Biochimica et Biophysica Acta, 448, (1976) 284–289
© Elsevier/North-Holland Biomedical Press

BBA 77452

THE TRANSPORT OF CALCIUM AND SODIUM IONS ACROSS BILAYERS INDUCED BY NEUTRAL SYNTHETIC LIGANDS USED IN SPECIFIC ELECTRODES

GENEVIEVE AMBLARD and CLAUDE GAVACH

Groupe de Recherche de Physico Chimie des Interfaces, Route de Mende, BP 5051, Montpellier Cedex (France)

(Received March 23rd, 1976)

SUMMARY

Neutral synthetic ligands of calcium and sodium which enter into the liquid membrane composition of the selective electrodes of these ions have been incorporated within the bilayer.

The membrane conductance measured shows that each of these two ligands behave as ion carriers for calcium or sodium ions. The selectivities with respect to the other alcaline or alcaline earth ions are similar to those observed by potentiometric measurements with thick liquid membrane selective electrodes.

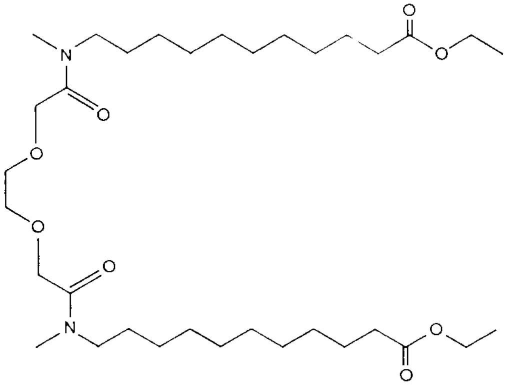
INTRODUCTION

The permeability of bimolecular lipid membrane to inorganic cations induced by neutral ionophores results from the formation of selective complexes between the carrier and the small ion. The transport of K^+ mediated by valinomycin has been the subject of numerous experimental and theoretical studies. In the field of analytical chemistry, the property of forming liposoluble complexes with ions shown by certain neutral antibiotics has been applied to the preparation of thick liquid membrane specific electrodes. Valinomycin thus yields an analytically useful sensor for K^+ [1, 2] with a discriminating factor of about 5000 with respect to Na^+, while the antibiotics of the nonactin group have been used for the determination of NH_4^+ [3] with a good selectivity factor with respect to Na^+ and K^+.

As a result of these successful attempts, Pretsch and coworkers [4] have systematically undertaken the synthesis of neutral substances capable of forming selective complexes with given inorganic ions for inclusion in the liquid membranes of ion selective electrodes. Thus specific sensors for Na^+, Ca^{2+}, and Ba^{2+} [4] can actually be prepared using a different neutral ligand in each case.

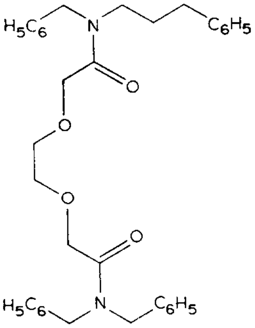
Certain results indicate that a mediated transport of Ca^{2+} across lipid black films or liposomes can be brought about by means of the X 537 and A 23187 ionophores which are charged substances [5, 6]. Again monensine [7], also a charged antibiotic, gives rise to a selective transport of Na^+ across bulk liquid membranes.

It seemed, therefore, interesting to investigate whether the class of neutral complexing molecules which shows ion selectivity at zero current when incorporated within the bulk membranes, could also behave as an ion carrier and induce a certain conductance across a bilayer lipid membrane. As cations, Ca^{2+} and Na^+, were chosen for this study because of their importance, together with K^+, in biological processes. The formulae for the calcium and sodium specific ion ligands L_I and L_{II} used in this study are respectively:



L_I

Scheme I



L_{II}

Scheme II

EXPERIMENTAL

The sample of calcium ligand L_I used was kindly given by Professor Simon, while the sodium ligand L_{II} was prepared by his method of synthesis from triethylene glycol and dibenzyl amine [8]. The product obtained was finally purified by chromatography using a silica column.

