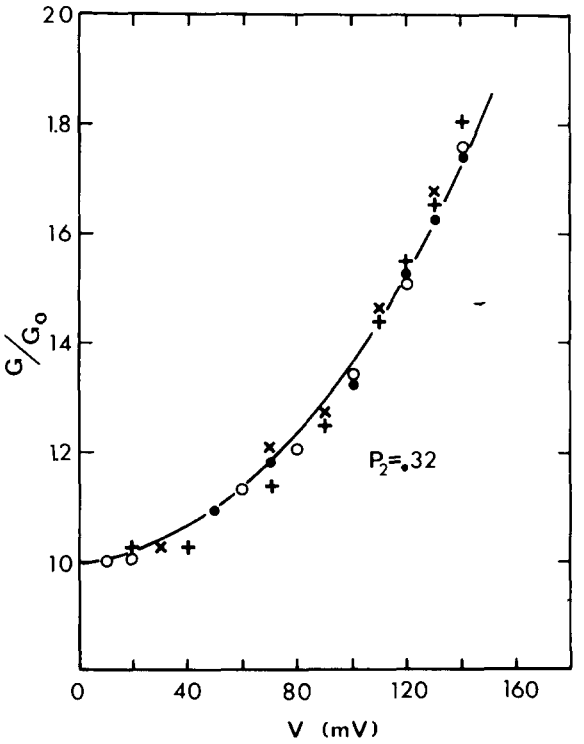

Fig 5. Conductance-voltage relationships in monoolein membranes for 1.0 N LiCl (×), NaCl (○), KCl (+) and $MgCl_2$ (●). Ordinate· The observed membrane conductance, G, normalized to G_0, the membrane conductance at the limit of zero-current. Abscissa. The applied potentials. Points are experimental, solid curve is the theoretical expectation for an 'equilibrium-domain' carrier, drawn to Eqn. 1 in the text, for $P_2 = 0.32$.

the process of permeation is the translocation of the ion-carrier complex across the membrane rather than the interfacial complexation reactions, (i.e., 'equilibrium-domain' systems [14]), the conductance-voltage relationship is theoretically expected to be hyperbolic [7,12,13,21]. Obeying the following expresson [13]:

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

where ϕ is the applied potential in units of F/RT, and P_2 is the parameter indicating the 'barrier-width' [12]. The solid curve in Fig. 5 is drawn to Eqn. 1 above with a value of 0.32 for P_2. The good agreement observed between the measured and theoretically expected conductances indicates that for the present system the rate-limiting step is indeed located within the membrane and that there are no kinetic limitations on the interfacial ion-ionophore association and dissociation reactions [13].

II. Membrane potentials

IIA. Assessing the sign and magnitude of a permeant ion. If, under given conditions, only one type of permeant species is present in the system, membrane zero-current potentials generated under a concentration gradient of a single salt (hence the term membrane 'dilution potentials'), should generally obey the following expression [9,19]:

$$V_0 = \frac{RT}{F}\frac{n}{z}\ln\frac{a_i'}{a_i''} \qquad (2)$$

where (′) and (″) represent the aqueous phases on each side of the membrane, a_i' and a_i'' the activities of the permeant ion in the aqueous phases, respectively, n the total ion stoichiometry, and z the net charge of the permeant species. A plot of the observed potentials vs. the logarithm of the ratio of the activities of the permeant ion on both sides of the membrane, should yield the term (n/z) which will have the sign of the charge. If, as in the present case, n, the total ion stoichiometry has been determined independently, the absolute magnitude of z, the net charge, can also be obtained.

Typical results are illustrated in Fig. 6, for two species, Li^+ and Cl^-. For Li^+, we have measured dilution potentials of Li_2SO_4, choosing this salt rather

