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TRANSPORT KINETICS OF HYDROPHOBIC IONS IN LIPID BILAYER MEMBRANES

CHARGE-PULSE RELAXATION STUDIES

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SUMMARY

A modified version of the charge-pulse relaxation technique with improved time resolution was applied to the study of transport kinetics of hydrophobic ions (tetraphenylborate, dipicrylamine) through lipid bilayer membranes. Besides a better time resolution the charge-pulse method has the additional advantage that the perturbation of the membrane can be kept small (voltage amplitudes between 1 and 10 mV). The results of the analysis support the model proposed earlier, according to which the overall transport takes place in three consecutive steps, adsorption of the ion from water to the interface, translocation to the opposite interface, and desorption into the aqueous phase. The translocation rate constant k_i and the partition coefficient γ of the hydrophobic ion between water and the membrane were measured for lecithins with different mono-unsaturated fatty acid residues. Increasing the chain length of the fatty acid from C_{16} to C_{24} resulted in a decrease of k_i by a factor of about 9 in the case of tetraphenylborate and by a factor of about 17 in the case of dipicrylamine.

INTRODUCTION

Certain large organic ions, such as tetraphenylborate or dipicrylamine, are strongly hydrophobic and are able to permeate through lipid bilayer membranes [1–5]. By studying the electrical properties of artificial lipid membranes in the presence of these ions information on the structure and dynamics of the membrane may be obtained. Some years ago, a detailed mechanism for the transport of hydrophobic ions through lipid membranes was proposed [6]. Based on the results of electrical relaxation studies it has been suggested that the transport occurs in three distinct steps, namely, (i) adsorption of the ion from the aqueous phase to the membrane-solution interface, (ii) translocation to the opposite interface and (iii) desorption into the aqueous solution. From the time course of the current transient following a sudden displacement of voltage it has been inferred that ions such as tetraphenylborate or dipicrylamine are located in deep potential energy minima in the membrane-solution

interface. These conclusions have been confirmed in recent studies by Bruner [7] and by Andersen and Fuchs [8].

Previous kinetic studies with hydrophobic ions have been carried out with the voltage-jump technique in which a voltage is suddenly applied to the membrane and the time course of the membrane is recorded. The time resolution of this method is often insufficient at low values of membrane conductance. In this paper, we describe the application of an alternative relaxation technique, the so-called charge-pulse method. Besides a better time resolution the charge-pulse method has the additional advantage that the perturbation of the membrane can be kept to a minimum (voltage amplitudes of 1–10 mV).

In the charge-pulse experiment, the membrane is charged up to an initial voltage by a current pulse of 10–100 ns duration. At the end of the pulse, the external circuit is switched to virtually infinite resistance. Redistribution of ions within the membrane and ion transport across the membrane then lead to a decay of the voltage V_m. From the time course of V_m, information on the transport kinetics may be obtained. The charge-pulse method has occasionally been used in electrophysiology [10] and has been further developed in the course of investigations on electrode kinetics [11–14]. Recently, this method has been applied to the study of the electrical properties of lipid bilayer membranes in the presence of hydrophobic ions [15, 16] and of ion carriers [17]. In these studies, the slow phase of the voltage decay has been recorded, i.e. that part of the voltage relaxation which is mainly determined by the stationary conductance of the membrane. In this paper, we show that at an increased time resolution also the early phase of the voltage relaxation may be recorded which reflect fast concentration changes of the permeable ion in the membrane-solution interfaces and which contains information on the rates of the elementary transport steps.

THEORY

We assume that the system is in equilibrium at times $t < 0$ and that the membrane capacitance is charged up by a brief current pulse at $t = 0$ to an initial voltage V_m^0. The decay rate of the voltage V_m is related to the time course of the concentrations N' and N'' of the permeable ion in the left-hand and right-hand potential minimum (N' and N'' are expressed in mol/cm^2). The rate of change of N' and N'' is determined by the absorption rate from the aqueous phase to the membrane (rate constants k'_{am} and k''_{am}), the desorption rate (rate constant k'_{ma} and k''_{ma}), as well as by the rate of translocation across the central barrier (rate constants k'_i and k''_i):

$$\frac{dN'}{dt} = k'_{am}c - k'_{ma}N' - k'_iN' + k''_iN'' \tag{1}$$

$$\frac{dN''}{dt} = k''_{am}c - k''_{ma}N'' - k''_iN'' + k'_iN' \tag{2}$$

(compare Fig. 1). c is the concentration of the permeable ion in the aqueous phase. At equilibrium ($V_m = 0$), the interfacial concentrations are equal and are given by the partition coefficient γ:

$$N' = N'' = N_t/2 \qquad (V_m = 0) \tag{3}$$

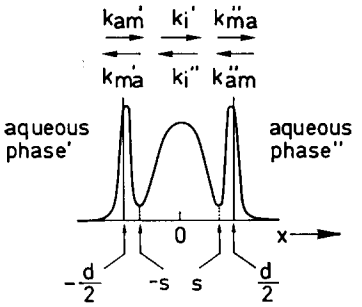

Fig. 1. Rate constants for the transport of hydrophobic ions through lipid bilayer membranes. The potential profile of the ion in the membrane is indicated schematically.

$$\frac{N_t}{2c} = \frac{k_{am}}{k_{ma}} = \gamma \frac{d}{2} \tag{4}$$

N_t is the total equilibrium concentration of the permeable ion in the membrane and d is the membrane thickness. Implicit in Eqns. 1–4 is the assumption that the ionic strength is kept sufficiently high (by addition of an inert electrolyte) so that interfacial potentials due to the adsorbed ions may be neglected. This assumption is strictly valid only at the lower concentrations c of the permeable ions which have been used in our experiments; however, it may be regarded as a reasonable approximation also at the higher values of c. In general, all the rate constants k'_{am}, k''_{am}, k'_{ma}, k''_{ma}, k'_i and k''_i are functions of voltage V_m. For convenience, we introduce the reduced voltage u:

$$u = \frac{\Psi' - \Psi''}{RT/F} = \frac{V_m}{RT/F} \tag{5}$$

Ψ' and Ψ'' are the electrical potentials in the left-hand and right-hand solution, respectively, R is the gas constant, T the absolute temperature and F the Faraday constant. We assume that the fraction α of the total voltage u drops across the central barrier; accordingly, the voltage $u(1-\alpha)/2$ acts on either interfacial barrier. In the following equations we restrict ourselves to small voltages ($|V_m| \ll 25$ mV, or $|u| \ll 1$); with in this limit, the rate constants are given by (see Appendix A):

$$k'_i = k_i[1+\alpha z u/2] \tag{6}$$

$$k''_i = k_i[1-\alpha z u/2] \tag{7}$$

$$k'_{am} = k_{am}[1+(1-\alpha)zu/4] \tag{8}$$

$$k''_{am} = k_{am}[1-(1-\alpha)zu/4] \tag{9}$$

$$k_{ma} = k_{ma}[1-(1-\alpha)zu/4] \tag{10}$$

$$k''_{ma} = k_{ma}[1+(1-\alpha)zu/4] \tag{11}$$

z is the valency of the permeable ion.

