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Application of a fast charge-pulse technique to study the effect of the dipolar substance 2,4-dichlorophenoxyacetic acid on the kinetics of valinomycin mediated K^+-transport across monoolein membranes.

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Abstract

We report on a modified charge-pulse relaxation technique applied at planar lipid membranes. The method has an improved time resolution of 20–30 ns. It is based on the capacitive coupling of a voltage-jump to the membrane. The method was used to study the fast relaxation processes induced by valinomycin/K^+ in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D). The change of the rate constants of the ion carrier valinomycin was analysed as a consequence of the adsorption of the dipolar substance 2,4-D to the membrane/water interface of monoolein membranes. The effect of 2,4-D can be explained solely via the influence of the introduced dipole potential, V_D. The latter was found to act (primarily) on the inner membrane barrier experienced by the positively charged carrier–ion complex and on the interfacial barriers responsible for complex formation and dissociation. No evidence for a change of the microviscosity of the membrane interior was obtained.

Keywords: Dipole potential; Ion carrier; Lipid membrane; Charge-pulse technique

1. Introduction

The kinetics of ion transport across planar lipid membranes have been studied by a variety of different methods. Usually the time dependence of the electric current across the membrane or of the membrane voltage is followed after an external stimulus such as a voltage-jump, a charge-pulse, a T-jump or a photo-generated concentration-jump (see [1] for a review). The time resolution, which has so far been achieved under favourable conditions, is of the order of 2 μs (V-jump, [2]), 40 ns (charge-pulse technique, [3]) and 10 μs (T-jump, [4]). To study charge separation in bacteriorhodopsin and in photosynthesis, photoelectric measurements with a time resolution in the ps region [5,6] have been published. These experiments, however, were performed with suspensions of biological membranes (e.g. from halophilic bacteria), i.e. not with the planar bilayer

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system. The excellent time resolution was obtained by capacitive coupling of the membrane suspension to the electrodes used for the detection of the electrical signals.

The method of capacitive coupling was also applied throughout the present study to improve the time resolution of the charge-pulse method at planar lipid membranes. So far the optimal time resolution of 40 ns was obtained only at the extremely small values of the membrane resistance observed at the electrical breakdown of the membrane [3]. The technique which is suggested in the present paper allows signal detection in the time range well below 100 ns over the complete range of the membrane resistances normally encountered.

The technique is applied to study the kinetics of valinomycin-induced K^+-transport across monoolein membranes in the presence of dipolar substances. The adsorption of compounds such as the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) or phloretin have been found to change the electrical membrane properties. As a result the membrane conductance induced by macrocyclic ion carriers of the valinomycin type is increased up to several orders of magnitude [7–11]. The effect has been explained on the basis of a modified dipole potential of the membrane/water interface, though a contribution of additional membrane properties, such as a change of the membrane fluidity, could not be excluded. The present communication reports on kinetic experiments which were performed in order to study the influence of the dipolar substance 2,4-D on the single rate constants of valinomycin-mediated K^+-transport. It is shown that the action of 2,4-D can be explained solely on the basis of the electrostatic effect induced by the changed dipole potential.

2. Charge-pulse technique

The principle of this method consists in the application of a brief charge-pulse to the membrane and the subsequent detection of the membrane voltage under virtually infinite external resistance. The decay of the voltage only proceeds by charge transport through the conductive pathways of the membrane under this condition. Therefore, the time dependence of the decay contains information about the kinetics

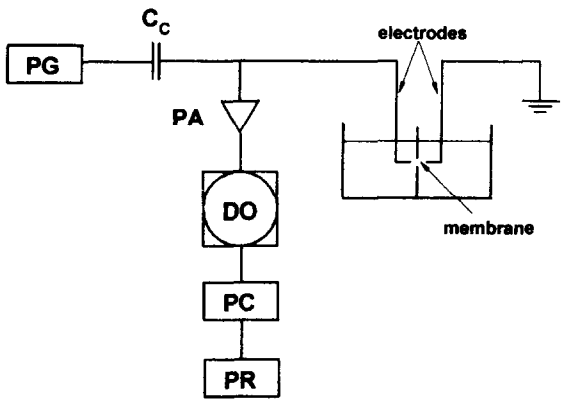

Fig. 1. A pulse-charge technique based on capacitive coupling (capacity C_c) of a voltage-jump from a pulse-generator PG to a lipid membrane. The voltage difference between the two electrodes is detected by the high-impedance preamplifier (PA). The signal is stored in a digital oscilloscope (DO), transferred to a personal computer (PC) and printed (PR).

of charge movement across the membrane. Ref. [1] provides a review about the development of this method.

So far application of the charge-pulse was achieved by a brief current pulse of 10–100 ns duration. At the end of the pulse the external circuit is switched to virtually infinite resistance either by means of a fast FET switch [12] or by using a fast diode (reverse resistance $> 10^{11}$ Ω) [13]. The time resolution achieved by this method was reported to be of the order of 100 ns.