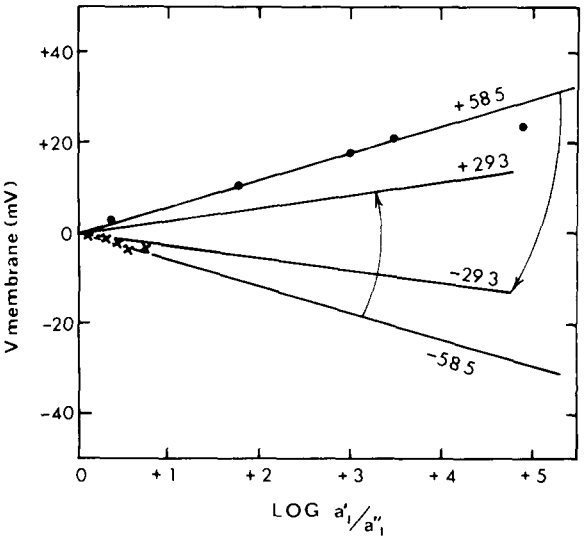

Fig. 6. Zero-current 'dilution potentials' of monoolein membranes for Li_2SO_4 (•) and $MgCl_2$ (×). Ordinate. Observed membrane potentials. Abscissa: Logarithm of the ratio of activities of the monovalent ion (in each case), on the concentrated (′) and the dilute (″) sides of the membrane. Points are experimental, lines are theoretical expectations, drawn to Eqn. 2 in the text, the numbers on each line giving the respective slopes. Arrows indicate the slope that the data should have followed if the divalent counterion were the permeant ion in each case (see text for further details).

than LiCl, since there were indications from zero-current conductances that sulphate is an impermeant ion in the present system. The observed potentials are plotted in Fig. 6 together with the expectations for Li^+ or for the counterion sulphate being the transported ion. The theoretical plots were drawn to Eqn. 2, calculating the expected slopes as follows: Recalling that the ion stoichiometry in these permeant complexes has been determined from conductance data to be 1, if Li^+ ($z = +1$) is the transported ion, the (n/z) term will have the value of +1. If the sulphate ($z = -2$) is the transported ion, the (n/z) term will have the value of −0.5. This will result (for 2.3 $RT/F = 58.5$) in theoretically expected slopes of +58.5 and −29.3, for Li^+ and SO_4^{2-}, respectively. The observed potentials fit the theoretical curve for Li^+, unambiguously. Similar results have been obtained for the other alkali cations. We can therefore deduce that this ionophore forms 2 : 1 ionophore-cation permeant species, carrying a net-charge of +1, and

exclude the participation of the sulphate anion in the permeant complex.

In the case of Cl^-, we have also plotted (in Fig. 6) the membrane 'dilution potentials' generated under gradients of $MgCl_2$, together with the theoretically expected slopes for the two ions present. These, following the procedure described above for the case of lithium, being +29.3 for Mg^{2+} and −58.5 for Cl^-. The data points clearly fit with chloride being the permeant ion, and exclude the participation of magnesium in the permeant complex. This finding implies that the AS701 ionophore has anion-carrying ability, forming permeant 2 : 1 carrier-anion complexes. Further support for the fact that the monovalent cations and anions are the permeant ions in this system, while the two divalent ions magnesium and sulphate are impermeant, comes from data of membrane zero-current potentials measured in salt mixtures, presented in the following section.

IIB. Membrane zero-current potentials in salt mixtures. Two types of experiments were carried out, typical results of which are illustrated in the upper and lower sections of Fig. 7. In the first type of experiment the concentrations of the solutions on each side of the membrane were equal for a given alkali cation, termed the major ion, Cl^- being the counter ion. Membrane potentials were generated by adding aliquots of LiCl to one side only, Li^+ termed the minor ion. In a variation of this type of experiment, for estimating Cl^- permeability, the two sides of the membrane contained equal concentrations of $MgCl_2$, Cl^- being the major ion. The observed potentials are plotted in the upper part of Fig. 7, vs. the logarithm of the ratio of the minor to the major ion activities. The solid curves are the theoretically expected potentials drawn to the following Goldman-Hodjkin-Katz type equation, for the permeability ratios listed in Table I:

$$V_0 = \frac{RT}{F} \ln \frac{a'_{Li} + (P_i/P_{Li})\, a'_i + (P_{Cl}/P_{Li})\, a''_{Cl}}{(P_i/P_{Li})\, a''_i + (P_{Cl}/P_{Li})\, a'_{Cl}} \qquad (3)$$

where (′) and (″) represent the aqueous phases on both sides of the membrane, a'_{Li} is the lithium activity on the (′) side of the membrane, a'_i, a''_i, a'_{Cl} and a''_{Cl} are the major ion and chloride activities on the (′) and (″) sides of the membrane, respectively, (P_i/P_{Li}) and (P_{Cl}/P_{Li}) are the permeability ratios of

