Biochimica et Biophysica Acta, 511 (1978) 499—508

BBA 78105

THE INFLUENCE OF pH ON THE CONDUCTANCE OF LIPID BIMOLECULAR MEMBRANES IN RELATION TO THE ALKALINE ION TRANSPORT INDUCED BY CARBOXYLIC CARRIERS GRISORIXIN, ALBORIXIN AND MONENSIN

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(Received December 28th, 1977)

Summary

The influence of the pH on the stability and stoichiometry of the complexes formed by carboxylic-antibiotics such as grisorixin, alborixin and monensin with alkaline and alkaline earth cations has been investigated. The maximum values of bimolecular lipid membrane conductance are obtained with grisorixin and potassium ion. The conductance-pH curves show a very pronounced maximum in the neutral pH range. The results are analysed on the basis of a dimeric form of the ionophore in the complex and the possibility of having several charged complexes resulting from an heterogeneous reaction, the number of each complexed form depending on the pH of the bulk solutions.

Introduction

Certain antibiotics extracted from bacteria and fungi are able to form hydrophobic complexes with inorganic cations. Such substances can then be used as ionophoric carriers which induce the passage of cations across lipid bilayers, and principally those of the alkaline group, which are not permeant through unmodified bilayers.

The majority of publications on carrier-mediated transport across bimolecular lipid membranes deal with neutral ionophores such as valinomycin and nonactin. Their electrical behaviour has been studied extensively, whereas inorganic

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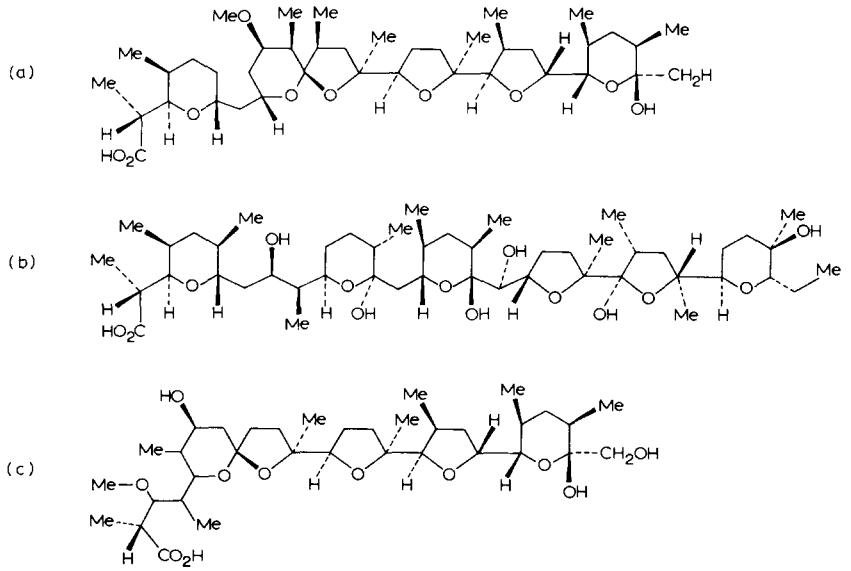

Fig. 1. Chemical formulae of the studied ionophores: (a), grisorixin; (b), alborixin; (c), monensin.

ion transport mediated by carboxylic ionophores has not retained attention to the same extent except in the case of calcium [1—5] and hydrogen ions [6—10]. Further, with protons, the ionophores act as uncouplers of the oxidative phosphorylation. The most studied among the carboxylic ionophores, complexing alkaline cations, is nigericin. In natural membranes this carrier modifies the pH gradient and also leads to uncoupling the oxidative phosphorylation [11—16], while in thick liquid membranes it exchanges K^+ with H^+ under the influence of the concentration gradient [17]. Moreover, in bimolecular lipid membranes it affects the conductance [18—21].

This paper deals with some experimental results obtained for the conductance of bimolecular lipid membranes in which antibiotics of the nigericin group have been incorporated, i.e., grisorixin, alborixin and monensin. The aqueous solutions on either side of the membrane are identical and contain alkaline or earth alkaline chlorides at different pH, particular attention being paid to the influence of pH on the conductance of the bimolecular lipid membrane.