The technique applied throughout the present study is illustrated in Fig. 1. This method differs from the previous one by the kind of charge-pulse generation. Instead of a current pulse of high amplitude, the charge-pulse is achieved by capacitive coupling of a rapidly rising voltage-jump to the membrane. The principle of the method is explained on the basis of the simplified equivalent circuit shown in Fig. 2. The membrane is represented by a parallel arrangement of a capacitance C_m and a resistance R_m. The time resolution of the method is substantially influenced by the magnitude of the series resistance R_s including the aqueous phases adjacent to the membrane, the output impedance of the pulse generator, and the electrodes. The application of a voltage-jump at time $t = 0$ to the circuit yields the following result (cf. Appendix A): The time depen-

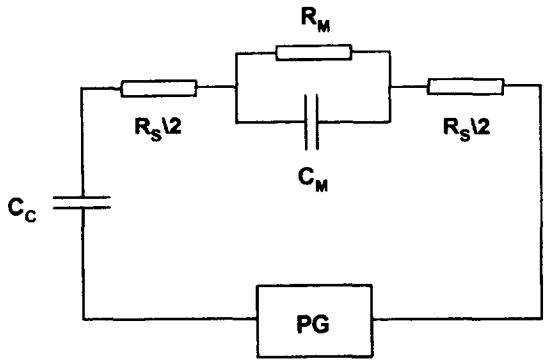

Fig. 2. Equivalent circuit of the arrangement according to Fig. 1. The membrane is represented by the capacitance, C_m, and the resistance, R_m. The series resistance, R_s, includes the aqueous phases adjacent to the membrane and the electrodes (PG = pulse-generator).

dence of the sum of voltages ($V_m + V_s$) measured by a high impedance preamplifier is given by Eq. (1):

$$V_m + V_s = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2) \quad (1)$$

with

$$\tau_1 = R_s C_c C_m/(C_m + C_c),\ \tau_2 = R_m(C_m + C_c)$$

$$\alpha_1 = VC_m/(C_m + C_c) \text{ and } \alpha_2 = VC_c/(C_m + C_c)$$

The expressions for τ_1, τ_2, α_1 and α_2 are approximations, which hold for $R_s \ll R_m$. For $C_c \ll C_m$ and for $t \gg R_s C_c$, Eq. (1) is reduced to

$$V_m + V_s \approx V_m = V(C_c/C_m)\exp(-t/R_m C_m) \quad (2)$$

Thus the time constant, $\tau_2 = R_m C_m$, which determines the exponential decay of V_m according to Eq. (2) corresponds to the normal result of a charge-pulse experiment (if the membrane is approximated by a simple RC element). The time constant, $\tau_1 = R_s C_c$, of the initial voltage spike determines the time resolution of the method. Assuming $R_s = 100\ \Omega$, $C_c = 10$ pF, $C_m = 1$ nF, and a voltage-jump amplitude of $V = 500$ mV, there is an initial voltage spike of $\alpha_1 = 495$ mV, which decays with a time constant $\tau_1 = 1$ ns to $\alpha_2 = 5$ mV. The subsequent decay of V_m provides information about the kinetics of ion translocation across the membrane (or in the case of the simple equivalent circuit about the time constant $R_m C_m$).

In the experimental reality the decay of the initial voltage spike is distorted and delayed because of the limited band width of the instrumentation applied. Instead of a few ns, the decay lasts for 20 ns at least. Fig. 3 illustrates the result obtained for a membrane formed from dioleoyllecithin in the presence of the K^+-carrier valinomycin. The conditions were chosen

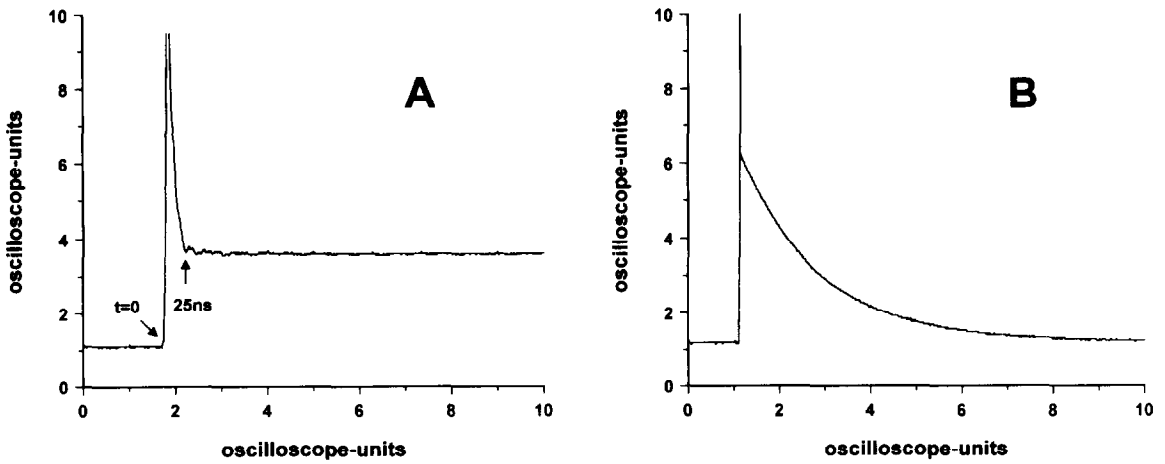

Fig. 3. The decay of $V_m(t)$ at two different time scales. The membrane was formed from dioleoyllecithin/n-decane with 10^{-3} M valinomycin in the membrane-forming solution and with 1 M KCl in the aqueous phase ($T = 298$ K). The measurement was performed using a coupling capacity $C_c = 5.7$ pF. The diameter of the membrane was about 0.6 mm ($C_m = 1000$ pF). The series resistance, R_s, was minimised (cf. 'Materials and Methods'). (A) Ordinate: 2 mV/division, abscissa: 50 ns/division. (B) Ordinate: 1 mV/division, abscissa: 100 μs/division.

