Biochimica et Biophysica Acta, 774 (1984) 193–198
Elsevier

BBA 72167

ELECTROGENIC AND SELECTIVE TRANSPORT OF BIOGENIC AMINES ACROSS LIPID BILAYER MEMBRANES MEDIATED BY A NEUTRAL IONOPHORE (AS701)

RIMONA MARGALIT [a], AMIRA ZEEVI [a] and ABRAHAM SHANZER [b]

[a] *Department of Biochemistry, The G.S. Wise Life Science Center, Tel-Aviv University, Tel-Aviv 69978, and* [b] *Department of Organic Chemistry, Weizman Institute of Science, Rehovot (Israel)*

(Received April 9th, 1984)

Key words: Biogenic amine; Ionophore AS701; Electrogenic transport; (Lipid bilayer)

The neutral noncyclic imide and ether containing ionophore (AS701), a selective carrier for Li^+ among alkali cations, was found to be capable of mediating the transport of NH_4^+ and of biogenic amines (catechols and indoles) across lipid bilayer membranes also. Ionophore-mediated electrical properties of planar lipid bilayers were studied under experimental conditions where the positively-charged amine species was dominant. The ionophore was found to act as a selective carrier of the biogenic amines, mediating their electrogenic transport across the membrane, forming 2:1 carrier-amine permeant complexes, carrying a net-charge of +1. Selectively among the amines corresponding to the following sequence: tryptamine (35) > Li^+ (1) > serotonin (0.60) > dopamine (0.19) > norepinephrine (0.13) > epinephrine (0.05) > NH_4^+ (0.05). The molecular factors involved in determining these selectivities are assessed.

Introduction

Carboxylic ionophores such as X-537A have been shown to mediate selective transport of biogenic amines across lipid bilayer membranes, the major ionophore-amine permeant complex being electrically-silent [1–4]. We have recently found that selective transport of biogenic amines across such membranes can also be mediated by the neutral ionophore AS701 (Fig. 1) the transport being, in this case, electrogenic. This ionophore has been originally designed to be, and indeed is, a selective carrier for Li^+ among alkali cations [5,6].

We have studied the activities of this ionophore with ammonium and with two types of aromatic amines: Catechols and indoles. The catechol group included dopamine, norepinephrine, epinephrine and the structurally-related phenylethylamine. The indole group included tryptamine and serotonin.

The studies, to be reported here, were conducted at the pH range of 4–7, where the protonated species are dominant (the pK values ranging from 8.5 up [7]). Since the ionophore is neutral, its complex with the major amine species should carry a net-charge across the membrane and be detected by the experimental system which was set-up to measure electrical properties of the membrane. We will show that this, indeed, was the case. We shall also show that this ionophore is a carrier of biogenic amines, that there is selectivity among them and that it is possible to assess the molecular factors involved in determining the selectivity.

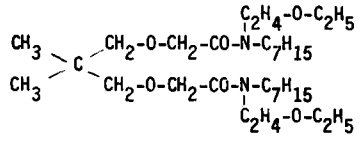

Fig. 1. Structural formula of AS701.

Materials and Methods

The ionophore was synthesized by A. Shanzer. The biogenic amines and the lipids (glycerol-monoleate (GMO) and phosphatidylcholine (Asolectin)) were purchased from Sigma Chemical Co. Bacterial phosphatidylethanolamine (PE) was purchased from Supelco.

Membranes were formed on the aperture (usually 1 mm diameter) of a teflon cell, from lipid/decane solutions (25–50 mg/ml). Steady-state electrical properties of the membranes were measured using previously described methods [5,6,8,9].

Results

I. Zero-current membrane conductances

In the absence of the ionophore, regardless of the salt present, we have found the level of membrane conductance (at the limit of zero-current) to be rather low, of the order of 5–50 nS. This is in agreement with the findings of others [10,11] for self-diffusion of protonated biogenic amines at pH values of 7 and below. Upon introducing and increasing the concentration of the ionophore in the system, we have found the membrane conductance to increase, up to several orders of magnitude. Typical data are illustrated in Fig. 2 for glycerol monooleate membranes in the presence of several amine species (each, separately) and in Fig. 3 in the presence of tryptamine, the most effective among the amines studied, for asolectin and PE membranes, also.