Some crystallographic data concerning solid ion · ionophore complexes are also given. The formulae of these ionophores including that of grisorixin are given in Fig. 1.

The ionophores are not cyclic but open molecules. Nevertheless, a cyclic form is stabilized by two factors: hydrogen bonding between the carboxylic groups and between several OH groups and charge transfer bondings within the cavity.

Experimental

Experimental details concerning the method of preparation of bilayers and measurements of their limiting conductance have been given in a preceeding

paper [22]. The bilayer-forming solution was composed of a 7 mM glyceryl-monooleate (Sigma) in decane (Fluka purissimum) in which known quantities of ionophore were dissolved. Grisorixin and alborixin were prepared as previously described [23,24]. The monensin sample which was purified by thin layer chromatography was generously given by Eli Lilly. Between pH 5 and 10, the aqueous solutions were buffered with Tris and Bis Tris buffers. The pH of each solution is measured using a Tacussel (Minisis) pH meter. Before undertaking measurements we checked that the presence of the buffers does not modify the membrane conductance in the absence of ionophores.

The structure of the grisorixin salt is determined by X-ray diffraction [25–27] on a solid crystal. The data concerning monensin have been given by Pinkerton and Steinrauf [28].

Results

The effect of the pH of the bulk aqueous solutions is determined by measuring the limiting conductance at zero time and zero voltage (λ^{00}); the amount of inophore added to the lipid hydrocarbon solution, and the concentration of inorganic cation in the two aqueous solutions are both kept constant and only the pH is varied either by the addition of HCl or the corresponding hydroxyde, or a buffer. The ionic strength of the solutions is equal to 0.1 M, except for the

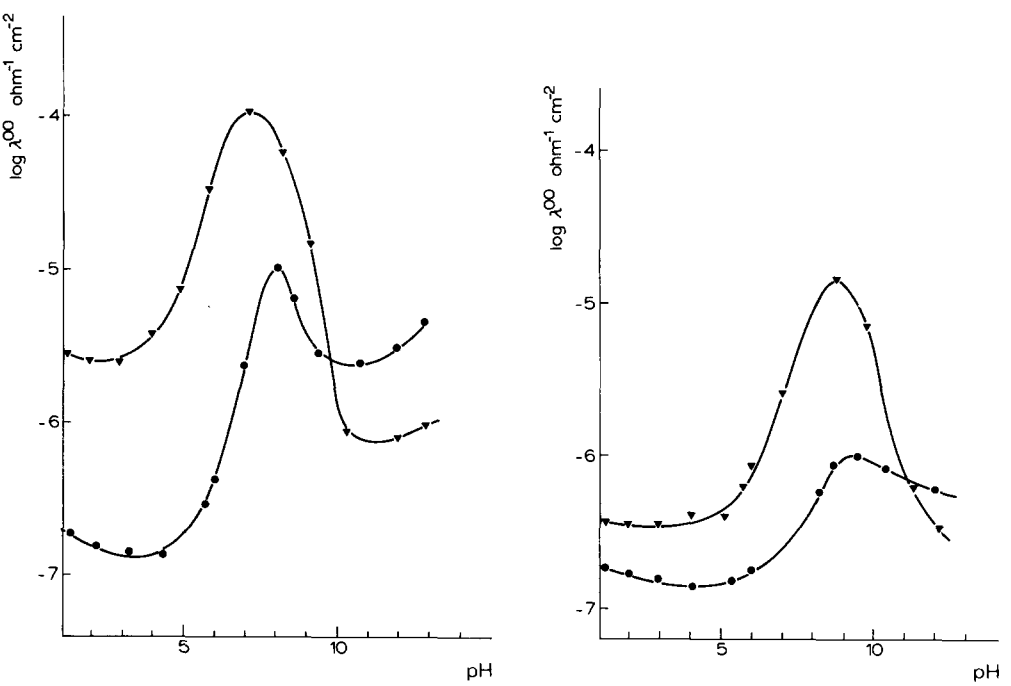

Fig. 2. Variation of λ^{00} with the pH of the aqueous solutions 0.1 M in KCl and 0.1 M NaCl and $3.5 \cdot 10^{-3}$ M in grisorixin in the membrane-forming solution. ▼——▼, KCl; ●——●, NaCl.