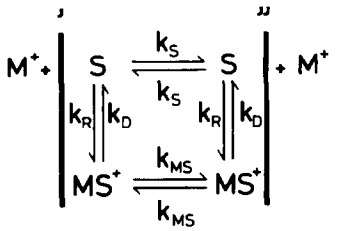

Fig. 4. Reaction scheme of the transport of cations M^+ mediated by macrocyclic carriers S of the valinomycin-type [1].

to ensure a simple 'RC-behaviour' of the membrane (i.e. the amplitudes of the specific relaxation of valinomycin-induced transport are comparatively small).

In view of the limited band width of the instrumentation the fast relaxation process (i.e. time τ_1 in Eq. (1)) can only be determined, if the series resistance R_s is increased. A good agreement between theory and experiment was obtained in this case (data not shown). Though this fast exponential term determines the time resolution of the method, it is not of relevance for the general applicability of the method, since the information about the dynamics of transport phenomena inside the membrane is derived from the decay of the voltage after completion of the initial voltage spike (see below). Fig. 3 indicates that the minimal time, where the onset of membrane-specific relaxation processes can be detected, is of the order of 20–30 ns with our present instrumentation.

Application of the method is demonstrated by studying the kinetics of ion transport in the presence of macrocyclic ion carriers of the valinomycin-type. Fig. 4 shows the reaction scheme usually applied in this case (cf. refs. [1,14–16] for a review). It may be analysed using the circuit shown in Fig. 2.

The membrane resistance has to be replaced by the reaction scheme of Fig. 4 in this case. In order to simplify the treatment, we neglect the series resistance, R_s (i.e. we neglect description of the initial voltage spike, cf. Eq. (1)). Then, application of Kirchhoff's laws yields the following equations:

$$V_c + V_m = V \tag{3}$$

V is the amplitude of the voltage-jump applied to the circuit, V_c is the voltage across the coupling capacity, V_m is the membrane voltage, and

$$J = C_c \frac{dV_c}{dt} = C_m \frac{dV_m}{dt} + F(N'_{MS}k'_{MS} - N''_{MS}k''_{MS}) \tag{4}$$

The term in brackets represents the flux of carrier-mediated transport of cations M^+ (N'_{MS} and N''_{MS} are the interfacial concentrations of complexes MS^+).

Combining Eqs. (3) and (4), and taking into account that (after the voltage jump) $dV/dt = 0$, one obtains

$$\frac{dV_m}{dt} = -\frac{F}{(C_m + C_c)}(N'_{MS}k'_{MS} - N''_{MS}k''_{MS}) \tag{5}$$

This result agrees with the analysis of the carrier model by the previous charge-pulse technique, if the denominator $(C_m + C_c)$ is replaced by C_m (cf. Eq. (9) of ref. [12]). Eq. (5) may be integrated together with the differential equations for N'_{MS} and N''_{MS} derived from the carrier scheme (Fig. 4). The result obtained by Benz and Läuger [12] is

$$V_m(t) = V_m^0(a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)),\ a_1 + a_2 + a_3 = 1 \tag{6}$$

The three relaxation times, τ_1, τ_2, and τ_3 of Eq. (6) and the two independent relaxation amplitudes, a_1 and a_2, depend on the four rate constants k_R, k_D, k_S, and k_{MS} of the carrier model and on the carrier concentration, N_0, inside the membrane. The reader should consult ref. [12] for the detailed equations and for the procedure, which allows determination of the model parameters. The equations hold for sufficiently small membrane voltages of the order of a few mV.

The validity of Eq. (6) in order to explain the time-dependent decay of V_m in the presence of valinomycin is illustrated by Fig. 5. The experiment was performed in the presence of 2,4-D in order to accelerate the decay of the process (see below). The three relaxation times of 346 ns, 5.59 μs and 75.93 μs are clearly separated under these conditions.

3. Materials and methods

Planar lipid membranes of the Mueller–Rudin type were formed from 1% solutions of either

monoolein (Nu Check Prep or Sigma) or of 1,2-dioleoyllecithin (Avanti Polar Lipids) in n-decane (Fluka, standard for gas chromatography) containing an appropriate concentration of valinomycin (Boehringer). The aqueous phase contained 1 M KCl and variable amounts of 2,4-dichlorophenoxyacetic acid (2,4-D) (Aldrich). The experiments were performed at pH 2 to ensure that 2,4-D was largely in the neutral form [17].

The principle of the charge-pulse technique was described in detail in the last section (cf. Fig. 1). The following electronic equipment was used: Pulsgenerator Philips PM 5785 (rise time < 1 ns), digital oscilloscope Tektronix 7603/7D20 (70 MHz band width, 1024 words memory size, 8 bit solution) and an IBM compatible 486-PC. Two different kinds of preamplifiers were applied: The faster one (Tektronix Differential Probe P6046) had a band width of 100 MHz but a comparatively small input resistance of $R_i = 1\ \mathrm{M}\Omega$. Its application is limited to membranes of comparatively large conductance (the condition $R_i \gg R_m$ must be fulfilled in order to avoid that the