Bilayer membranes were prepared using a 7 mM glyceryl monooleate solution in *n*-decane to which were added small amounts (always less than 1 %, by volume) of an L_I ethanolic solution.

It was thus possible to attain concentrations of L_I in the lipid hydrocarbon mixture as high as 10^{-2} M.

The sodium ligand not being soluble in *n*-decane and only slightly soluble in water, very small volumes (less than 2 ‰) of an ethanolic solution of L_{II} were added to the aqueous solution whose concentration never exceeded 10^{-6} M, above which precipitation was observed.

The lipid bilayers were prepared by the bubble technique [9], in which a very small volume of the lipid-hydrocarbon solution was deposited on a hole of 1.2 mm diameter in the wall of a "teflon" cup.

The two aqueous solutions on both sides of the bilayer were identical and contained a single electrolyte, the anion of which was the chloride ion. Two identical Ag-AgCl electrodes, 1.5 cm^2, were immersed in each solution. The limiting conductance at zero voltage and zero time was measured by means of the charge pulse technique previously described [10].

RESULTS AND DISCUSSION

Figs 1 and 2 represent the logarithm of the limiting conductance λ^{00} versus the logarithm of calcium, magnesium and barium chloride concentrations in the aqueous phase when the lipid bilayer contains the L_I ligand; the concentration of ligand L_I in the membrane forming solution is $1.86 \cdot 10^{-3}$ M for Fig. 1 and $1.2 \cdot 10^{-3}$ M for Fig. 2. In both cases the highest membrane conductances are obtained with Ca^{2+}.

For a ligand concentration $1.2 \cdot 10^{-3}$ M in the membrane forming solution the bilayer conductance for 0.1 M aqueous solutions of Na^+, K^+ and Li^+ have been measured. They are respectively 2.10^{-7}, 6.10^{-8} and 2.10^{-8} $\Omega^{-1} \cdot cm^{-2}$.

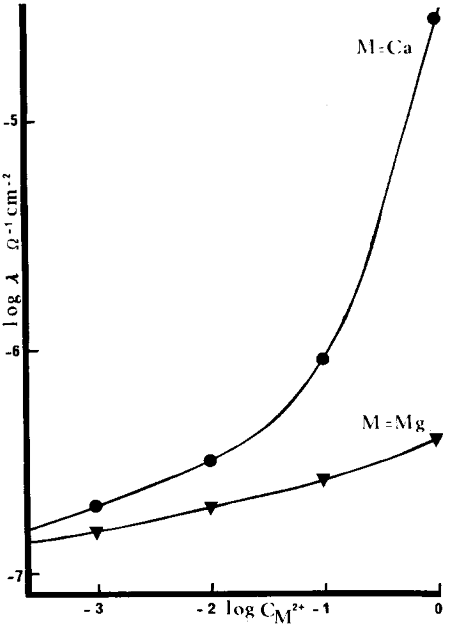


Fig. 1. Variation of log λ^{00} with the logarithm of the concentration of Ca^{2+} and Mg^{2+} in the aqueous solution. Concentration of L_I in the glyceryl monooleate-decane solution, $1.86 \cdot 10^{-3}$ M.

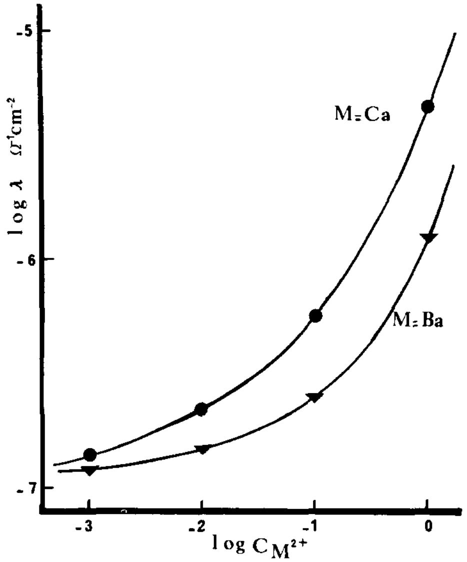


Fig. 2. Variation of log λ^{00} with the logarithm of the concentration of Ca^{2+} and Ba^{2+}. Concentration of L_I in the glyceryl monooleate-decane solution, $1.2 \cdot 10^{-3}$ M.