Fig. 3. Variation of λ^{00} with the pH of the aqueous solutions 0.01 M KCl and 0.01 M NaCl and $3.5 \cdot 10^{-3}$ M in grisorixin in the membrane-forming solution. ▼——▼, KCl; ●——●, NaCl.

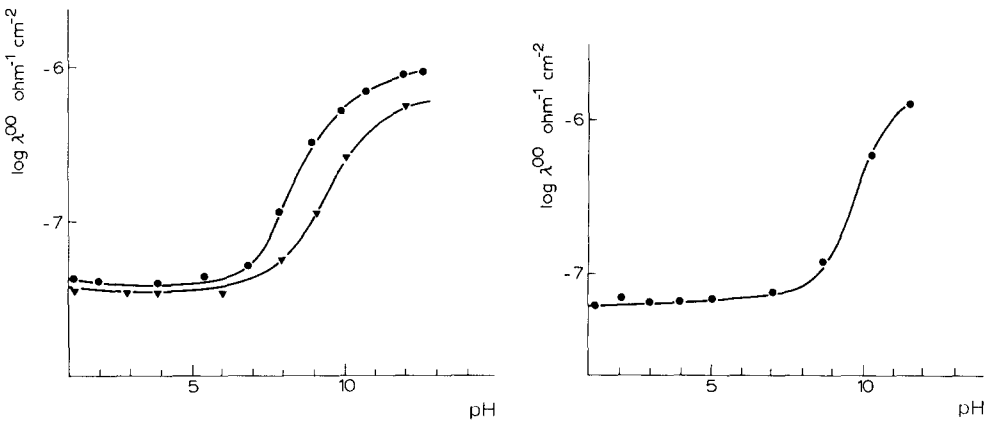

Fig. 4. Variation of λ^{00} with the pH of the aqueous solution 0.1 M LiCl and 0.1 M CsCl and $3.5 \cdot 10^{-3}$ M in grisorixin in the membrane-forming solution. ▼———▼, CsCl; ●———●, LiCl.

Fig. 5. Variation of λ^{00} with the pH of the aqueous solution 0.1 M $CaCl_2$ and grisorixin $3.5 \cdot 10^{-3}$ M in the membrane-forming solution.

0.1 M solution of alkaline or alkaline earth cations in which case it is brought up to 0.3 M.

In Figs. 2—5 are plotted the variation of λ^{00} with the pH of the aqueous solutions: here the ionophore is the grisorixin ($3.5 \cdot 10^{-3}$ M in the bilayer-forming solution); the aqueous solutions are KCl 0.1 M and NaCl 0.1 M. (Fig. 2) or 0.02 M (Fig. 3); 0.1 M LiCl (Fig. 4), 0.1 M CsCl (Fig. 4) and 0.1 M $CaCl_2$ (Fig. 5). The variation of the bilayer conductance in the presence of alborixin, at the same concentration in the aqueous solution and in the membrane-forming solution, is plotted in Fig. 6. The concentrations are again 0.1 M in K^+

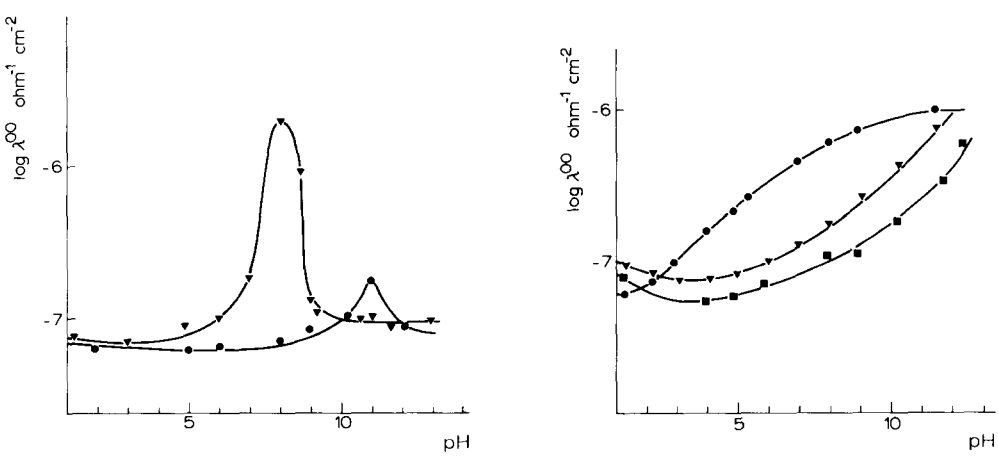

Fig. 6. Variation of λ^{00} with the pH of the aqueous solutions 0.1 M KCl and 0.1 M NaCl and alborixin $3.5 \cdot 10^{-3}$ M in the membrane-forming solution. ▼———▼, KCl; ●———●, NaCl.