Several conclusions can be immediately drawn: The ionophore-induced increase of membrane conductance clearly indicates the formation of a charge-carrying membrane-permeant species. The span of conductance increase among different amine species at similar concentrations (Fig. 2) indicates cation-specificity (all salts have the same anion). The phenomena is not restricted to glycerol monooleate (Fig. 3). Furthermore, the trend and magnitude of the lipid-composition effect is as expected for carrier-mediated transport of positively-charged species [5,6,9]. Additional support for the protonated amine, being the ion complexed and carried across the membrane, will be presented in a later section. As also seen in Figs. 2 and 3, the data for each amine system fit to a slope of 2, indicating a 2nd power dependence of conductance on ionophore concentration, similar to

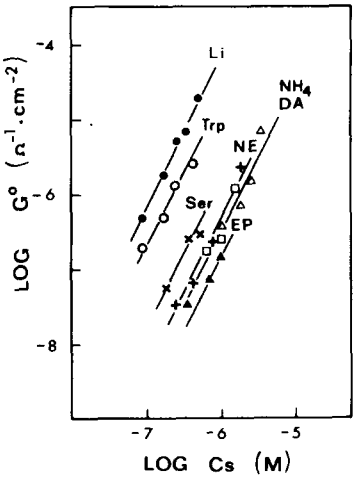

Fig. 2. Dependence of zero-current conductance of glycerol monooleate membranes on ionophore concentration, in the presence of chloride salts of the following ions: Li^+ (●), tryptamine (○), serotonin (×), dopamine (△), NH_4^+ (□), norepinephrine (+) and epinephrine (▲). Concentrations of biogenic amines are 20 mM (in 1.0 M $MgCl_2$). Other concentrations: 1 M LiCl and 0.1 M NH_4Cl. Ordinate: Logarithm of membrane conductance. Abscissa: Logarithm of aqueous ionophore concentration. Points are experimental, lines drawn to a slope of 2. Abbreviations used on the figure: TRP, tryptamine; Ser, serotonin; DA, dopamine; NE, norepinephrine; EP, epinephrine.

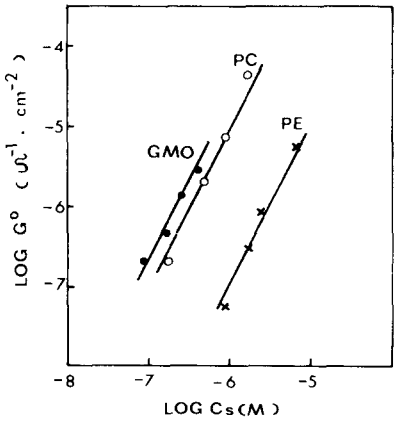

Fig. 3. Dependence of membrane zero-current conductance on AS701 concentration, in the presence of tryptamine (10 mM in 1.0 M $MgCl_2$), on membrane lipid composition. Ordinate and abscissa are as in Fig. 2. Points are experimental, lines drawn to a slope of 2. GMO, glycerol monooleate; PC, Asolectin; PE, phosphatidylethanolamine.

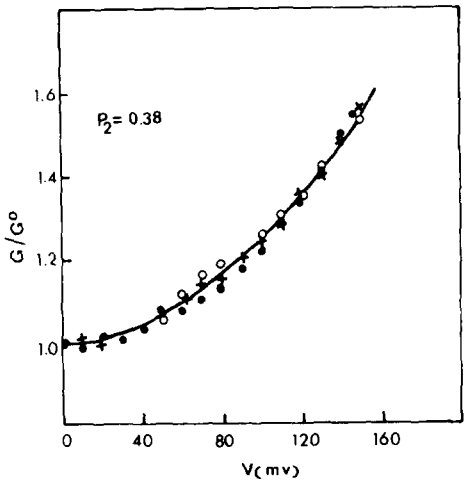

Fig. 4. Conductance-voltage relationships of asolectin membranes induced by AS701, in the presence of 1.0 M LiCl (●), 5 mM tyramine (○) or 10 mM tryptamine (+). Ordinate: The observed membrane conductance G normalized to G_0, the membrane conductance at the limit of zero-current. Abscissa: The applied potentials. Points are experimental, solid curves drawn according to the following equation:

the case of alkali cations, as examplified by the data for LiCl, reproduced in Fig. 2 [5,6].