The selectivity factor K_{ji}^{Pot} related to the interference effect of a foreign ion (j) affecting the response of an ion selective electrode specific of the ion (i) is defined by the classical Nicholsky equation:

$$E = \frac{RT}{Z_i F} \text{Log} \left\{a_i + K_{ji}^{Pot} a_j^{Z_j/Z_i}\right\}$$

Ammann et al. have determined the values of K_{ji}^{Pot} for a thick liquid membrane calcium electrode including the L_I ligand [11].

For the five cations which have been studied here these authors have found the following sequence for $K_{jCa^{2+}}^{Pot}$, the selectivity factor:

$K^+ \simeq Ba^{2+} > Na^+ > Li^+ > Mg^{2+}$

One notices that this sequence is somewhat different from those obtained for the bilayer conductances for the same concentrations of alkaline or alkaline earth chloride in the aqueous phase.

The curve in Fig. 3 shows the variation of λ^{00} with the concentration of L_I ligand in the lipid-decane solution when the aqueous phase is a 1 M solution of $CaCl_2$. The linear part of this curve has a slope of approximately 3.7. If one assumes, as Eisenman does [12], that the membrane conductance is proportional to the number of ligand-ion complexes, inside the bilayer lipid membrane and that in this layer the equilibrium complex formation obeys the same law as in a thick membrane, then the value of the slope suggests that the complex between Ca^{2+} and L_I must be composed of an ion of the metal associated with more than one molecule of L_I. This observation is also similar to the one made in the case of another class of neutral synthetic complexing ligands [12].

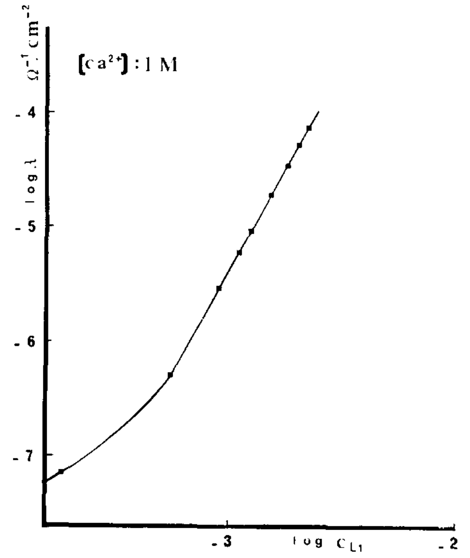


Fig. 3. Variation of the conductivity with the concentration of L_I in the membrane. Aqueous solution, 1 M $CaCl_2$.

Fig. 4 represents the variation of the monoglyceryl oleate-decane bilayer conductances when the aqueous solutions contain NaCl at different concentrations and the L_{II} ligand at a fixed concentration of 2.10^{-7} M. Although the exact concentration of L_{II} inside the bilayer is not known, we may expect that it is only slightly affected by the NaCl concentration in the aqueous solution. The measured conductances increase with the NaCl concentration but the values are lower than those obtained with the L_I ligand added to the membrane and Ca^{2+} in the aqueous phase; in any case one can draw no conclusion from this observation because the L_{II} concentration inside the bilayer is presumably smaller than those of L_I.

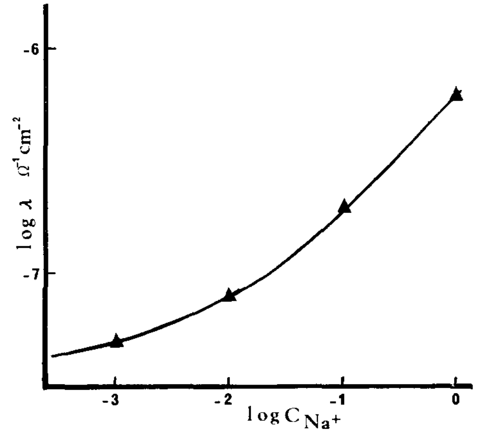


Fig. 4. Variation of log λ^{00} with the logarithm of the concentration of Na^+ in the aqueous solution. Concentration of L_{II} in this phase, $2 \cdot 10^{-7}$ M.