The decay of the membrane voltage V_m depends on the rates of ion transport over the two interfacial barriers and over the central barrier. In general, the distance 2 s between the two energy minima is different from the distance d over which V_m drops (compare Fig. 1). This means that geometrical and electrostatic parameters enter into the expression which relates dV_m/dt to the rates of ion transport. It is shown in Appendix A that under rather general conditions the contribution of the central barrier to dV_m/dt is proportional to the same parameter α which also describes the voltage dependence of k'_i and k''_i (Eqns. 6 and 7); likewise, the contribution of the interfacial barriers depends on the factor $(1-\alpha)/2$. The rate of change of V_m is thus given by (compare Appendix A):

$$\frac{dV_m}{dt} = -\frac{zF}{C_m}\left[\frac{1-\alpha}{2}(k'_{am}c - k'_{ma}N') + \alpha(k'_i N' - k''_i N'') + \frac{1-\alpha}{2}(k''_{ma}N'' - k''_{am}c)\right] \quad (12)$$

C_m is the electrical capacity per unit area of the membrane. In the limit $\alpha = 1$, Eqn. 12 reduces to $dV_m/dt = -J/C_m$, where $J = -zF(k'_i N' - k''_i N'')$ is the current density in the membrane. Eqns. 1, 2 and 12 (together with Eqns. 5–11) represent a system of three linear differential equations for the unknown functions $N'(t)$, $N''(t)$ and $V_m(t)$. As shown in Appendix B, the solution for $V_m(t)$ is of the form

$$V_m(t) = V_m^0(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}) \quad (13)$$

The relaxation times τ_1, τ_2 and the relaxation amplitudes a_1, a_2 are given by the following expressions:

$$\frac{1}{\tau_1} \equiv \lambda_1 = p + \sqrt{p^2 - 2k_{ma}k_i bN_t} \quad (14)$$

$$\frac{1}{\tau_2} \equiv \lambda_2 = p - \sqrt{p^2 - 2k_{ma}k_i bN} \quad (15)$$

$$a_1 = \frac{\lambda_1 - (k_{ma} + 2k_i)}{\lambda_1 - \lambda_2} \quad (16)$$

$$a_2 = 1 - a_1 \quad (17)$$

$$p = \frac{k_{ma}}{2}[1 + (1-\alpha)^2 bN_t] + k_i(1 + \alpha^2 bN_t) \quad (18)$$

$$b = \frac{z^2F^2}{4RTC_m} \quad (19)$$

$1/b$ is the surface density of elementary charges which is needed to charge the membrane capacitance C_m to a voltage of $4\,RT/z^2F$ ($1/b \cong 4 \cdot 10^{-13}$ mol/cm^2 for $|z| = 1$). From the experimentally determined values of the relaxation times τ_1, τ_2 and the relaxation amplitude a_1 the kinetic parameters k_{ma}, k_i and N_t may be evaluated according to Eqns. 14–16 if α has been determined in a separate experiment (see below). The consistency of the analysis may be tested by measuring τ_1, τ_2 and a_1 at different ion concentrations c and checking whether the calculated values of k_{ma}, k_i, and N_t/c become independent of c (this test has to be performed at sufficient low

concentrations c where saturation phenomena are absent). For a discussion of Eqns. 14–17 it is useful to introduce the steady-state time constant τ_m of the membrane which is equal to the product of the specific ohmic membrane resistance R_m^0 in the steady state, times the specific membrane capacitance C_m. It may be shown that R_m^0 is independent of α and is given by Eqn. 17 of Ketterer et al. [6]. Thus,

$$\tau_m = R_m^0 C_m = \frac{1}{bN_t}\left(\frac{1}{k_{ma}} + \frac{1}{2k_i}\right) \tag{20}$$

It is interesting to specialize Eqns. 14–17 to the case of low concentrations c of the permeable ion where the relation $bN_t \ll 1$ holds. In this limit, Eqns. 14–17 reduce to

$$\tau_1 \approx \frac{1}{k_{ma}+2k_i} \tag{21}$$

$$\tau_2 \approx \tau_m \tag{22}$$

$$a_1 \approx 0,\ a_2 \approx 1 \tag{23}$$

In this case the amplitude a_1 vanishes so that only the slow relaxation process is observed which corresponds to the steady-state decay of a voltage across a parallel combination of R_m^0 and C_m. This means that at low ion concentrations the charge-pulse experiment merely yields information on the steady-state conductance of the membrane. At higher concentrations, however, even the slow relaxation process is influenced by a redistribution of ions between the two energy minima. Accordingly, τ_2 (Eqn. 15) is in general different from $\tau_m = R_m^0 C_m$.

Finally, we apply Eqns. 14–19 to the case where the desorption rate constant k_{ma} is much smaller than the translocation rate constant k_i. For $k_{ma} \ll k_i$ Eqns. 14–19 reduce to

$$\tau_1 \approx \frac{1}{2k_i(1+\alpha^2 bN_t)} \tag{24}$$

$$\tau_2 \approx \frac{1+\alpha^2 bN_t}{k_{ma} bN_t} \tag{25}$$

$$a_1 \approx \frac{\alpha^2 bN_t}{1+\alpha^2 bN_t} \tag{26}$$

MATERIALS AND METHODS

Optically black lipid membranes were formed from glycerylmonooleate (monoolein) and from five diacyl-L-α-phosphatidylcholines with mono-unsaturated fatty acid residues of different chain lengths: dipalmitoleoyl-, dioleoyl-, di-Δ^{11}-eicosenoyl-, dierucoyl-, dinervonoylphosphatidylcholine (di-(16 : 1)-, di-(18 : 1)-, di-(20 : 1)-, di-(22 : 1)-, di-(24 : 1)-lecithin). The lecithins were prepared according to a modified

version of the synthesis of Baer et al. [18, 19]. Monoolein was obtained from NuCheck Prep, Elysian, Minn. (U.S.A.); it contained about 90 % of the α-isomer. The purity of all lipids was checked by thin-layer chromatography. *n*-Decane was from Merck (standard for gas chromatography). Tetraphenylborate (Merck, analytical grade) and dipicrylamine (Fluka, purissimum) were used as concentrated stock solutions in water or in ethanol. Small amounts of these stock solutions were added to the aqueous solutions to get a final concentration between 10^{-10} and 10^{-6} M. The ethanol content in the aqueous phase never exceeded 0.1 % (v/v). The aqueous solutions in the membrane experiments were unbuffered (pH 6) and always contained 0.1 M NaCl as an inert electrolyte. The membranes were formed usually from a 1–2 % (w/v) lipid solution in *n*-decane.

The cell used for bilayer formation was made from teflon and was surrounded by a thermostatted metal block as described earlier [19]. The circular hole in the teflon wall between the two aqueous compartments had a diameter between 1 and 3 mm; in most cases a 2 mm hole was used. The area of the black film was found to have no influence on the relaxation times and amplitudes. All measurements were performed 30 min after the membrane had turned completely black. This waiting period was needed in order to obtain stable values of the ion concentration N_t in the membrane. After this period both k_i and N_t remained constant within the limit of experimental error.