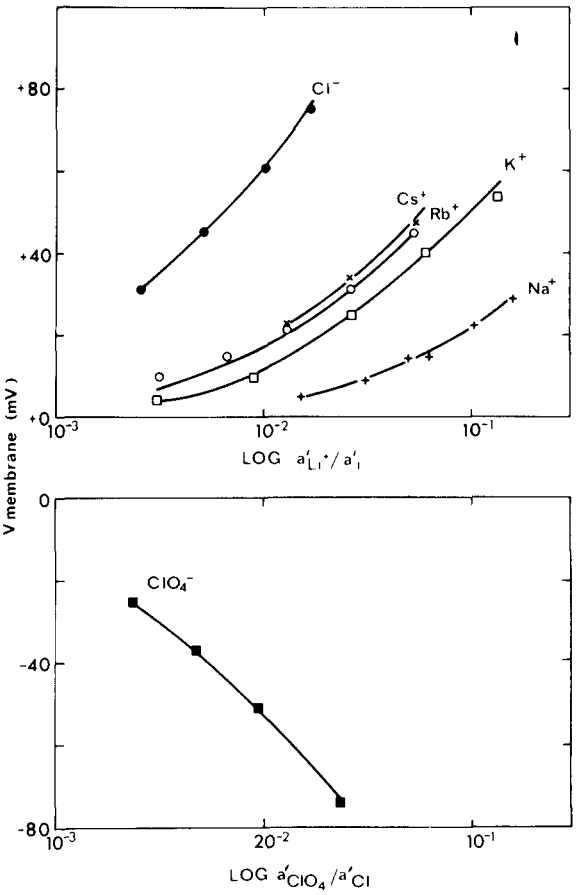

Fig. 7. Zero-current potentials of monoolein membranes in salt mixtures. Ordinate Observed membrane potentials. Abscissa. Logarithm of the ratio of activities of the minor to the major ion (for definitions of major and minor ion see text) Upper section One-sided additions of LiCl to 1.0 N chloride salts of the indicated alkali cations. Points are experimental, curves are theoretical expectations, drawn to Eqn. 3 in the text, for the permeability ratios listed in Table I Lower section· One-sided additions of $Mg(ClO_4)_2$ to 1.0 N $MgCl_2$. Points are experimental, curve is theoretical expectation, drawn to Eqn. 4 in the text, with a value of 700 for the permeability ratio of ClO_4^- to Cl^-

the major ion to Li^+ and chloride to Li^+, respectively.

It is clear from the upper section of Fig. 7, that for all ion species studied there is good agreement beween the experimentally-observed and the theoretically-expected potentials. We have found similar good agreement and similar values of permeability ratios for lower concentrations of major-ion salts (data not shown), indicating these are truely concen-

TABLE I

PERMEABILITY AND CONDUCTANCE RATIOS OF THE 2 : 1 AS701-ION COMPLEXES

Ion	P_I/P_{Li}	G_I^0/G_{Li}^0
Li^+	1	1
Na^+	0 07	0 112
K^+	0.016	0.018
Rb^+	0.0095	0.005
Cs^+	0.0083	0.005
ClO_4^-	0.74	0.64
Cl^-	0.001	0.0005

tration-independent permeability ratios. The adherence of the data points to the theoretical curve, even at the limit of higher membrane potentials is as expected for 'equilibrium-domain' carriers [14], and in agreement with the conductance-voltage behavior we have observed for this system (see Fig. 5).

In the second type of experiment, illustrated in the low section of Fig. 7, the aqueous solutions on both sides of the membrane held equal concentrations of $MgCl_2$, and membrane potentials here generated by adding aliquots of $Mg(ClO_4)_2$ to one side only. If only anions are permeant, the membrane potentials should follow the expression given below.

$$V_0 = -\frac{RT}{F} \ln \frac{a'_{Cl} + (P_{ClO_4}/P_{Cl})\, a'_{ClO_4}}{a''_{Cl}} \qquad (4)$$

The data points are seen to be in good agreement with the solid curve, drawn to Eqn. 4, with a value of 700 for the permeability ratio of ClO_4^- to Cl^-.

Discussion

I. Selectivity among cations

The zero-current conductance data of the type illustrated in Fig. 2 already indicate selectivity among the alkali cations, in this system. The stoichiometric analysis has established the permeant cationic species to be 2 : 1 carrier-monovalent cation complexes. Furthermore, the studies on anion-carrying abilities, to be discussed below, have shown Cl^- to have poor permeability in this system. The alkali cation complex can, therefore, be assumed to be the dominant permeant species present in alkali chlorides.