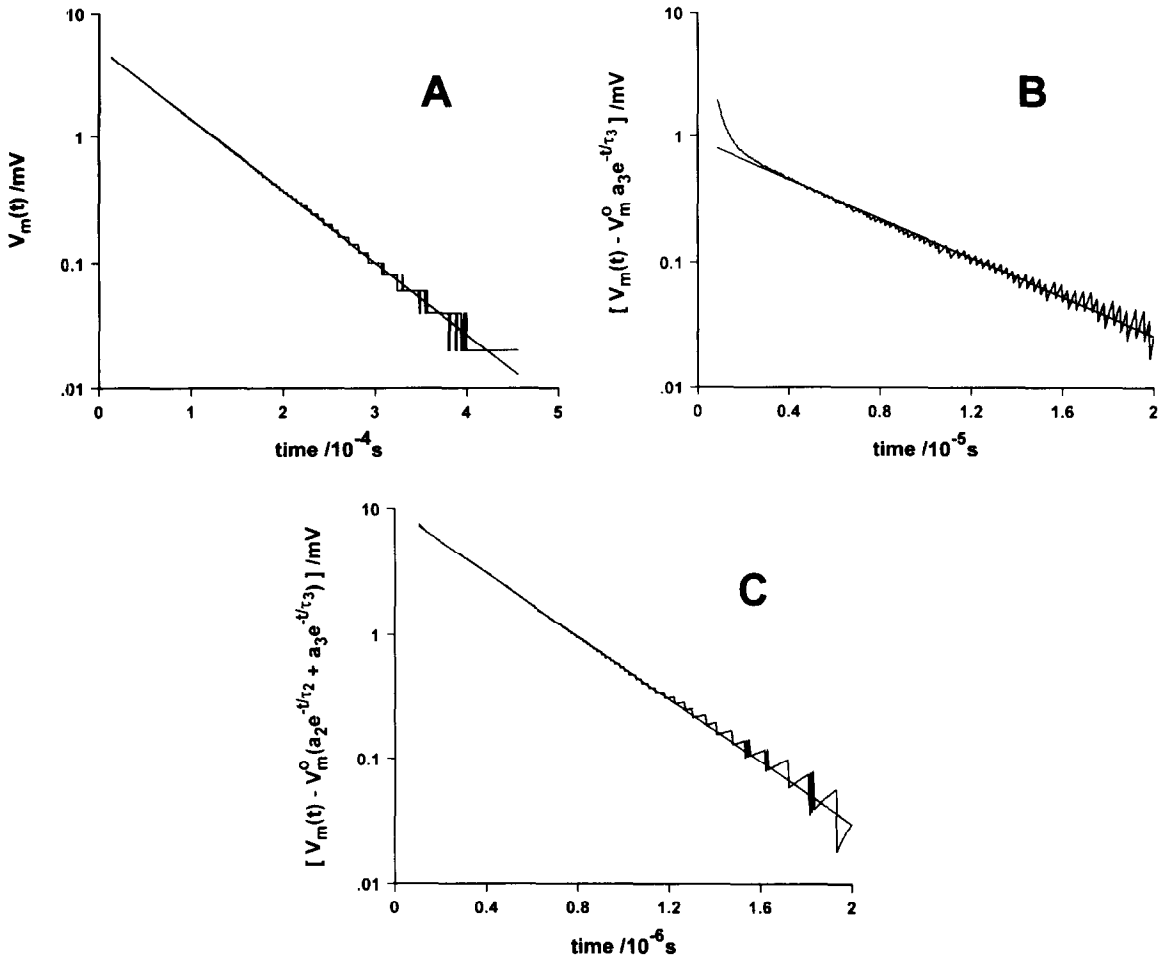

Fig. 5. The decay of $V_m(t)$ in the presence of valinomycin/K^+ and 2,4-D. Three different time scales are applied to demonstrate the validity of Eq. (6). For $t > 20$ μs the decrease of V_m is only due to the third exponential term (A: $\tau_3 = 75.93$ μs, $\alpha_3 = 0.33$). The two faster relaxation terms (B: $\tau_2 = 5.59$ μs, $\alpha_2 = 0.06$ and C: $\tau_1 = 346$ ns, $\alpha_1 = 0.61$) were determined by successive subtraction of the slower terms. The fit (solid lines) was optimised by a nonlinear curve fitting procedure (cf. Materials and Methods). The experiment was performed with 5.0×10^{-4} M valinomycin in the membrane forming solution (1% monoolein in decane), 1 M KCl and 0.3 mM 2,4-D in the aqueous phase (pH 2, $T = 25$°C).

decay of V_m is influenced by R_i). In most cases a preamplifier was used which was built in our workshop (band width 20 MHz, input resistance 10^{11} Ω, gain 10 fold). The time resolution obtained with it is about 100 ns (cf. Fig. 5).

In order to optimise the time resolution of the method, the series resistance, R_s, must be kept as low as possible (cf. Eqs. (1) and (2)). This was achieved by using L-shaped low-impedance platinised platinum electrodes, which — by way of two manipulators — were moved as close as possible (distance < 1 mm) to the membrane. The time resolution (defined as the completion of the initial voltage spike, cf. Fig. 3) is of the order of 20–30 ns in this case. It may be further improved by using electronic equipment with an increased band width.

The diameter of the hole across which the membrane was formed was typically 0.5–1 mm. The coupling capacity C_c usually applied was of the order of 5–20 pF.