II. Conductance-voltage behavior

The rate-limiting step in the transport of alkali cations by this carrier is the translocation of ion-carrier species across the membrane [9,12–14]. Conductance-voltage measurements for the amines, illustrated by the typical data in Fig. 4, show that for these also the conductances increase hyperbolically with the applied potential. This increase was found to be independent of salt over the 0.01–1.0 M concentration range and of the rate of voltage increase over the 0.01–0.20 Hz range employed. This is as expected for 'equilibrium domain' [13] systems and shows that in this carrier system the

$$\frac{G}{G_0} = 2P_2 \frac{\sin h(\phi/2)}{\sin(P_2\phi)} \qquad \phi = FV/RT \qquad (2)$$

and the magnitude of P_2 listed on the figure.

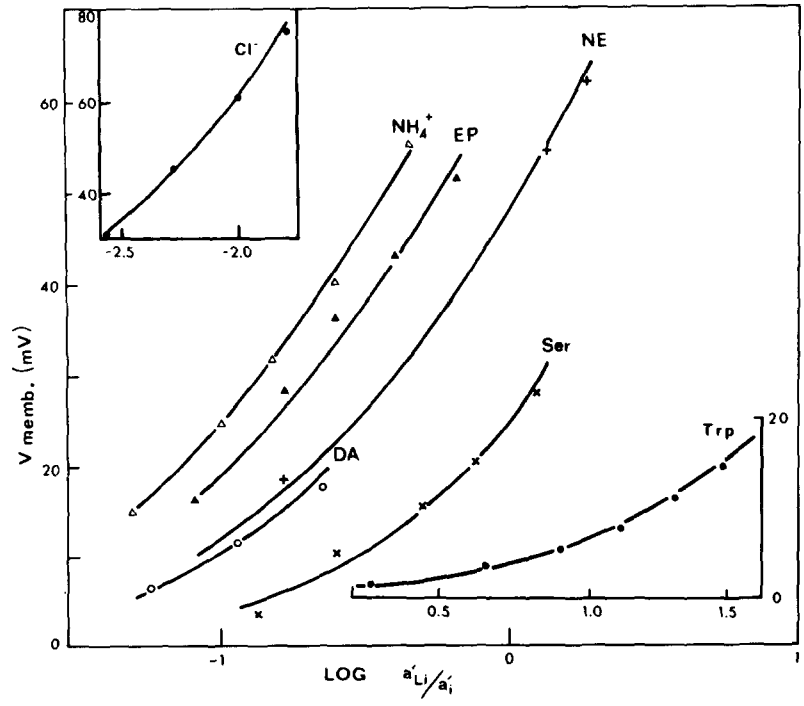

Fig. 5. Zero-current potentials of glycerol monooleate membranes, in the presence of AS701 and salt mixtures (for abbreviations see legend to Fig. 2). Ordinate: Observed membrane potentials. Abscissa: Logarithm of the ratio of the activities of Li^+ to the ion indicated. Point are experimental. Solid curves drawn according to Eqn. 1 in the text, for the permeability ratios listed in Table I. Inset: Zero-current potentials of glycerol monooleate membranes in the presence of AS701 and $LiCl/MgCl_2$ mixtures. Data reproduced from Ref. 6.

TABLE I

BIOGENIC AMINES: HYDROPHOBICITIES AND IONOPHORE-MEDIATED CONDUCTANCE AND PERMEABILITY RATIOS

Permeability data for phenylethylamine and for tyramine are from asolectin membranes. Permeability and conductance data of all other species are from glycerol monooleate membranes.

Ion	$\frac{P_{amine}}{P_{Li}}$	$\frac{G^0_{amine}}{G^0_{Li}}$	Log partition water/octanol [a]				
			Total	Indole	Benzene	Phenol	Catechol
Tryptamine	35 ±3	32	1.98	2.25			
Phenylethylamine	35 ±2		1.88		2.15		
Tyramine	3.0 ±0.14		1.19			1.46	
Lithium	1	1					
Serotonin	0.60 ± 0.10	0.89	0.60	0.87			
Dopamine	0.19 ± 0.03	0.18	0.61				0.88
Norepinephrine	0.13 ± 0.02	0.17	−0.43				0.88
Epinephrine	0.05 ± 0.01	0.05	−0.01				0.88
NH_4^+	0.05 ± 0.003	0.035					
Ethylamine			−0.27				
Ethanolamine			−1.31				

[a] Data taken from Ref. 15.

same type of mechanism operates for the alkali cations and for the biogenic amines.