Fig. 7. Variation of λ^{00} with the pH of the aqueous solutions 0.1 M NaCl, KCl and CsCl and monensin $3.5 \cdot 10^{-3}$ M in the membrane-forming solution. ▼———▼, KCl; ●———●, NaCl; ■———■, CsCl.

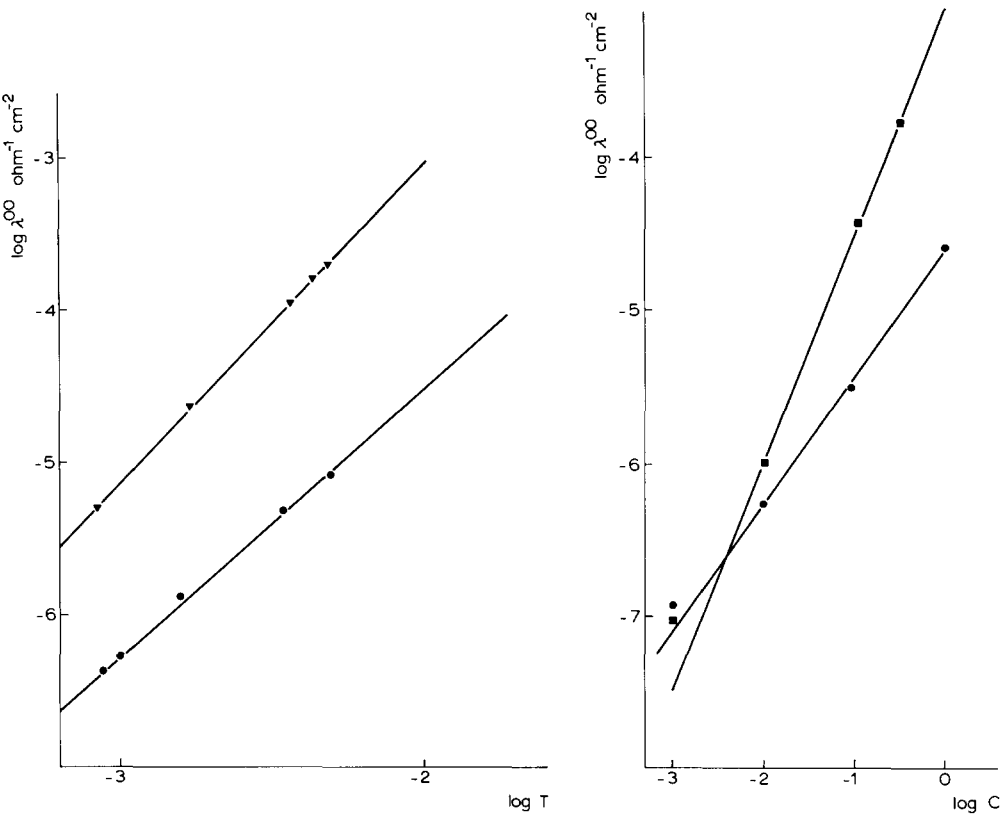

Fig. 8. Variation of the membrane-limiting conductance λ^{00} with the logarithm of the grisorixin concentration at fixed pH and at fixed concentration of cations in the aqueous solution. ▼———▼, pH 7.5, KCl 0.1 M; ●———●, pH 2.5, KCl 0.1 M.