The charge pulse experiments were performed using a voltage source (output voltage 10 mV–6 V) in series with a fast FET switch. The membrane was charged up to a voltage between 0.7 and 8 mV by a current pulse of about 50 ns duration. The impedance of the switch in the "open" position was larger than 10^{12} Ω. The switch was triggered by a separate battery-operated pulse generator. The voltage decay across the membrane was measured with a high-impedance voltage-follower (Analog Devices 42 K, $R_i > 10^{12}$ Ω) and recorded with a storage oscilloscope (Tektronix 7633 with amplifier 7A22). The time resolution of the whole set-up was close to 1 μs. The charge pulse was applied to the membrane through Ag/AgCl electrodes. Because of the high impedance of membrane and detecting system pairs of electrodes had to be selected carefully in order to keep the zero-current asymmetry voltage small (usually less than 0.1 mV).

A number of tests with dummy circuits were carried out in order to check the performance of the set-up. In one set of experiments, the cell was replaced by a resistor (representing the resistance of solutions and electrodes) in series with a parallel combination of a resistor and a capacitor (the "membrane"). In a second set of test experiments, the arrangement of cell, membrane and electrodes was the same as used later in the experiments with hydrophobic ions, but the membrane was formed in a solution containing only 0.1 M NaCl and the membrane conductance (corresponding to experiments with hydrophobic ions) was simulated by introducing an external resistor R_e in parallel to the membrane. The magnitude of R_e was varied in such a way that the resulting time constants of the circuit covered the whole range of the experiments with the hydrophobic ions (50 μs to several seconds). In all these cases, the decay of the voltage V_m was purely exponential (this was checked by plotting ln V_m versus time); an example is shown in Fig. 2. In this way, it was established that the electrodes themselves did not introduce voltage transients in the time range considered here and that the distortion of the signal by the amplifier system was negligible.

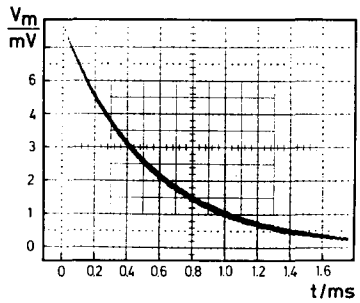

Fig. 2. Oscillographic record of a test experiment with a monoolein/*n*-decane membrane in 0.1 M NaCl (resistance of solutions plus electrodes about 250 Ω). The membrane capacitance C_M was 10.4 nF. An external resistance $R_e = 51.5$ kΩ was introduced in parallel to C_M. At time $t = 0$ a charging pulse of 50 ns duration was applied to the membrane. The decay of the membrane voltage V_m is purely exponential with a time constant of 540 μs which agrees with the calculated time constant $\tau = R_e C_M = 536$ μs.

As discussed in the previous section, the decay of V_m is governed by two relaxation times τ_1 and $\tau_2 > \tau_1$:

$$V_m(t) = V_1 \exp(-t/\tau_1) + V_2 \exp(-t/\tau_2) \tag{27}$$

Under most conditions of our experiments, τ_1 and τ_2 were found to be sufficiently different (by a factor of the order of 100 or more). In this case τ_1 and τ_2 as well as V_1 and V_2 could be obtained simply by recording $V_m(t)$ at two different time scales and by plotting the voltage signals from both relaxation processes on a logarithmic scale versus time. In the other cases, the relaxation times were evaluated in the following way. For long times ($t \gg \tau_1$) the relation $V_m(t) \approx V_2 \exp(-t/\tau_2)$ holds; accordingly, a plot of ln $V_m(t)$ at long times t gives a straight line from which τ_2 and V_2 are obtained. If then the logarithm of the difference $V_m(t) - V_2 \exp(-t/\tau_2)$ is plotted as a function of t, again a straight line results which gives τ_1 and V_1.

The voltage-jump experiments were carried out as described earlier [6].

RESULTS

In all cases studied here, two relaxation processes with widely separated time constants have been observed. An example is given in Fig. 3 where the voltage decay after a charge pulse is shown at two different time scales. A logarithmic plot of the data of Fig. 3 is represented in Fig. 4; it is seen that both processes are purely exponential within the experimental error limits. This behaviour was found in the experiment with tetraphenylborate (except at the lowest concentration); in the presence of dipicrylamine, however, deviations in the late process were observed, the voltage at long times decaying slower than expected from an exponential law.

The occurrence of two decay processes with distinctly separated relaxation times may be explained, in principle, by the assumption that the rate constant of translocation, k_i, is much larger than the rate constant of desorption, k_{ma} or that the diffusion in the aqueous phase is slow and therefore rate limiting. In both cases the fast process arises from the translocation of permeable ions across the central barrier, and the slow process from the exchange between the interfacial adsorption sites and

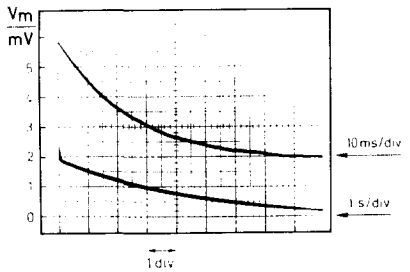

Fig. 3. Decay of the membrane voltage V_m after a charge-pulse. Dioleoyllecithin/*n*-decane membrane in 0.1 M NaCl and $3 \cdot 10^{-8}$ M tetraphenylborate, $T = 25$ °C. At time $t = 0$, the membrane capacitance was charged up to a voltage of 5.9 mV by a current pulse of 50 ns duration. The decay of V_m was recorded with two different sweep times as indicated on the right side of the oscillogram.

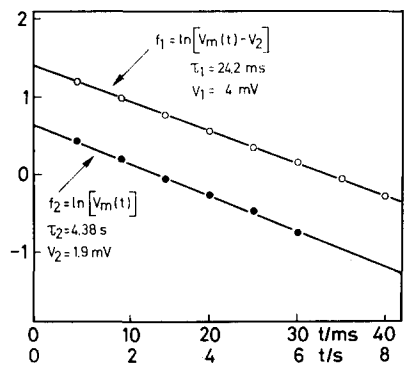

Fig. 4. Logarithmic plot of the data of Fig. 3 at two different time scales. V_m is expressed in mV.

the aqueous solutions. In accordance with the results of voltage-jump experiments [6–8] we indeed believe that the fast process is governed by the redistribution of ions between the two potential energy minima (rate constant k_i). It is unlikely, however, that the slow process is exclusively determined by exchange between the adsorption sites and water. This is because the latter process is limited by the diffusion rate in the unstirred aqueous layers adjacent to the membrane. Indeed, it may be shown (Appendix C) that in the charge-pulse experiment the characteristic time for the diffusional exchange of ions between water and the interface is at least of the order of $\tau = \beta^2/D_w$, where $\beta = N_t/2_c$ is the interfacial distribution coefficient and D_w is the diffusion coefficient of the ion in water (Hladky, S. B., personal communication). From the analysis of the fast process (see below), β is found to be about $2 \cdot 10^{-2}$ cm for tetraphenylborate (dioleoyllecithin membranes, $c \leqslant 300$ nM). With $D_w = 5 \cdot 10^{-6}$ cm^2/s [35], τ becomes about 80 s whereas the experimentally observed time constants of the slow process (τ_2) are in the range of 2–4 s (Table II). For dipicrylamine the discrepancy between τ_2 and τ is even larger. For this reason, only the first relaxation process can be analyzed in the terms of the model described in the previous section. As this process is much faster than all subsequent decay processes, one can use the approximate equations 24 and 26 for the evaluation of k_i and N_t.