III. Membrane zero-current potentials in salt mixtures

To assess the selectivity, if any, among the biogenic amines, we have measured the zero-current potentials of glycerol monooleate membranes, generated under salt gradients at a constant concentration of AS701 in the system, using the previously described experimental design [5].

Typical results are illustrated in Fig. 5. The points are the measured membrane potentials, at given salt gradients. The solid curves are the theoretical expectations, drawn according to the following equation for the permeability ratios listed in Table I.

$$V_0 = \frac{RT}{F} \ln \frac{a'_{Li} + (P_i/P_{Li}) a'_i + (P_{Cl}/P_{Li}) a''_{Cl}}{(P_i/P_{Li}) a''_i + (P_{Cl}/P_{Li}) a'_{Cl}} \quad (1)$$

Parameters in one of the aqueous phases are indicated by ('), those in the other by (''). The activities of Li^+, Cl^- and the positively-charged amine are given by a_{Li}, a_{Cl} and a_i, respectively. P_{Cl}/P_{Li} and P_i/P_{Li} are the permeability ratios of the corresponding complexes.

Chloride is included among the permeant ions contributing to the observed membrane potential (Eqn. 1), eventhough the AS701 ionophore renders the bilayers only slightly permeable to this anion [5] (see Fig. 5). Still, consideration of chloride was necessary, since its concentration in the medium was high relative to that of the amines.

The good agreement observed in Fig. 5 between the experimentally-observed and theoretically-expected potentials supports an ion stoichiometry of 1 and identification of the protonated amine as the major ion transported in solutions of biogenic amines.

The permeability ratios, obtained from the type of data illustrated in Fig. 5, are listed in Table I together with the corresponding conductance ratios, with which they are seen to be in good agreement. The data clearly show that there is selectivity among the amines, ranging over several orders of magnitudes.

Discussion

I. AS701 as an electrogenic and selective carrier of biogenic amines

The conductance and potential data presented in the previous section clearly show, that AS701 acts as a carrier of the protonated biogenic amine species (i.e., the monovalent cation), mediating

their electrogenic transport across the membrane quite similar to its activity with alkali cations [5]. Examples of this similarity can be evidenced from the following data: The second power dependence of membrane conductance on ionophore concentration setting a carrier stoichiometry of 2 for the permeant complex. The decrease in conductance from glycerol monooleate to PE membranes, indicating carrier-mediated transport of positively-charged species. The 'equilibrium domain' type of mechanism. The fit of the zero-current membrane potentials with the theoretical expectations of a stoichiometry of 1 and a charge of +1 for the complexed and transported ion. These smilarities allow us to extend the 2 : 1 carrier/ion composition deduced for the permeant complexes of this ionophore with alkali cations, to include its complexes with positively-charged biogenic amines.

The conductance, and more so the membrane potential data show that there is considerable selectivity among the biogenic amines in this carrier system. The permeability ratios, listed in Table I span over several orders of magnitude. Three amines tryptamine, phenylethylamine and tyramine are more selective than lithium, the ion for which this ionophore was originally designed.

The 'equilibrium domain' type of mechanism, observed for the present case type means, in terms of selectivity, that the major determining factor should be the amine-ionophore binding equilibrium [9,12,13]. Hence, the assessment of the molecular factors involved in the binding, should give insight into the factors determining the selectivity.

II. Selectivity among the biogenic amines: molecular factors involved

Four molecular factors could be involved in the amine-ionophore binding: Electrostatic interactions, H-bonding, hydrophobic interactions (all three favorable) and steric hindrance (which is expected to be unfavorable).

Starting with the unfavorable factor, it does not seem to be major. If there is steric hindrance due to the bulky aromatic residues, then it is masked (or compensated for) since several aromatic amines exceed lithium in selectivity (recall Table I). Minor steric hindrance, at the cationic head, is indicated by the sequence norepinephrine > epinephrine, these amines differing only by a methyl residue there.