To determine the selectivity among the monovalent cations we have measured membrane zero-current potentials in salt mixtures. The permeability ratios obtained from this type of data, listed in Table I, correspond to the sequence: $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. The conductance data follow a similar selectivity sequence, with reasonably good agreement between corresponding permeability and conductance ratios, as can be clearly seen in Table I. This selectivity sequence is similar to the sequence observed for Simon's carrier (ETH 149) but differs in the magnitudes of permeability ratios [8,9,19].

II. Anion-carrying abilities

To find whether the present carrier has anion-carrying abilities, we have tested two anions: chloride, an anion present in biological media, frequently used as the counter ion in studies with alkali cation salts. Perchlorate, found to be highly permeable in the Simon carrier system [8–10] and used as the Li^+ counterion in some of the experiments performed in vivo. We have found the anionic permeant species to be 2 : 1 carrier-anion complex.

The selectivities of the ionophore among these anions and Li^+ are listed in Table I, where the corresponding permeability and conductance ratios of the anions to Li^+ are seen to be in agreement. Chloride is seen, as already noted, to have poor selectivity in this system. In contrast, perchlorate is almost as good as Li^+. The combined selectivity sequence for the ions studied is, therefore $Li^+ > ClO_4^- > Na^+ > K^+ > Rb^+ > Cs^+ > Cl^-$.

III. Findings of the present study which have physiological relevance

Selectivity. We have found that the AS701 ionophore renders lipid bilayer membranes selectively permeable to Li^+. Furthermore, the ionophore is considerably less selective to those physiological ions tested so far (Na^+, K^+, Mg^{2+}, I^- and SO_4^{2-}), than to Li^+. Additional ions regularly present in biological media are currently tested.

Mechanism. The low ionophore stoichiometry of 2, observed for the permeant complexes of AS701, together with the small size of this molecule, suggest a carrier rather than a channel mechanism. It seems unlikely that two ionophore molecules would suffice to form a channel long enough to span the bilayer.

This is further supported by several findings of this study which indicate this system has characteristics expected of carriers, operating in the 'equilibrium-domain': A substantial decrease in the magnitude of membrane conductance, induced by the AS701-cation complexes, with increase in the membrane surface dipole-potential [7,15–20]. Permeability and conductance ratios which are, each, salt-concentration independent, over a wide enough range [12–14, 19]. Good agreement between corresponding permeability and conductance ratios [14,19] and hyperbolic conductance-voltage behavior [7,12,13,19]. These findings suggest that the AS701 ionophore, in mediating selective permeation of lithium through biological membranes, will act in a carrier mechanism, with the rate-limiting step in the process being the translocation of the carrier-ion complex across the membrane.

We suggest the properties of the Li^+-selective molecule, characterized in the present study, have sufficient physiological relevance to encourage the study of this system, at the molecular level as well as in the in vivo systems studied by Shanzer and his colleagues.

Conclusions

We conclude that:

(1) The ionophore AS701 can act in lipid bilayer membranes as a selective carrier of monovalent cations, operating in the 'equilibrium-domain', forming 2 : 1 carrier-cation membrane-permeating complexes.

(2) This molecule can also act in lipid bilayer membranes as an 'equilibrium-domain' carrier of monovalent anions, forming 2 : 1 carrier-anion membrane-permeating complexes.

(3) The selectivity among the ions studied corresponds to the sequence: $Li^+(1) > ClO_4^- > (0.74) > Na^+(0.07) > K^+(0.016) > Rb^+(0.0095) > Cs^+(0.0083) > Cl^-(0.001)$. Both Mg^{2+} and SO_4^{2-} were found to be impermeant ions under the conditions of the present study.

(4) Li^+ is sufficiently more selective than those ions present in biological media, which we have tested so far: $Li^+(1) > Na^+(0.07) > K^+(0.16) > Cl^-(0.001) \gg Mg^{2+}$; SO_4^{2-}. This reduces considerably the possible interference of these ions in the Li^+-permeability this carrier can induce in vivo and the interference of AS701 in the natural transport processes of these ions.

Acknowledgement

We thank David Samuel for stimulating discussions throughout this work and for his valuable comments on the manuscript.

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