For the calculation of k_i and N_t from Eqns. 24 and 26, the parameter α has to be known. α has been determined from voltage-jump experiments [6–8] by analyzing the voltage dependence of the initial membrane conductance λ or the voltage dependence of the relaxation time τ of the membrane current. With the same notation as used in Appendix A it is easily shown that the following relations hold (see also ref. 8):

$$\left(\frac{\lambda}{\lambda_0}\right)_{t=0} = \frac{k_i' - k_i''}{z\alpha u k_1} = \frac{\sinh(z\alpha u/2)}{z\alpha u/2} f(u) \tag{29}$$

$$\frac{\tau_0}{\tau} \approx \frac{k_i' + k_i''}{2k_i} = f(u)\cosh(z\alpha u/2) \tag{30}$$

λ_0 and τ_0 are the ohmic limits ($[zu] \ll 1$) of the specific membrane conductance λ and the relaxation time τ, respectively. As pointed out by Haydon and Hladky [30] and by Andersen and Fuchs [8] the function $f(u)$ represents a small correction at moderate voltages ($[V_m] < 200$ mV, or $[u] \leq 8$) and may be approximated by $f(u) \approx \exp(-\omega u^2)$; ω is a function of membrane thickness d and ranges between 0.0072 ($d = 4.8$ nm) and 0.0089 ($d = 6.5$ nm) in our experiments [8].

The results of the voltage-jump experiments are summarized in Table I. In the case of tetraphenylborate where the membrane conductance is relatively low,

TABLE I

RESULTS OF VOLTAGE-JUMP EXPERIMENTS WITH MEMBRANES MADE FROM MONOOLEIN AND FROM LECITHINS WITH DIFFERENT MONO-UNSATURATED FATTY-ACID RESIDUES

The measurements have been carried out 20–30 min after the membranes have been turned completely black. The aqueous phases contained 0.1 M NaCl, $T = 25$ °C. The values of the membrane thickness d have been taken from refs. 20 and 21. n is the number of membranes used for each set of experimental conditions. Mean values are given together with the standard deviations. See text for further explanations.

Fatty acid	d (nm)	n	τ_0 (ms)	λ_0 ($t = 0$) (μS/cm^{-2})	α (from τ)	α (from λ)	k_1 (s^{-1})	N_t (pmol/cm^{-2})
100 nM tetraphenylborate, lecithin membranes								
16:1	4.8	5	35±10	157± 28		–	9 ±2*	1.1 ±0.2
18:1	5.0	5	74±11	43± 9	$0.88 \leq \alpha \leq 0.95$		7.2±1.1	3.0 ±0.5
20:1	5.2	5	180±15	17± 5	$0.86 \leq \alpha \leq 0.97$		2.9±0.7	2.9 ±0.3
22:1	5.7	6	265±20	10± 2	$0.85 \leq \alpha \leq 0.95$		1.8±0.4	3.3 ±0.5
24:1	6.5	5	350±40	11± 2	$0.88 \leq \alpha \leq 0.94$		1.3±0.3	4.1 ±0.4
10 nM dipicrylamine, lecithin membranes								
16:1	4.8	5	0.51±0.02	970±150	0.89±0.02	0.98±0.01	987±39	0.53±0.11
18:1	5.0	6	1.11±0.21	748±100	0.87±0.03	0.96±0.02	462±95	0.89±0.14
20:1	5.2	5	2.83±0.40	380± 45	0.89±0.02	0.98±0.01	175±17	1.18±0.25
22:1	5.7	4	6.49±0.36	184± 25	0.87±0.02	0.98±0.01	77± 7	1.22±0.25
24:1	6.5	5	15.2 ±1.4	72± 10	0.89±0.01	0.97±0.02	33± 4	1.18±0.20
10 nM dipicrylamine, monoolein membranes								
18:1	4.8	5	0.70±0.10	75± 15	0.78±0.04	0.85±0.05	720±140	0.056±0.013

* This value was calculated using $k_{ma} \approx 10$ s^{-1}.

TABLE II

RESULTS OF CHARGE-PULSE RELAXATION EXPERIMENTS WITH DIOLEOYLLECITHIN AND MONOOLEIN MEMBRANES

0.1 M NaCl plus various concentrations of tetraphenylborate or dipicrylamine, 25 °C. Together with the mean values the standard deviations are indicated. n is the number of membranes used for each set of experimental conditions. For the calculation of the partition coefficient γ (Eqn. 4) a membrane thickness $d = 5.0$ nm for dioleoyllecithin and $d = 4.8$ nm for monoolein has been used [20, 21].

c (nM)	n	τ_1 (ms)	τ_2 (s)	a_1	k_1 (s^{-1})	N_t (pmol/cm^{-2})	$\gamma \times 10^3$
Tetraphenylborate; dioleoyllecithin membranes							
10	7	48 ±7	—	0.36±0.06	6.8±1.5	0.28 ±0.12	56
30	6	23 ±6	4.0 ±1.5	0.67±0.05	7.0±0.2	1.00 ±0.16	67
100	6	10 ±2	2.6 ±1.8	0.87±0.05	6.5±1.3	3.3 ±0.3	66
300	8	4.2±1.3	2.8 ±1.2	0.95±0.02	6.0±1.8	9.4 ±2.3	63
1000	7	4.0±1.1	3.2 ±1.0	0.97±0.02	3.7±1.1	16.0 ±2.9	32
3000	6	3.9±1.2	2.6 ±1.3	0.98±0.01	2.7±1.2	24.2 ±4.0	16
Dipicrylamine; dioleoyllecithin membranes							
1	6	0.98 ±0.17	—	0.16±0.04	425± 72	0.088±0.025	177
3	7	0.72 ±0.13	—	0.35±0.07	460± 90	0.25 ±0.05	167
10	15	0.41 ±0.09	0.21±0.11	0.65±0.04	431± 53	0.86 ±0.12	173
30	10	0.17 ±0.04	0.23±0.10	0.86±0.05	412±110	2.9 ±0.6	190
100	9	0.086±0.018	0.18±0.09	0.94±0.03	352± 42	7.3 ±0.5	145
300	5	0.094±0.022	0.23±0.11	0.95±0.02	268± 44	8.8 ±1.0	59
Dipicrylamine; monoolein membranes							
10	5	0.65 ±0.10	1.36±0.75	0.09±0.01	675±105	0.062±0.008	13

TABLE III

RESULTS OF CHARGE-PULSE RELAXATION EXPERIMENTS WITH MEMBRANES MADE FROM LECITHINS WITH DIFFERENT MONO-UNSATURATED FATTY ACID RESIDUES

0.1 M NaCl plus 100 nM tetraphenylborate or 10 nM dipicrylamine, 25 °C. The values of the membrane thickness d have been taken from ref. 20. See legend of Table II for further explanations.