On the basis of the experimental data (recall Table I) the first two favorable factors can also be excluded from having a dominant role. For the electrostatic interactions this is shown by the low selectivity of NH_4^+. For H-bonding involving hydroxy residues of the amines, this is shown by the high selectivities of tryptamine and phenylethylamine, the two best amines having no hydroxy residues. As to H-bonding involving $-NH$ residues of the amines, which could have a significant role in binding, we suggest it coud not have a significant role in selectivity since the free energy change of such interactions should be of similar magnitude for all amines studied.

To get some insight into the ability of the present amines to engage in hydrophobic interactions with nonpolar regions of the ionophore we have compared their coefficients of partition from water into octanol (which is an accepted measure of hydrophobicity) with their selectivities. The partition data, also listed in Table I, are taken from the extensive review of Leo et al. [15]. Scanning, first, the total hydrophobicities of the amine molecules, two issues become clear: (a) The sequence of hydrophobicities is quite similar to the sequence of selectivities. (b) The differences in the magnitudes of hydrophobicity (note that the data is given in the logarithmic form) are large enough to account for the observed differences in selectivity. We do not suggest a quantitative correlation, rather, that the hydrophobicities are in the right direction in terms of sequence and magnitudes. When the contributions of the aromatic as opposed to those of aliphatic regions of each amine to the total hydrophobicity of the molecule are considered (these data are also given in Table I, in the logarithmic form, which is the additive one), then another issue becomes apparent. Taking tryptamine and serotonin (which differ structurally only in their aromatic rings), they show differences in their total and in their ring hydrophobicities, and also in their selectivities. The same thing is found for the group phenylethylamine, tyramine and dopamine. On the other hand, dopamine and norepinephrine (which differ structurally only in their aliphatic chains and in their total hydro-

phobicities) show no difference in their ring hydrophobicities (obviously) and little difference in their selectivities. Taking these pieces of data together strongly suggests that hydrophobic interactions are the major molecular factor determining the selectivities observed among the biogenic amines in the present carrier system, with the aromatic regions carrying the major weight of it.

References

1 Schadt, M. and Haeusler, G. (1974) J. Membrane Biol. 18, 277–294

2 Kinsel, F.J., Melnik, I.E., Lindenbaum, S., Sternson, A.L. and Ovchinnikov, A.Yu. (1982) Biochim. Biophys. Acta 684, 233–240

3 Kinsel, F.J., Melnik, I.E., Sterenson, A.L., Lindenbaum, S. and Ovchinnikov, A.Yu. (1982) Biochim. Biophys. Acta. 692, 377–383

4 Kinsel, F.J., Melnik, I.E., Lindenbaum, S., Sternson, A.L. and Ovchinnikov, A.Yu. (1982) Int. J. Pharmacol. 12, 97–106

5 Margalit, R. and Shanzer, A. (1981) Biochim. Biophys. Acta 649, 441–448

6 Margalit, R. and Shanzer, A. (1982) Pflügers Arch. 395, 87–92

7 Dawson, R.M., Elliot, D.C., Elliot, W.H. and Jones, K.M. (1969) in Data for Biochemical Research, 2nd Edn. Clarendon Press, London

8 Szabo, G., Eisenman, G. and Ciani, S. (1969) J. Membrane Biol. 1, 346–382

9 Margalit, R. and Eisenman, G. (1981) J. Membrane Biol. 61, 209–221

10 Bean, C.R., Shepherd, C.W. and Chan, H. (1968) J. Gen. Physiol. 52, 495–508

11 Gutknecht, J. and Walter, A. (1981) Biochim. Biophys. Acta 649, 149–154

12 Krasne, S. and Eisenman, G. (1976) J. Membrane Biol. 30, 1–44

13 Ciani, S. (1976) J. Membrane Biol. 30, 45–63

14 Hladky, S.N. (1979) in Current Topics in Membrane Transport (Bronner, F. and Klienzellar, A., eds.), Vol. 12, pp. 54–264, Acaemic Press, New York

15 Leo, A., Hansch, C. and Elkins, D. (1971) Chem. Rev. 71, 525–616