With the same amount of ligand L_{II} introduced in the aqueous phase, the measured conductances for other alkaline chlorides 10^{-1} M in the aqueous phase (KCl, LiCl, CsCl) have the same value of $3.10^{-7}\ \Omega^{-1}\cdot cm^{-2}$, while with alkaline earth cations at the same concentration the bilayer conductance values are still smaller and respectively: $10^{-8}\ \Omega^{-1}\cdot cm^{-2}$ and $3.10^{-9}\ \Omega^{-1}\cdot cm^{-2}$ for Ca^{2+} and Mg^{2+}. Ligand L_{II} has also been incorporated in a bulk membrane by Ammann et al. [13] in the preparation of a sodium selective electrode and they found the following sequence for the selectivity factors $K_{jNa^+}^{Pot}$ determined as has been described above for the Ca^{2+} selective membrane electrode:

$$Ca^{2+} \simeq K^+ > Li^+ > Cs^+ > Mg^{2+}$$

In this case too, one notices a difference between a thick liquid membrane and a bilayer both containing the ligand L_{II}. Nevertheless, the principal results obtained show that the highest bilayer conductances values are reached with Ca^{2+} and Na^+ when the membrane contains the ligand which is responsible for the properties of selective electrode studied by Ammann and coworkers [11, 13].

At this stage, it would be somewhat difficult to draw additional conclusions from these observations because of the difference in the nature of the factors bearing on these two membrane systems.

The potentiometric selectivity constant is affected not only by the formation constants of the complex in the organic solvent and the standard free enthalpy of transfer between the water and the solvent but also by the diffusion coefficient of the anion which is present inside the bulk membrane. On the contrary for bilayer lipid membranes it is classically admitted that the anions are excluded from the membrane and that complex formation takes place only at the interface.

ACKNOWLEDGEMENTS

We are grateful to Professor Simon (Swiss Federal Institute of Technology, Zurich) for his gift of a sample of the ligand.

REFERENCES

1 Pioda, L. A. R. and Simon, W. (1969) Chimia 23, 72–73

2 Frant, M. S. and Ross, J. W. (1970) Science 167, (3920) 987–988

3 Scholer, R. L. and Simon, W. (1970) Chimia 24, 372–374

4 Pretsch, E., Ammann, D. and Simon, W. (1974) Res. Dev. 25, (3) 20–24

5 Kafka, M. S. and Holz, R. W. (1976) Biochim. Biophys. Acta 426, 31–37

6 Hyono, A., Hendriks, Th., Daemen, F. J. M. and Bonting, S. L. (1975) Biochim. Biophys. Acta 389, 34–46

7 Choy, E. M., Evans, D. F. and Cussler, E. L. (1974) J. Am. Chem. Soc. 96 (22), 7085–7090

8 Ammann, D., Pretsch, E. and Simon, W. (1973) Helvetica Chim. Acta 56 (5), 1780–1787

9 Szabo, G., Eisenman, G. and Ciani, S. (1970) Biochim. Biophys. Acta 307, 429–443

10 Gavach, C. and Sandeaux, R. (1975) Biochim. Biophys. Acta 413, 33–344

11 Ammann, D., Pretsch, E. and Simon, W. (1972) Tetrahedron Lett. 24, 2473–2476

12 Szabo, G., Eisenman, G., Laprade, R., Ciani, S. M. and Krasnes, S. (1973) in Membranes, Lipid Bilayer and Antibiotics, (Eisenman, G., ed.), Vol. 2, p. 215, Marcel Dekker, New York

13 Ammann, D., Pretsch, E. and Simon, W. (1974). Anal. Lett. 7 (1) 23–32