Fatty acid	d (nm)	n	τ_1 (ms)	τ_2 (s)	a_1	k_1 (s^{-1})	N_t (pmol/cm^{-2})	$\gamma \times 10^3$
100 nM tetraphenylborate								
16:1	4.8	6	19±3	0.14±0.02	0.57±0.04	8.3±1.0	0.97±0.15	20
18:1	5.0	6	10±2	2.6 ±1.8	0.87±0.05	6.5±1.3	3.3 ±0.3	66
20:1	5.2	5	28±2	19	0.84±0.04	2.9±0.6	2.5 ±0.3	48
22:1	5.7	5	37±5	22	0.89±0.03	1.5±0.3	1.5 ±0.6	61
24:1	6.5	5	48±5	25	0.91±0.02	0.9±0.2	3.8 ±0.5	59
10 nM dipicrylamine								
16:1	4.8	8	0.32±0.02	0.05±0.02	0.45±0.05	856±43	0.39±0.07	82
18:1	5.0	15	0.41±0.09	0.21±0.11	0.65±0.04	431±53	0.86±0.12	173
20:1	5.2	6	0.84±0.07	16	0.71±0.06	172±35	1.08±0.20	208
22:1	5.7	7	1.42±0.07	12	0.74±0.03	88± 9	1.16±0.15	203
24:1	6.5	7	2.85±0.30	20	0.79±0.03	38± 6	1.29±0.10	200

the evaluation of α from τ_0/τ or from λ/λ_0 was rather inaccurate so that only upper and lower limits could be given; in the further calculations a value of $\alpha = 0.9$ was used throughout for tetraphenylborate. In the experiments with dipicrylamine, the values of α as determined from λ/λ_0 were consistently higher than the values from τ_0/τ; the origin of this difference is unclear. In this case, the mean of both values has been used for the calculation of the rate constants. For all systems studied here, except for monoolein membranes, α was found to be close to unity; the introduction of α into the model represents a relatively small correction in these cases. With other lipids, considerably smaller values of α may be obtained, however, [8]. Furthermore in the experiments with lecithins of different chain length (Table I) α was found to be independent of membrane thickness. This observation can be explained by the assumption that the binding site of the ion moves slightly towards the center of the membrane as the chain length is increased. For comparison with previous data [6] and with the results of the present charge-pulse experiments we have also evaluated from the voltage-jump measurements the rate constant k_i and the concentration N_t of adsorbed ions, according to the analysis of Ketterer et al. [6]. These data are included in Table I.

From the experimental values of τ_1, a_1 and α the rate constant k_i and the partition coefficient $\gamma = N_t/cd$ have been evaluated according to Eqns. 24 and 26 (Table II and III). The results of the previous voltage-jump relaxation studies are compared with the present charge-pulse data in Table IV. It is seen that the values of the parameters determined by the two different methods are in satisfactory agreement (compare also Table I).

TABLE IV

COMPARISON BETWEEN THE VOLTAGE-JUMP RELAXATION DATA OF KETTERER et al. [6] AND THE RESULTS OF THE PRESENT CHARGE-PULSE RELAXATION STUDIES

Dioleoyllecithin membranes, 25 °C. Aqueous concentrations of dipicrylamine or tetraphenylborate between 10 and 100 nM.

Ion	k_1 (s^{-1})		$\gamma \times 10^3$	
	This study	Ketterer et al.	This study	Ketterer et al.
Tetraphenylborate	6.5	9	66	120
Dipicrylamine	430	380	173	80

For both dipicrylamine and tetraphenylborate k_i becomes smaller at high concentrations, as shown in Table II. A similar result has been obtained recently by Bruner [7] from voltage-jump studies with dierucoyllecithin membranes in the presence of dipicrylamine. For both ions, the partition coefficient γ decreases with increasing aqueous ion concentration c at large values of c (compare Fig. 5). This saturation behaviour, together with the decrease of k_i with increasing c, seems to explain the peculiar concentration dependence of membrane conductance in the presence of dipicrylamine or tetraphenylborate [6–8].

An interesting result of this study concerns the influence of chain length on the kinetics of ion transport [7, 19, 22]. Variation of the fatty acid chain length of the lecithin from C_{16} to C_{24} decreases k_i by a factor of about 9 in the case of tetra-

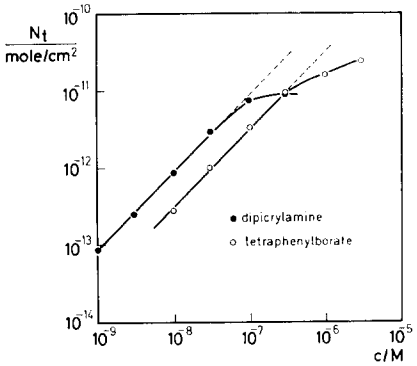

Fig. 5. Total concentration N_t of hydrophobic ions per unit area of the membrane as a function of the aqueous ion concentration c. Dioleoyllecithin membranes, 25 °C. The aqueous phase contained 0.1 M NaCl.

phenylborate and by a factor of about 17 in the case of dipicrylamine (Table III). This effect on k_i may be qualitatively explained by the increase in the hydrocarbon layer thickness d of the membrane.

The temperature dependence of the kinetic parameters is summarized in Table V for dipicrylamine in dipalmitoleoyllecithin and dioleoyllecithin membranes. γ is found to be a decreasing function of temperature, whereas k_i strongly increases with temperature.

The partition coefficient γ of dipicrylamine is more than ten times smaller in monoolein membranes as compared with dioleoyllecithin membranes (Table II). A possible explanation for this large difference is the existence of a dipolar potential in the membrane-solution interface which tends to make the membrane interior positive with respect to the aqueous solution. As the absolute value of the dipolar potential is smaller for monoolein membranes than for dioleoyllecithin membranes [25], the partitioning of anions into the membrane is favoured in the latter case.

TABLE V

TEMPERATURE DEPENDENCE OF THE KINETIC PARAMETERS OF DIPICRYLAMINE IN MEMBRANES MADE FROM TWO DIFFERENT LECITHINS

The aqueous phase contained 0.1 M NaCl and 10 nM dipicrylamine. See legend of Table II for further explanations. For the parameter α (Eqns. 14–16) the values obtained at 25 °C have been used.

