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NEGATIVE HYDROPHOBIC IONS AS TRANSPORT-MEDIATORS FOR POSITIVE IONS

EVIDENCE FOR A CARRIER MECHANISM

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Summary

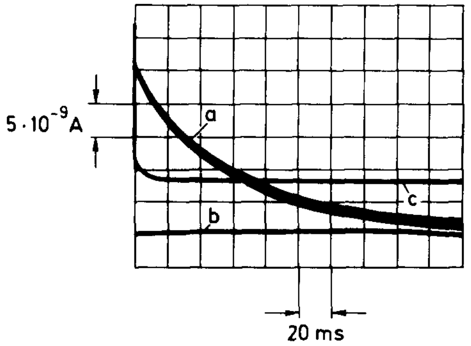
The permeability of hydrophobic cations, such as tetraphenylarsonium, across biological membranes and artificial lipid membranes is strongly increased in the presence of trace amounts of hydrophobic anions like tetraphenylborate (Liberman, Y.A. and Topaly, V.P. (1969) Biofizika 14, 452—461). Voltage-jump relaxation experiments performed on thin lipid membranes support the idea that the anions, A^-, act as carriers for the cations, B^+, by the formation of neutral ion pairs, A^-B^+. Their permeability is not affected by the electric dipole potential, which hinders the movement of free cations, B^+.

The distribution of hydrophobic ions is frequently used as an indirect method to estimate the magnitude of the electrical potential difference across biological membranes and to study the potential dependence of membrane phenomena in bioenergetics [1]. Lipophilic cations, such as dimethyldibenzylammonium or triphenylmethylphosphonium, which are added to the external medium of a cell suspension, accumulate in the electrically negative cell interior. From the concentration ratio of the cations on both sides of the membrane, the potential difference is obtained by using Nernst's equation. The equilibration time of lipophilic cations across the membrane is relatively long. It may, however, be considerably reduced, if trace amounts of lipophilic anions such as tetraphenylborate are simultaneously present. The phenomenon was first reported by Liberman and Topaly [2], who found that the relatively small conductance of artificial (black) lipid membranes is increased in the presence of the dimethyldibenzylammonium cation or the triphenylmethylphosphonium cation by several orders of magnitude on addition of small amounts of the tetraphenylborate anion. A possible mechanism underlying this effect is suggested below on the basis of voltage-jump experiments performed with black lipid films.

Lipophilic anions are able to permeate through thin hydrophobic barriers much more readily than lipophilic cations [2—4]. This is an effect of the electrical dipole potential at the membrane/water interfaces which, due to the positive sign of the dipole potential at the membrane part of the interface, favours the adsorption of anions. The combined effect of anions and cations, however, exceeds the sum of the individual effects by far, i.e., the membrane conductance, $\lambda^{+,-}$, observed in the presence of both cations and anions is much larger than the sum $(\lambda^+ + \lambda^-)$, where λ^+ and λ^- represent the conductances measured in the presence of only one sort of hydrophobic ionic species [2]. Liberman and Topaly [2] only performed steady-state experiments and explained them qualitatively on the basis of indirect electrostatic effects via ion adsorption to the membrane. The kinetic experiments illustrated in Fig. 1 rather point to a direct mutual interaction of cations and anions.

The electric current following the application of a voltage jump to a lipid membrane in the presence of the tetraphenylborate anion decreases from a relatively large value down to virtually zero. This current relaxation is typical for lipophilic anions and is usually interpreted as a voltage-dependent redistribution of the anions between the two membrane interfaces; i.e., the anions move across the membrane and accumulate at the positive interface [3—6]. If the same experiment is performed in the presence of the cation, tetraphenylarsonium, instead of the tetraphenylborate anion, the current level coincides with the base-line, i.e., the effect of the cation is not resolved within the range of current sensitivity applied. Therefore, the same current relaxation would be expected in the presence of both cations and anions as was observed with the anions alone, if cations and anions act independently. Instead, curve c of Fig. 1 shows a constant current level (following the initial current spike), i.e., the current decay, indicative of the component of free anions, has disappeared. This is only true at high tetraphenylarsonium cation concentrations. If the tetraphenylarsonium cation concentration is increased at a constant concentration of $1 \cdot 10^{-7}$ M tetraphenylborate anion, the decaying current component is continuously reduced to virtually zero at $1 \cdot 10^{-3}$ M. At the same time, the steady-state current component is increased up to the level shown in curve c.