The decay of $V_m(t)$ in the presence of ion carriers extends over several orders of magnitude in time (cf. Fig. 5). Therefore, in view of the limited memory size of the digital oscilloscope applied, the different time ranges of $V_m(t)$ had to be measured successively. The complete signal was subsequently assembled in the computer. Signal analysis in terms of Eq. (6) was obtained using the software package Asyst

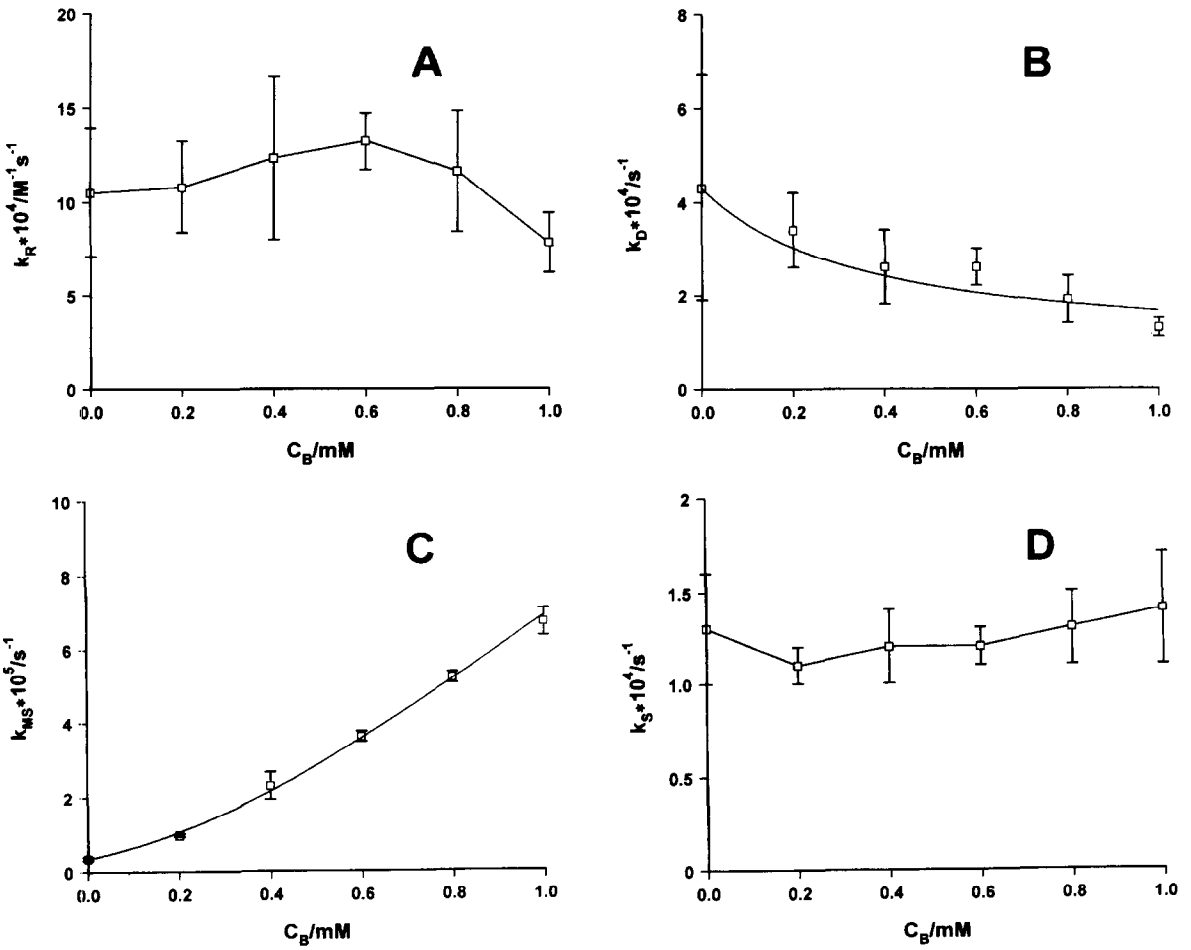

Fig. 6. The rate constants of valinomycin-induced K^+-transport across monoolein membranes in the presence of 1 M KCl and varying concentrations of the herbizide 2,4-D in water (pH 2, $T = 10°C$). The values were calculated from the relaxation data summarised in Table 1. The solid lines in B and C were calculated according to Eqs. (8), (9), and (13) assuming $s = 0$ (values for $b\beta$, a, and βN_D see Table 2).

(Keithly Instruments). The analysis was performed in the following way: The slowest relaxation time was obtained from the exponential decay of $V_m(t)$ observed at sufficiently long times. The two faster relaxation times were determined by successive subtraction of the slower exponential terms from the original data. As a final step, the complete fit was optimised by using the nonlinear curve fitting procedures supplied by Asyst. The additional minimisation of chi-square, i.e. of the sum of squares of deviations of the experimental data from the fitting function, was found to provide a significant improvement, if the two faster relaxation times differed by less than a factor of three.

4. Results and discussion

The charge-pulse technique was used to study the influence of the herbicide 2,4-D on the single rate constants of valinomycin-induced K^+-transport across monoolein membranes. The substance has been found to adsorb to the membrane/water interface and to modify the interfacial dipole potential [7,8]. We have been studying the kinetics of the adsorption process in previous publications [17,18]. Applying a laser T-jump technique and using the membrane conductance (induced by macrocyclic ion carriers) as a sensor for the adsorbing molecules, we were able to estimate the surface density of the ligands as a function of their concentration in water. The data were interpreted on the basis of Langmuir's isotherm. In addition we have been able to calculate the contribution, b, of a single adsorbed ligand molecule to the dipole potential. The calculation was based on an electrostatic model which allowed to correlate the rate constants of the carrier model with the ligand concentration in water. The present investigation was intended to provide an experimental test for this model.