T (°C)	n	τ_1 (ms)	τ_2 (ms)	a_1	k_i (s^{-1})	N_t (pmol/cm^{-2})	$\gamma \times 10^3$
Dipalmitoleoyllecithin							
10	7	0.82±0.03	30± 15	0.53±0.03	280± 23	0.52±0.05	108
25	8	0.32±0.02	50± 20	0.45±0.05	856± 43	0.39±0.07	82
40	6	0.17±0.02	70± 30	0.39±0.04	1790±300	0.31±0.08	67
Dioleoyllecithin							
10	5	0.60±0.07	130± 75	0.74±0.03	212± 9	1.20±0.20	240
25	15	0.41±0.09	210±110	0.65±0.04	431± 53	0.86±0.12	173
40	6	0.24±0.05	480±180	0.52±0.05	1090±284	0.52±0.08	104

This view is consistent with the observation that in the presence of hydrophobic cations monoolein membranes have a higher conductance than dioleoyllecithin membranes, whereas the reverse is true for hydrophobic anions (Benz, R., to be published).

DISCUSSION

The charge-pulse relaxation technique has been used so far only at limited resolution for the study of quasi-stationary conductance processes in thin lipid membranes. In this paper we have shown that a more sensitive version of this method may give detailed information on the transport kinetics of hydrophobic ions in lipid membranes. In a parallel study we have applied the charge-pulse technique to the kinetic analysis of carrier-mediated ion transport [23]. The principal advantage of this method, compared with the conventional voltage-jump relaxation technique, is the increased time resolution at low membrane conductivities. Furthermore, charge-pulse experiments may easily be performed at low voltage-amplitudes (of the order of 1–10 mV) where distortions of the membrane structure are minimal.

The results of the present analysis support the previously proposed mechanism which describes the transport of hydrophobic ions through lipid bilayer membranes as occurring in three subsequent steps: adsorption to the interface, translocation across the central barrier to the opposite interface, and desorption into the aqueous solution. From the analysis of the fast voltage decay after a charge pulse, the rate constant k_i of translocation, as well as the partition coefficient γ between water and the membrane could be determined. The value of the desorption rate constant K_{ma}, however,had to be left open, the principal difficulty in the evaluation of k_{ma} being the slow diffusion rate in water. Whereas diffusion theory predicts time constants of the order of 100 s for the slow voltage decay process, the observed decay times of this process were much smaller. This means that there must be a different process that is able to supply charge carriers to the membrane at a much higher rate than diffusion of the hydrophobic ions in water. The nature of this process remains unclear, however. One possibility is that the concentration difference of the hydrophobic anion A^- which has been built up in the membrane during the fast process decays by a back transport of the protonated form HA or of an ion-paired form M^+A^- where M^+ is the cation of the added inert electrolyte (Hladky, S. B., personal communication). We have carried out a few experiments with aqueous solutions of different composition which, however, do not support this possibility. In the pH range between 4 and 8, the time course of the voltage decay was found to be independent of pH within the limits of error for both dipicrylamine and tetraphenylborate. Likewise, variation of NaCl concentration between 1 and 10^{-2} M or substitution of NaCl by LiCl failed to produce any significant effect on $V_m(t)$. These experiments also make it unlikely that the supply of A^- is buffered in the aqueous phase, for instance by the reaction $H^+ + A^- \rightleftarrows HA$. There remains the possibility that the intrinsic membrane conductance is increased in an unspecific way by the presence of the hydrophobic ions. In order to give a decay time of $\tau_m = 0.1$ s, the intrinsic membrane conductance needs to be $\lambda = C_m/\tau_m \cong 4 \cdot 10^{-6}$ S/cm^{-2}. This is a rather low conductance which could originate from slight perturbations of membrane structure.

From the distribution coefficient $\beta = N_t/2_c$ of the hydrophobic ion between

water and adsorption site the free energy ΔG of adsorption may be estimated according to

$$\Delta G \cong -RT \ln (\beta/l_a) \tag{31}$$

l_a is a characteristic length which is approximately equal to the distance between adjacent energy minima of the ion in the aqueous phase. ($l_a c = N_a$ is the concentration in mol/cm^2 of the ion in an energy minimum on the aqueous side of the membrane so that $\beta/l_a = N_t/2N_a$). With $l_a \cong 1$ nm, ΔG becomes approx. -29 kJ/mol. ΔG may be compared with the free energy change ΔG^* associated with the transfer of tetraphenylmethane (which may be considered as the electrically neutral equivalent of tetraphenylborate) from water into a hydrocarbon.

From the approximate value of the hydrocarbon/water partition coefficient of tetraphenylmethane, $\gamma^* \cong 10^6$ [8], ΔG^* may be estimated to be $\Delta G^* = -RT \ln \gamma^* \cong -34$ kJ/mol. A difference between ΔG and ΔG^* is to be expected because ΔG contains, in addition to ΔG^*, at least two other energy terms, namely, the contribution of the image-force interaction which tends to make ΔG less negative than ΔG^*, and the contribution of the dipole potential in the membrane-solution interface [25] which tends to make ΔG more negative than ΔG^*.

For dipicrylamine and dioleoyllecithin membranes, the adsorption energy is estimated to be $\Delta G \cong -32$ kJ/mol. From the data of Table V the enthalpy of adsorption, ΔH, may be evaluated according to $\Delta H = \Delta G + T\Delta S = RT^2(\mathrm{d} \ln \gamma/\mathrm{d}T)$. This gives $\Delta H \cong -14$ kJ/mol and $T\Delta S = 18$ kJ/mol. The positive value of ΔS means that the adsorption of dipicrylamine to the membrane is partly entropy driven, a conclusion that has already been drawn by Bruner [7].

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APPENDIX A

Influence of barrier shape on the voltage relaxation

(a) *Derivation of Eqns.* 6–11. We assume that the potential energy profile of the permeable ion has two minima at positions $x = \pm s$ which are separated by a barrier of arbitrary shape (Fig. 1). The ion flux Φ over the barrier is then given by the generalized Nernst-Planck equation [27]:

$$\Phi = -D\left(\frac{\mathrm{d}C}{\mathrm{d}x} + zC\frac{\mathrm{d}\phi}{\mathrm{d}x} + C\frac{\mathrm{d}w}{\mathrm{d}x}\right) \tag{A.1}$$

$D(x)$ is the diffusion coefficient and $C(x)$ the concentration of the permeable ion in the membrane; $\phi(x) = F\Psi(x)/RT$ is the reduced electrical potential and $w(x)$ the potential energy (expressed in units of RT) of the ion at $u = 0$. $w(x)$ accounts for the combined effects of the electrical image force, the hydrophobic interaction of the ion with the membrane, the dipole potential in the membrane-solution interface and energy terms arising from the distortion of the membrane structure around the ion. We locate the zero point of $w(x)$ in the aqueous phase and the zero point of $\phi(x)$ at $x = 0$.