The disappearance of the current signal of the anions and the concomitant increase of the steady-state membrane conductance are difficult to understand on the basis of modified interfacial potentials through ion adsorption. They are, however, easily explained on the basis of the reaction scheme illustrated in Fig. 2. It assumes the formation of ion pairs, A^-B^+, which act as neutral 'carrier complexes' for the cations, B^+. The underlying physical principle consists of a drastic reduction of the transport barrier for the neutral ion pairs, A^-B^+, compared with the free cations, B^+. The movement of the latter is strongly hindered through the large electric dipole potential difference at the membrane/water interface [2—4]. The neutral complexes, however, can be influenced by an electric field only through



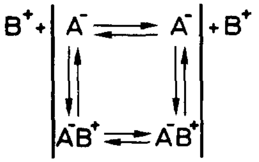


Fig. 1. Relaxation of the electric current following a voltage jump of 30 mV to a membrane formed from a 1% solution of dioleoyl phosphatidylcholine in decane (membrane diameter 2 mm, T = 25°C). See Refs. 5 and 7 for details of the technique. Curve a was obtained in the presence of $1\cdot10^{-7}$ M tetraphenylborate anion and 0.1 M NaCl, curve c after addition of tetraphenylarsonium cation ($1\cdot10^{-3}$ M), curve b represents the base-line.

Fig. 2. Transport of lipophilic cations, B^+, through ion-pair formation with anions, A^-.

second-order phenomena. The model is comprised of the following two conditions:

(1) A steady-state current consists of a net transport of B^+ across the membrane.

(2) Inside the membrane the current is carried by free anions, A^-, which move in the opposite direction to the complexes, A^-B^+.

Condition 1 was tested by measurement of diffusion potentials at identical concentrations of the tetraphenylborate anion but differing concentrations of the tetraphenylarsonium cation on both sides of the membrane. The sign and magnitude of the open circuit potential found under these conditions was in fair agreement with condition 1.

According to condition 2, a certain amount of free anions is indispensable for the maintenance of a constant current. The concentration of A^- should decrease with increasing concentration of B^+. This was tested by an experiment. The model shown in Fig. 2 is formally identical with that which has successfully been used to describe the kinetics of valinomycin-mediated K^+ transport [7]. There is only a difference with respect to the charge of the free carrier and the carrier complex. If this is accounted for, a similar kinetic treatment to that in the case of macrocyclic ion carriers [7] leads to the following equation for the total membrane concentration, w, of free anions, A^- ($w = 2N_A$, N_A = interfacial membrane concentration of A^-):

$$w = \left| \frac{I}{\dfrac{\mathrm{d}\ln I}{\mathrm{d}t}} \right|_{t=0} \cdot \frac{4RT}{F^2 U} \tag{1}$$

where I = current, t = time, T = temperature, R = gas constant, F = Faraday constant and U = membrane voltage.