The time dependence of the membrane voltage, $V_m(t)$, following a charge-pulse in the presence of macrocyclic ion carriers, is given by Eq. (6). The data shown in Fig. 5 illustrate that Eq. (6) is also valid in the presence of 2,4-D. Table 1 summarises the relaxation data as a function of the aqueous concentration of 2,4-D. There is a pronounced shortening of the fast relaxation time τ_1, while τ_2 is less affected. At the same time the relaxation amplitude α_2 is strongly decreased, while α_1 is increased correspondingly. Therefore, the overall effect of 2,4-D consists in a shift of the relaxation curve to shorter times.

Table 1
Relaxation times and amplitudes as a function of 2,4-D concentration, c_D, in water. The data refer to valinomycin-induced K^+-transport across monoolein membranes ($c_K = 1$ M, pH 2, $c_{Val} = 5.0 \times 10^{-4}$ M, $T = 10°C$)

c_D (mM)	τ_1 (μs)	τ_2 (μs)	τ_3 (μs)	α_1	α_2	α_3
0	5.09	10.33	311	0.129	0.380	0.491
0.2	2.54	7.60	412	0.395	0.118	0.487
0.4	1.21	6.63	566	0.428	0.050	0.522
0.6	0.68	6.10	549	0.501	0.043	0.456
0.8	0.56	7.09	749	0.416	0.030	0.554
1.0	0.39	9.31	632	0.482	0.025	0.492

Fig. 6 illustrates the consequences of this effect for the rate constants of valinomycin-induced K^+-transport. A pronounced increase of the translocation rate constant k_{MS} for the positively charged ion–carrier complex MS^+ is obtained, while the corresponding rate constant, k_S, remains unaffected. The same holds for the rate constant of complex formation, k_R. The rate constant of dissociation, k_D, shows a small but significant decrease.

The results will be analysed on the basis of the simple electrostatic model shown in Fig. 7. The electric potential difference, V_D, introduced by ad-

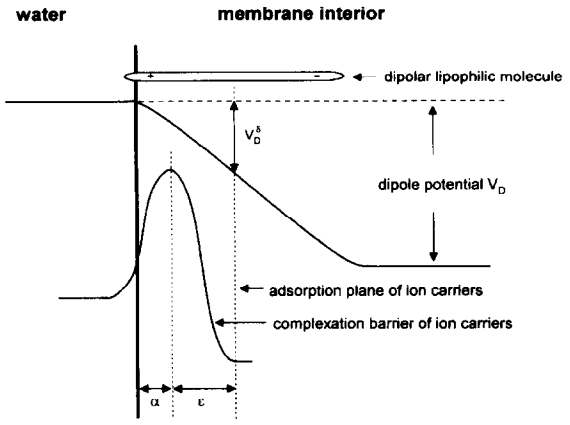

Fig. 7. The dipole potential, V_D, induced by oriented adsorption of dipolar molecules to the membrane/water interface and its effect on the complex formation of macrocyclic ion carriers.

sorption of dipolar molecules is assumed to decay over a certain distance inside the membrane. If the adsorption plane of the carrier complexes, MS^+, is located at the inner part of the interface, the heterogeneous equilibrium constant $K_h = k_R/k_D$ of interfacial complex formation will experience the potential difference, V_D^δ $(0 \le V_D^\delta \le V_D)$. The difference, $V_D - V_D^\delta$, is assumed to act on the inner membrane barrier for the positively charged complexes MS^+ (not shown). The height of the inner energy barrier will be lowered by $F(V_D - V_D^\delta)$, giving rise to a change of the translocation rate constant, k_{MS}, i.e.

$$k_{MS} = k_{MS}^0 \exp\left(-F\left(V_D - V_D^\delta/RT\right)\right) \quad (7)$$

where F is the Faraday constant, R is the gas constant, and T is the temperature.

The effect of V_D^δ on the complex formation and dissociation may be accounted for by an unsymmetrical barrier (characterised by the distances α and ϵ) between the aqueous phase and the adsorption plane of the ion carriers. If V_D is approximated by a linear decay, one obtains

$$k_R = k_R^0 \exp\left(-sFV_D^\delta/RT\right) \quad (8)$$

$$k_D = k_D^0 \exp\left[(1-s)FV_D^\delta/RT\right] \quad (9)$$

with

$$s = \frac{\alpha}{\alpha + \epsilon} \quad (10)$$

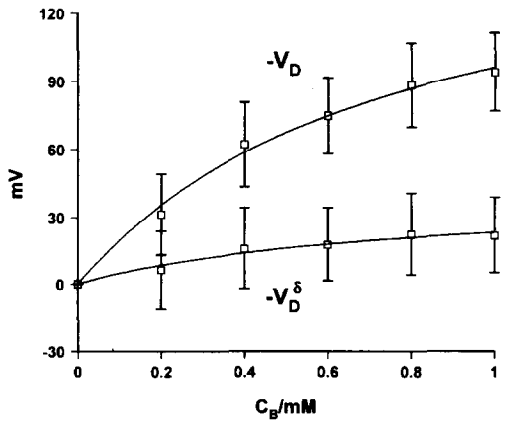

Fig. 8. V_D and V_D^δ as function of the concentration of 2,4-D in water. The solid lines were calculated according to Eqs. (11–13) (values for $b\beta$, a, and βN_D see Table 2).