Introducing $h(x) \equiv \exp[z\phi(x)+w(x)]$, Eqn. A1 may be integrated to give

$$\Phi = \frac{C(-s)h(-s)-C(s)h(s)}{\int_{-s}^{s} \frac{h(x)}{D(x)}\,dx} \tag{A2}$$

We now assume that the membrane is symmetric and behaves as a linear dielectric; this means that both $w(x)$ and $\phi(x)$ are symmetric functions with respect to $x = 0$. We may therefore set $w(-s) = w(s) = w_m$ and $\phi(-s) = \alpha u/2$, $\phi(s) = -\alpha u/2$. The voltage drop between the two adsorption planes is then equal to αu; as the dielectric constant is a function of position, α is, in general, different from $(1-2s/d)$. We further assume that the energy minima are deep $(-w_m \gg 1)$ and that the central barrier is high $([w(0)-w_m] \gg 1)$. Under the conditions where most of the ions are located in the vicinity of $x = \pm s$, it is reasonable to introduce interfacial concentrations N' and N'' which may be set proportional to the volume concentrations at $x = \pm s$ [8]: $N' = \vartheta C(-s)$, $N'' = \vartheta C(s)$. Accordingly, the equation for the ion flux assumes the form

$$\Phi = k_i' N' - k_i'' N'' \tag{A3}$$

Comparing Eqns. A2 and A3 it is seen that

$$k_i' = k_i e^{z\alpha u/2} f(u) \tag{A4}$$

$$k_i'' = k_i e^{-z\alpha u/2} f(u) \tag{A5}$$

where the function

$$f(u) = \frac{\int_{-s}^{s} \frac{\exp[w(x)]}{D(x)}\,dx}{\int_{-s}^{s} \frac{\exp[w(x)+z\varphi(x)]}{D(x)}\,dx} \tag{A6}$$

accounts for the shape of the central barrier. k_i is the value of k'_i nad k''_i for $u = 0$ and is given by:

$$k_i = \frac{1}{\vartheta \int_{-s}^{s} \frac{\exp[w(x)-w_m]}{D(x)}\,dx} \tag{A7}$$

Similar treatments of ion transport over barriers may be found elsewhere [8, 27–34]. We now apply Eqns. A4–A6 to the limiting case $|zu| \ll 1$, $|z\phi| \ll 1$ where the approximation $\exp(z\phi) \approx 1+z\phi+(z\phi)^2/2$ may be used. As $\exp w(x)$ is an even function and $\varphi(x)$ an odd function of x, the relation

$$\int_{-s}^{s} \phi(x) \exp[w(x)]dx = 0 \tag{A8}$$

holds. Writing $\phi(x) = u \cdot \chi(x)$ where $\chi(x)$ is independent of u, it is easy to show that $f(u)$ has the expansion

$$f(u) \approx 1 + Bu^2 \tag{A9}$$

where B is a constant. This proves Eqns. 6 and 7 and also, by a completely analogous argument, Eqns 8–11. It is thus seen that up to terms of the order of u the rate constants k'_i and k''_i depend only on the effective voltage αu but are independent of the detailed shape of the barrier.

(b) *Derivation of Eqn. 12.* For a derivation of Eqn. 12 we consider the general case of a dielectric film in which the dielectric constant is a function of the coordinate x normal to the film surface. We assume that minima of the potential energy of the permeable ion are located at position $x_\nu (\nu = 1,2,\ldots,n)$ and that the membrane surfaces are located at x_0 and x_{n+1}. If in the absence of permeable ions an external voltage V_m is applied between x_0 and x_{n-1}, then the fraction $\alpha_\nu V_m$ of this voltage drops across the ν-th barrier which separates the minima at $x_{\nu-1}$ and x_ν:

$$\alpha_\nu V_m = \int_{x_{\nu-1}}^{x_\nu} E(x)\mathrm{d}x \tag{A10}$$

$E(x)$ is the electric field strength due to the external voltage. In this case where the interior of the film does not contain free charges, the dielectric displacement D^* is independent of x and is equal to $C_m V_m$:

$$D^* = \varepsilon_0 \varepsilon(x) E(x) = C_m V_m \tag{A11}$$

$\varepsilon(x)$ is the dielectric constant and ε_0 is the permittivity of free space. Eqn. A11 together with Eqn. A10 yields

$$a_\nu = C_m \int_{x_{\nu-1}}^{x_\nu} \frac{\mathrm{d}x}{\varepsilon_0 \varepsilon(x)} \tag{A12}$$

In the special case $\varepsilon(x) \equiv \varepsilon$, Eqn. A12 reduces to $\alpha_\nu = (x_\nu - x_{\nu-1})/d$, where d is the membrane thickness.

We now allow ions to adsorb to the planes located at positions x_ν but assume that the amount adsorbed remains low so that ion-ion interactions may be neglected. The dielectric displacement D^* now varies with x but remains constant within the intervals $(x_{\nu-1} < x < x_\nu)$:

$$D^*(x) \equiv D^*_\nu \qquad (x_{\nu-1} < x < x_\nu) \tag{A13}$$

The external voltage V_m is given by

$$V_m = \int_{x_0}^{x_{n+1}} E(x)\mathrm{d}x = \int_{x_0}^{x_{n+1}} \frac{D^*(x)}{\varepsilon_0 \varepsilon(x)}\mathrm{d}x = \sum_{\nu=1}^{n+1} D^*_\nu \int_{x_{\nu-1}}^{x_\nu} \frac{\mathrm{d}x}{\varepsilon_0 \varepsilon(x)} \tag{A14}$$

Comparison with Eqn A12 yields

$$V_m = \frac{1}{C_m} \sum_{\nu=1}^{n+1} \alpha_\nu D^*_\nu \tag{A15}$$

Accordingly, the rate of change of V_m after a charge-pulse is related to the time derivatives $\mathrm{d}D^*\nu/\mathrm{d}t$ which may be obtained by the following consideration. As the electric current in the external circuit vanishes during the decay of V_m, the sum of the ionic

current $J = zF\Phi$ and of the displacement current $J_D = dD^*/dt$ must be zero. Applying this condition to the interval $(x_{\nu-1} < x < x_\nu)$ we get

$$zF\Phi_\nu + \frac{dD_\nu^*}{dt} = 0 \tag{A16}$$

Φ_ν is the net ion flux density over the ν-th barrier. Combining Eqn. A16 with Eqn. A15 we finally obtain:

$$\frac{dV_m}{dt} = -\frac{zF}{C_m}\sum_{\nu=1}^{n+1}\alpha_\nu\Phi_\nu \tag{A17}$$

We now apply this equation to the symmetrical potential profile of Fig. 1, setting $\alpha_2 = \alpha$, $\alpha_1 = \alpha_3 = (1-\alpha)/2$. In this case the ion fluxes are given by $\Phi_1 = k'_{am}c - k'_{ma}N'$, $\Phi_2 = k'_iN' - k''_iN''$, $\Phi_3 = k''_{ma}N'' - k''_{am}c$. This proves Eqn. 12.