Fig. 9. Variation of the limiting conductance λ^{00} with the logarithm of the KCl concentration in the aqueous solutions at fixed pH and grisorixin concentration. ■———■, pH 6.2, grisorixin $3.5 \cdot 10^{-3}$ M; ●———●, pH 3, grisorixin $3.5 \cdot 10^{-3}$ M.

or Na^+. With monensin, the shapes of the curves are different, even at the same ionophore and cation concentrations (Fig. 7). The variation of the membrane conductane at fixed pH and potassium concentration with the concentration in ionophore is shown in Fig. 8. At pH 2.5 and 7.5 the curves are straight lines with a slope of about 2. Fig. 9 shows the variation of λ^{00} with the cation concentration, the pH and the ionophore concentration being kept constant. The slopes of these latter curves depend on the value of the pH: less than 1 at pH 3 and 1.8 at pH 6.5.

In addition, a detailed analysis of the topology is also proposed. Figs. 10 and 11 represent the Van der Waals sections of the crystallographic structure of two solid complexes, these sections passing through the center of the cavity.

Discussion

The most important results of this set of measurements are the following.

(1) The high conductances of bimolecular lipid membranes in presence of grisorixin and potassium ions.

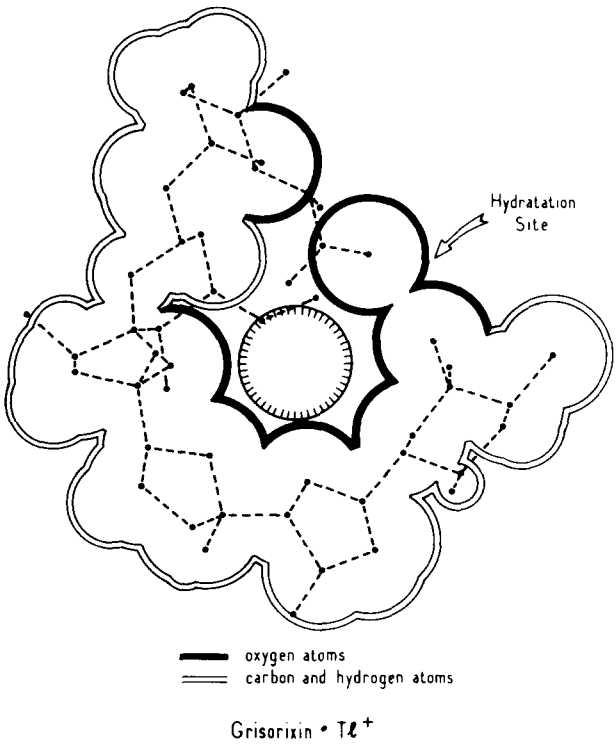

Fig. 10. Van der Waals section of the crystallographic structure of the grisorixin · Tl^+ solid complex.

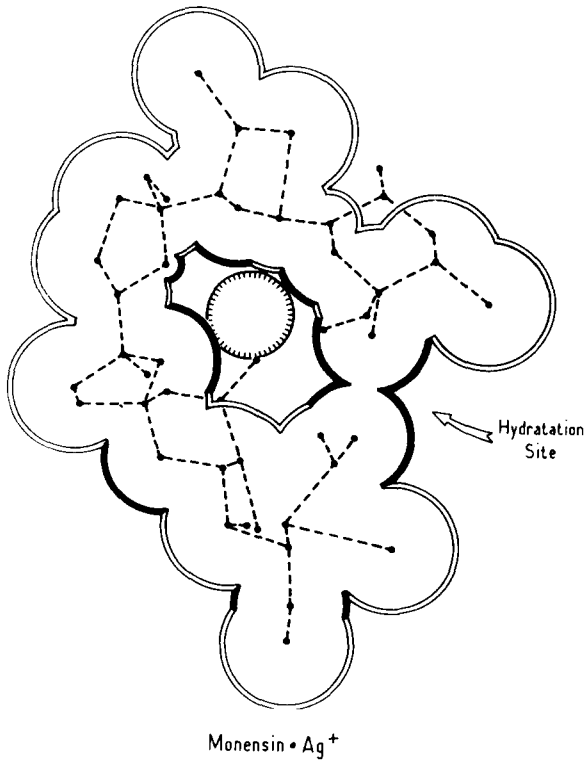

Fig. 11. Van der Waals section of the crystallographic structure of the monensin · Ag^+ solid complex.