Table 2
Parameters of adsorption of the herbicide 2,4-D to lipid membranes formed from monoolein (MO, present study) and to dioleoyllecitin (PC, [17])

	$b\beta$/[mV mM^{-1}]	a	β/N_D (mM^{-1})	V_D^{max} (mV)
MO	225	0.24	1.35	−167
PC	850	0.26	3.7	−239

Similar expressions have been applied by Hladky [19] to account for the dependence of k_R and k_D on the voltage difference, V, across the membrane. There is a comparatively weak dependence on V which is neglected in the present treatment. This is justified by the fact that V_D acts across a very thin layer (comparable to the dimensions of the dipolar molecule), whereas the membrane voltage, V, drops across the complete thickness of the membrane. Therefore, at comparably absolute values of V_D and V, the effect of V_D on the interfacial reaction is considerably larger.

Applying Eqs. (7–9), the dipole potential V_D as well as $V_D^\delta = aV_D$ may be obtained from the experimental values of the rate constants:

$$V_D = (RT/F)\ln\left(\frac{k_R^0 k_D k_{MS}^0}{k_R k_D^0 k_{MS}}\right) \quad (11)$$

$$V_D^\delta = aV_D = (RT/F)\ln\left(\frac{k_R^0 k_D}{k_R k_D^0}\right) \quad (12)$$

We have shown previously [17] that the interfacial concentration, N, of 2,4-D as a function of its concentration, c_B, in water obeys Langmuir's isotherm, i.e.

$$V_D = aV_D^\delta = -bN = -b\beta c_B/(1 + \beta c_B/N_D) \quad (13)$$

where β is the concentration independent partition coefficient at low-surface density of 2,4-D and N_D is the number of binding sites at the interface.

Combining Eqs. (11) and (12) with Eq. (13), theoretical expressions for V_D and V_D^δ are obtained as a function of 2,4-D concentration in water. The corresponding fit allows determination of the quantities $b\beta$, a, and β/N_D. From this the maximum dipole potential $V_D^{max} = -bN_D$ may be estimated (cf. Fig. 8 and Table 2). In a similar way combination of Eqs. (7–10) with Eq. (13) yields theoretical expressions for the dependence of the rate constants k_{MS},

k_R and k_D on c_B and allows estimation of s (cf. Fig. 6 and Table 2).

The behaviour of k_R and k_D can be explained only, if $s \ll 1$, i.e. if we assume a highly unsymmetrical barrier for the complexation reaction. The experimental result of a rather constant k_R and a decreasing k_D with increasing c_B is in contrast to the analysis of our previous T-jump studies [17]. There, a less general treatment of the effect of V_D on the complexation reaction was applied (though the present model could not be excluded). Discrimination between the two models is made difficult by the fact that the decrease of k_D is small, if compared with possible experimental errors of the analysis. The amplitude of the relaxation amplitude, α_2, is drastically reduced at high c_B (cf. Table 1). It cannot be excluded that the accuracy of the analysis in this region is influenced by systematic errors arising from 'unspecific' (i.e. carrier-independent) relaxations of the pure lipid membrane (see [20] for a discussion of this problem).

The main effect of the adsorption of 2,4-D, however, is the 20-fold increase of the translocation rate constant, k_{MS}. This is a clear confirmation of previous investigations [7,8,17] on these subjects, which were, however, only based on indirect methods such as the rise of the membrane conductance. The increase of k_{MS} deduced from these studies is several orders of magnitude, i.e. it is considerably larger than observed throughout the present investigation. This is a result of the different lipids used for membrane formation. While previous studies were performed with phospholipid membranes, the present analysis was based on monoolein membranes, which (contrary to phospholipid membranes) allow good resolution of all characteristic relaxation processes of valinomycin-induced K^+-transport [2,12]. On the other hand the dipole potential, V_D, introduced by adsorption of 2,4-D is substantially smaller for monoolein membranes (as may be concluded from a comparison of the V_D^{max} values, cf. Table 2). As a consequence the increase of the rate constant k_{MS} induced by 2,4-D is less prominent for monoolein.

The different behaviour of dioleoylphosphatidylcholine and monoolein may in principle be understood on the basis of the different intrinsic dipole potentials of the two lipids between membrane interior and water. The latter is of the order of 100–300 mV depending on the kind of lipid. The dipole potential has been found to be responsible for the enhanced permeability of negatively charged hydrophobic ions as compared with positively charged ions [21–24]. The intrinsic dipole potential generates a positive potential inside the membrane. This is contrary to the dipole potential, V_D, introduced by ligand binding (cf. Fig. 7). Therefore ligand binding may be assumed to be favoured by electrostatic interaction of the antiparallelly oriented intrinsic dipoles and of the ligand dipoles. The intrinsic dipole potential of monoolein membranes is smaller by about 100 mV as compared with membranes formed from dioleoylphosphatidylcholine [23]. Therefore electrostatic interactions will be reduced for monoolein giving rise to a smaller partition coefficient, β, and to a smaller V_D^{max}-value of 2,4-D.

Summarising our conclusions, the simple electrostatic model applied is sufficient to explain the main features of the effect of 2,4-D adsorption on valinomycin-induced K^+-transport. The change of the rate constants observed experimentally can be explained solely as an effect of the introduced dipole potential, V_D, on the inner membrane barrier and on the interfacial barriers, as was explained above in detail. This may be considered a surprising result in view of different other possibilities of interference between ligand adsorption and carrier-mediated transport: Properties such as the microviscosity of the membrane interior and of the membrane/water interface may depend on the concentration of the adsorbed ligand and may be considered to modify the rate constants of complexation and/or of translocation across the membrane. A more complex behaviour was, indeed, found for the dipolar substance phloretin and its analogues (unpublished). In the case of 2,4-D, however, there is no experimental evidence for such phenomena.