APPENDIX B

Derivation of Eqns. 13–17

Introducing the variables

$r = (N'+N''-N_t)/N_t$

$y = (N'-N'')/N_t$

$v = zu$

Eqns. 1, 2 and 12 may be written in the form (using Eqns. 3–11):

$$\frac{dr}{dt} = -k_{ma}r \tag{B1}$$

$$\frac{dy}{dt} = A_{11}y + A_{12}v \tag{B2}$$

$$\frac{dv}{dt} = A_{21}y + A_{22}v \tag{B3}$$

where

$$A_{11} = -(k_{ma}+2k_i); \qquad A_{12} = (1-\alpha)\frac{k_{ma}}{2} - \alpha k_i$$

$$A_{21} = 2bN_t[(1-\alpha)k_{ma} - 2\alpha k_i]; \quad A_{22} = -bN_t[(1-\alpha)^2k_{ma} + 2\alpha^2k_i]$$

In derivating Eqns. B1–B3 we have neglected terms proportional to rv or yv which are small to the second order. Eqn. B1 may be integrated at once to give

$$r(t) = \text{const} \cdot e^{-k_{ma}t} \tag{B4}$$

But from the boundary condition $r(o) = 0$ we see that $r(t) \equiv 0$, which means that the total concentration $(N'+N'')$ of the ion does not change during the experiment. This is a consequence of our assumption that the perturbation of the system is small $(|V_m^0| \ll RT/F)$ which allows the linearisation of the original differential equations.

At larger perturbations, however, the system is described by the complete set of three differential equations and, accordingly, by three relaxation times.

The solution of Eqns. B2 and B3 obeying the boundary conditions $y(o) = 0$, $v(o) = v_0$ is obtained by standard methods and has the form:

$$v(t) = v_0(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}) \tag{B5}$$

$$y(t) = \eta(e^{-t/\tau_1} - e^{-t/\tau_2}) \tag{B6}$$

where η is a constant. τ_1, τ_2 and $a_1 = 1 - a_2$ are given by Eqns. 14–16. With $v = zV_m F/RT$ and $v_0 = zV_m{}^0 F/RT$, Eqn. B5 transforms into Eqn. 13.

APPENDIX C

Diffusion polarisation in the charge-pulse experiment

In this section we estimate the maximum possible rate of exchange between the adsorption sites at the membrane surface and the aqueous solution in a charge-pulse experiment. For this purpose we consider the following case: (a) The translocation of permeable ions across the central barrier is fast compared with the exchange between adsorption sites and the bulk aqueous solution. (b) The rates of adsorption and desorption are much larger than the diffusion rate in water so that there is always partition equilibrium at the interface. (c) The perturbation of the membrane by the charge-pulse is small ($|V_m{}^0| \ll RT/F$).

Under these circumstances the initial voltage $V_m{}^0$ after the charge-pulse decays in a fast process (time constant τ_1) to a value $V_2 = (1 - a_1)\ V_m{}^0$ (compare Eqns. 24 and 26). Thereafter the membrane is in a quasi-stationary state so that the concentrations N' and N'' of the ion in the interfaces are given by the Boltzmann relation:

$$\frac{N'(t)}{N''(t)} = e^{-zu(t)} \tag{C1}$$

For a symmetrical system we may write:

$$N'(t) = \frac{N_t}{2}[1 + h(t)] \tag{C2}$$

$$N''(t) = \frac{N_t}{2}[1 - h(t)] \tag{C3}$$

If y is the distance normal to the membrane, the aqueous concentrations c' and c'' of the permeable ion on the left and right side are given by

$$c'(y,t) = c[1 + g(y,t)] \tag{C4}$$

$$c''(y,t) = c[1 - g(y,t)] \tag{C5}$$

From assumption b we see that

$$\frac{N'(t)}{c'(_o,\ t)} = \frac{N''(t)}{c''(_o,\ t)} = \frac{N_t}{2c} \equiv \beta \tag{C6}$$

or

$$h(t) = g(o,t) \tag{C7}$$

Furthermore, for small perturbations ($|u| \ll 1$) Eqns. C1–C3 yield

$$h(t) \approx -\frac{z}{2} u(t) \tag{C8}$$

The diffusion equation in the aqueous phase (left side) reads

$$\frac{\partial c'}{\partial t} = D_w \frac{\partial^2 c'}{\partial y^2} \tag{C9}$$

D_w is the diffusion coefficient of the permeable ion in water. Using Eqn. C4, this relation assumes the form

$$\frac{\partial g}{\partial t} = D_w \frac{\partial^2 g}{\partial y^2} \tag{C10}$$

This equation has to fulfill the boundary condition

$$g(o,o) \equiv g_0 = -\frac{z}{2} u_2 \tag{C11}$$

(compare Eqns. C7 and C8). $u_2 = V_2 F/RT$ is the voltage at zero time, i.e. the voltage after the fast initial decay. A second boundary condition is obtained in the following way: the decay rate of the membrane voltage V_m is related to the flux density Φ of ions across the central barrier and to the specific membrane capacity C_m:

$$\frac{dV_m}{dt} = \frac{RT}{F} \frac{du}{dt} = -\frac{zF}{C_m} \Phi \tag{C12}$$

This relation follows from Eqn. 12 with the approximation $\alpha \approx 1$. Φ is given by the condition that the rate of change of N' is equal to the flux of ions from the aqueous solution to the interface minus the flux of ions across the central barrier:

$$\frac{dN'}{dt} = D_w \left(\frac{\partial c'}{\partial y}\right)_{y=0} - \Phi \tag{C13}$$

Combination of Eqns. C4, C6–C8, C12 and C13 then yields (with $b \equiv z^2F^2/4RTC_m$):

$$\left(\frac{\partial g}{\partial t}\right)_{y=0} = \frac{bN_t}{1+bN_t} \cdot \frac{D_w}{\beta} \left(\frac{\partial g}{\partial y}\right)_{y=0} \tag{C14}$$

The solution of the diffusion equation C10 which fulfills the boundary conditions C11 and C14 may be obtained by the method of Laplace transformations [35]:

$$g(y,t) = g_0 \exp\left(\frac{y}{\sqrt{\tau D_w}} + \frac{t}{\tau}\right) \operatorname{erfc}\left(\frac{\beta y}{2\sqrt{D_w t}} + \sqrt{\frac{t}{\tau}}\right) \tag{C15}$$

$$\tau = \left(1 + \frac{1}{bN_t}\right)^2 \frac{\beta^2}{D_w} \tag{C16}$$

$$\operatorname{erfc} \rho = 1 - \frac{2}{\sqrt{\pi}} \int_0^{\rho_0} e^{-\eta^2} d\eta \tag{C17}$$

Using Eqns. C7, C8 and C11 the time course of the voltage u may be calculated from Eqn. C15:

$$u(t) = u_2 \exp\left(\frac{t}{\tau}\right) \operatorname{erfc}\left(\sqrt{\frac{t}{\tau}}\right) \tag{C18}$$

According to Eqn. C18, the voltage decays monotonously from the initial value u_2 to zero. From the tabulated values of the function $\exp(\rho^2)\operatorname{erfc}\rho$ [35] it is seen that τ is the time for the decay to about one half of the initial voltage ($u/u_2 \cong 0.43$ for $t = \tau$).

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