Thus, a measurement of the initial current I_0 and the initial time derivative $(\mathrm{d}\ln I/\mathrm{d}t)_{t=0}$, after the application of a voltage jump of amplitude U allows the determination of w. Eqn. 1 is only valid at low voltages (ohmic range of the membrane). Fig. 3 illustrates experimental data obtained with the tetraphenylarsonium cation and the two different anions, tetraphenylborate and dipicrylamine. At the highest cation concentrations, there is a drop of w down to a few percent of its initial value. This is, however, sufficient to maintain the steady-state current observed experimentally, as was found by a more detailed analysis not presented here. The dependence of w on c_B allows the estimation of the dissociation constant, K, for ion-pair formation in water, defined by:

$$\frac{c_A c_B}{c_{AB}} = K \tag{2}$$

Since $c_B \gg c_A^t$ (for the situation considered here), $c_A^t = c_A + c_{AB}$, and defining the partition coefficient between membrane and water as:

$$\beta_A = N_A/c_A \tag{3}$$

we obtain from Eqns. 2 and 3:

$$w = 2N_A = \frac{2\beta_A K\, c_A^t}{c_B + K} \tag{4}$$

Eqn. 4 shows a reasonably good fit to the data of the system, dipicrylamine anion/tetraphenylarsonium cation.

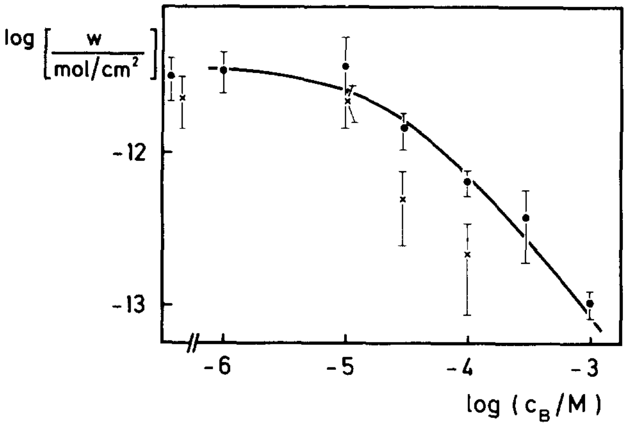


Fig. 3. The concentration, w, of anions, A^-, inside the membrane as a function of cation concentration, c_B. The data (mean values of five different membranes plus standard error) were obtained from the analysis of the current relaxation after a voltage jump by using Eqn. 1. (●) Membranes (dioleoyl phosphatidylcholine in decane) were formed in the presence of $1 \cdot 10^{-7}$ M dipicrylamine anion (plus 0.1 M NaCl) and the indicated concentration of tetraphenylarsonium cation. (×) The indicated amounts of tetraphenylarsonium cation were added to membranes preformed in a solution of $1 \cdot 10^{-7}$ M tetraphenylborate anion and 0.1 M NaCl. The data were obtained about 10 min after addition of tetraphenylarsonium cation. They do not represent equilibrium values, since there was a continuous change of the relaxation curves for at least 1 h after addition. The full line represents a fit of Eqn. 4 to the dipicrylamine anion data assuming $K = 2.5 \cdot 10^{-5}$ M.

Though there is satisfactory agreement between theory and experiment, the scheme illustrated in Fig. 2 only shows the most essential characteristics of how the transport of lipophilic cations seems to be 'facilitated' by the presence of anions through ion-pair formation. A more rigorous treatment of the problem must also include effects of ion adsorption on the interfacial potential. It may, however, be shown that, at the anion concentrations employed, this effect is of minor importance [5,6]. Another simplification applies to the place of ion-pair formation. In fact, the possibility cannot be excluded, at present, that the ion pairs diffusing across the membrane are formed within the unstirred layers adjacent to the membrane (and not at the interfaces as indicated in Fig. 2). One can show, however, that this is of no consequence for the validity of the presented analysis, i.e., Eqn. 1 and Fig. 3 remain correct. Preliminary spectrophotometric studies have provided additional evidence for a direct interaction of the dipicrylamine anion and the tetraphenylarsonium cation in water in the concentration range considered (work in progress).

The suggested mechanism could be of more general importance for the understanding of a facilitated permeation of hydrophobic ionic substances across cell membranes. The membrane permeability of such substances could be considerably enhanced by formation of neutral ion pairs with integral ionic membrane components of opposite charge.

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