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Appendix A: The time dependence of V_m after a voltage-jump to the equivalent circuit of Fig. 2

Application of Kirchhoff's laws yields

$$V = V_c + V_s + V_m \tag{A1}$$

$$J = \frac{V_s}{R_s} = C_c \frac{dV_c}{dt} = \frac{V_m}{R_m} + C_m \frac{dV_m}{dt} \tag{A2}$$

where V_c, V_s, and V_m are the voltages across the coupling capacity C_c, the series resistance R_s and the membrane resistance R_m, respectively.

Combining (A1) and (A2) leads to the differential equation

$$\frac{d^2V_m}{dt} + a\frac{dV_m}{dt} + bV_m = c\frac{dV}{dt} \tag{A3}$$

$$a = \left(\frac{1}{R_sC_c} + \frac{1}{R_mC_m} + \frac{1}{R_sC_m}\right),$$

$$b = \frac{1}{R_sC_cR_mC_m},\ c = \frac{1}{R_sC_m}$$

Eq. (A3) has to be solved assuming a voltage-jump at time $t = 0$, i.e.

$$V(t \geq 0) = V,\ V_m^0 = V_c^0 = 0$$

and from Eqs. (A1), (A2)

$$\left(\frac{dV_m}{dt}\right)t = 0 = \frac{V}{R_sC_m} \tag{A4}$$

The solution of the problem is obtained by standard techniques. The sum of voltages ($V_m + V_s$) detected by the high impedance preamplifier (cf. Fig. 1) reads

$$V_m + V_s = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2) \tag{A5}$$

$$-1/\tau_1 = \sigma - \sqrt{\delta},\ -1/\tau_2 = \sigma + \sqrt{\delta}$$

$$\sigma = -\frac{1}{2}\left(\frac{1}{R_sC_m} + \frac{1}{R_mC_m} + \frac{1}{R_sC_k}\right)$$

$$\delta = \frac{1}{4}\left(\frac{1}{R_sC_m} + \frac{1}{R_mC_m} - \frac{1}{R_sC_k}\right)^2 + \frac{1}{R_s^2C_mC_k}$$

$$\alpha_1 = \frac{-V}{(1/\tau_1 - 1/\tau_2)}\left(\frac{R_m + R_s(1 - R_mC_m/\tau_1)}{R_sR_mC_m}\right)$$

$$\alpha_2 = \frac{V}{(1/\tau_1 - 1/\tau_2)}\left(\frac{R_m + R_s(1 - R_mC_m/\tau_2)}{R_sR_mC_m}\right)$$

Assuming $R_s \ll R_m$, Eqs. (A5) are reduced to Eqs. (1).

References

[1] P. Läuger, R. Benz, G. Stark, E. Bamberg, P.C. Jordan, A. Fahr and W. Brock, Quat. Rev. Biophys., 14 (1981) 513.
[2] W. Knoll and G. Stark, J. Membr. Biol., 25 (1975) 249.
[3] R. Benz and U. Zimmermann, Biochim. Biophys. Acta, 597 (1980) 637.
[4] G. Stark, W. Brock and R. Awiszus, Rev. Sci. Instrum., 59 (1988) 1200.
[5] H.-W. Trissl, Biochim. Biophys. Acta, 806 (1985) 124.
[6] R. Simmeth and G.W. Rayfield, Biophys. J., 57 (1990) 1099.
[7] P. Smejtek and M. Paulis-Illangasekare, Biophys. J., 26 (1979) 411.
[8] P. Smejtek and M. Paulis-Illangasekare, Biophys. J., 26 (1979) 467.
[9] O.S. Andersen, A. Finkelstein, I. Katz and A. Cass, J. Gen. Physiol., 67 (1976) 749.
[10] E. Melnik, R. Latorre, J.E. Hall and D.C. Tosteson, J. Gen. Physiol., 69 (1977) 243.
[11] J. Reyes, F. Greco, R. Motais and R. Latorre, J. Membr. Biol., 72 (1983) 93.
[12] R. Benz and P. Läuger, J. Membr. Biol., 27 (1976) 171.
[13] R. Benz, Biophys. J., 54 (1988) 25.
[14] G. Szabo, G. Eisenman, R. Laprade, S.M. Ciani and S. Krasne, in G. Eisenman (Editor), Membranes, Vol. 2, Marcel Dekker, New York, 1973, p. 179.
[15] S.B. Hladky, Curr. Top. Membr. Transp., 12 (1979) 53.
[16] R. Benz, H.-A. Kolb, P. Läuger and G. Stark, Methods Enzymol., 171 (1989) 274.
[17] R. Awiszus and G. Stark, Eur. Biophys. J., 15 (1988) 299.
[18] R. Awiszus and G. Stark, Eur. Biophys. J., 15 (1988) 321.
[19] S.B. Hladky, Biochim. Biophys. Acta, 352 (1974) 71.
[20] G. Stark and B.F. Gisin, Biophys. Struct. Mech., 6 (1979) 39.
[21] Ye.A. Liberman and V.P. Topaly, Biophysics, 14 (1969) 477.
[22] G. Szabo, in M.R. Heinrich (Editor), Extreme Environment: Mechanisms of Microbial Adaption, Academic Press, New York, 1976, p. 321.
[23] A.D. Pickar and R. Benz, J. Membr. Biol., 44 (1978) 353.
[24] R.F. Flewelling and W.L. Hubbell, Biophys. J., 499 (1986) 541.