(2) The maximum in the conductance is very much more pronounced in the conductance-pH curves obtained here than in those given by Toro et al. [21] for nigericin. However, one must stress that the method of measuring the bimolecular lipid membrane conductance by the charge pulse technique, used in our case, enables one to determine the limiting conductance at zero time and zero voltage, while that used in the study on nigericin leads only to steady-state conductance values, generally lower than those of the initial conductances.

(3) In the case of a 1 : 1 complexation between grisorixin and potassium ions the highest conductance would be expected at lower pH where the presumed complex should be charged because of the neutralisation of the carboxylic group, but, in fact, the experimental results give lower values in this pH range.

(4) With grisorixin, 3.5 mM, the bimolecular lipid membrane conductance is in the same order of magnitude at the highest pH whatever the cation except for Na^+. In this pH range the grisorixin is probably present in anionic form making it possible for these ions to cross the bimolecular lipid membrane exactly like hydrophobic anions such as tetraphenylborate and dipicrylamine, whose transfer across bimolecular lipid membranes has been extensively studied.

The essential conclusion which can be drawn from these conductance-pH curves is that potassium and sodium ions are transported across bimolecular lipid membranes by means of charged complexes, the charge and the stoichiometry depending on the pH of the aqueous solutions. The transport of K^+ mediated by neutral carriers such as valinomycin and nonactin can be explained if one takes into consideration the possibility of a heterogenous complex-forming reaction between the antibiotic located within the membrane and K^+ in the aqueous solution [29—32], the complex being adsorbed on both sides of the bimolecular lipid membranes. X-ray diffraction studies show that the crystallized neutral complexes of carboxylic ionophore with monovalent cations have amphiphilic characteristics and on this point they differ notably from the charged macrocyclic complexes. The detailed analysis of the topology of these complexes reveals that the outer envelope of the ligand is not entirely hydrophobic but that it contains a hydrophilic pole constituted by the carboxylic group. The resulting strongly hydrophilic character is clearly shown in the examples given by the existence of a molecule of water within the solid crystal itself. These amphiphilic properties of this complex suggest the possibility of a strong adsorption on the bilayer surface with the carboxylic group in contact with the aqueous solutions. Thus, the complexing reaction takes place between ions in the diffuse or in the compact layer and the ligand located on the adsorption plane. Such a model of the interfacial complexation reaction raises the problem of the exact value of local pH which may well be quite different from that in the bulk of the solution. The mechanism of ion transport is, therefore, identical to that proposed for neutral macrocyclic ionophores, i.e., a heterogeneous complexation reaction taking place at the one surface, secondly a translocation of the complex between the two adsorption planes and, lastly, a heterogeneous dissociation at the other surface. The main difference between the two cases is that here the protons participate in the complex formation with the possibility of the formation of several complexes which may be

charged within the bulk of the solution. The ability of an ionophore to form a complex with aqueous ions depends on a series of factors which have all been analysed by Lehn [33] and which are the following: the ionic radii, the volume of the cavity, the energy of solvation of the ion, the interactions between ions and oxygen atoms of the ionophore and conformational changes within the ionophore molecule.

In addition, in view of the heterogenous complexing reaction, which is here assumed to take place, the amphiphilic character of the free ionophore and of the complex and their orientation in the adsorbed state must be also taken into consideration. It will be noticed that with grisorixin the conductance sequences for acid and neutral solution are the same as those for complex formation in methanol [34], i.e., a maximum value for K^+ preceeding those of Na^+ and other alkaline cations. The same result is observed with alborixin, but with monensin the bimolecular lipid membranes conductances measured with sodium are slightly greater than those obtained with potassium, exactly as in the case for the values of the complex-formation constants [35]. All these considerations concern only the formation of 1 : 1 complexes which are charged in its protonated form or otherwise neutral.

If one assumes that only one 1 : 1 complex is formed, the conductance of the bimolecular lipid membranes would decrease as long as the pH increases; indeed in some cases the membrane conductance does decrease slightly when the pH of the solution increases from 1 to 5. A transport model in the protonated complex originally proposed by Ferguson et al. [35] can be used to explain the bimolecular lipid membranes conductance in the low pH ranges though it cannot account for the maximum. For this purpose one must consider the general heterogenous complexing reaction involving the ligand L^- in its anionic form and the K^+ and H^+ in the solution, each component being affected with a stoichiometric coefficient. The general form of the complex formation reaction is:

$$x H^+_{(w)} + y K^+_{(w)} + z L^-_{(m)} \rightarrow [L_z K_y H_x]^{(x+y-z)^+}_{(m)}$$

The different possible forms of complexes which can be deduced from this general formula become rapidly numerous. For instance, assuming that all the coefficients do not exceed a value of 2, it is possible to have 15 different chemical species containing a ligand molecule:

Five neutral complexes: $[LH]$, $[LK]$, $[L_2HK]$, $[L_2H_2]$ and $[L_2K_2]$

Four negatively charged species: L^-, L_2^{2-}, $[L_2K]^-$, $[L_2H]^-$

Six positively charged complexes: $[LHK]^+$, $[LH_2]^+$, $[LK_2]^+$, $[L_2HK_2]^+$, $[L_2H_2K]^+$ and $[L_2H_2K_2]^{2+}$.

The existence of a maximum can then be accounted for by the formation of differently charged carriers depending on the pH of the solution. As the shope of the curves log λ^{00} in function of the logarithm of grisorixin concentration (Fig. 8) have a value of about 2, a dimeric form can be expected at the pH of the maximum pH and for acid pH. For the curve of log λ^{00} vs. log $[K^+]$ (Fig. 9), the slope is less than unity for acid pH, and between 1 and 2 for higher pH. This last result suggests that in the region of the maximum a complex contain-

ing two potassium ions is more stable than in the acid range.

Consequently the experimental results show that whatever the range of pH considered the complexes formed will always contain two molecules of the ionophore: the species L_2^{2-}, L_2K^-, L_2H^-, $[L_2HK_2]^+$, $[L_2H_2K]^+$ and $[L_2H_2K_2]^{2+}$ will thus be the only complexes which are capable of explaining the results in Fig. 8. At low pH the complex will exist in the forms: $[L_2H_2K]^+$, $[L_2H_2K_2]^{2+}$, $[L_2HK_2]^+$ and $[L_2H]^-$, but from Fig. 9, one deduces that the most likely complex must be one whose stoichiometry corresponds to a single cation, i.e., $L_2H_2K^+$.

At the pH of maximum conductance the most likely species are those containing two cations and the two types $[L_2H_2K_2]^{2+}$ and $[L_2HK_2]^+$ are both feasible. At high pH values the acidic ion complexes no longer exist and it is the free anions of the ionophore which will be responsible for the passage of the current.

Examining the curves in Figs. 2 and 3, one can observe that the maximum is displaced towards lower pH when the concentration of potassium or sodium increases. This fact is to be compared to the results obtained by Pressman [2] for the variation of the dissociation constant of nigericin, on the basis of the following complexation reaction:

$$HA + M^+ \leftrightharpoons M^+A^- + H^+$$

$$K'_a = \frac{[H^+] \cdot [A^-]}{[HA] \cdot [M^+]}$$

where K'_a is not a real acid dissociation constant but depends on the value of $[M^+]$ (the value of the pK_a for nigericin in ethanol solution at 30°C is 8.45 and becomes 5.75 when 10 mg of KSCN is added).

The assumption of the existence of one or more dimeric forms of grisorixin to account for the maximum in the conductance-pH curves seems inconstant with X-ray data which indicate the existence of a single monomeric form in the crystals obtained from an aqueous solution. Recently, however, the existence of dimeric forms with charged antibiotics has been also proposed: Toro et al. [21] with nigericin in black lipid membrane note the possibility of a dimeric form. Chiang and Paul [37] using Lasolocid A (X-537A) carboxylic ionophore, which have similar formulae to those of nigericin and grisorixin, found that the structure of the sodium complex crystal obtained from methanol has a monomeric form, while the transport in non-polar media takes place by means of a dimeric form. These facts suggest that the ionophore may well be a dimer in the lipid bilayer reverting to a monomeric structure in the polar medium, Boguslavisky et al. [38] have also shown the existence of a dimeric mechanism for nigericin within a bilayer lipid membrane.

The formation of at least two different forms of complex, which is assumed in order to account for the conductance pH curves, will be confirmed by the voltage step measurements in progress.

Acknowledgements

Thanks are due to Dr. Robert Hamill of the Lilly Research Co. for his generous gift of monensin.

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