ELSEVIER

Biochimica et Biophysica Acta 1235 (1995) 378–386

BBA Biochimica et Biophysica Acta

Shape of the potential energy barrier of the iodine-mediated halide transport

Karl-Heinz Klotz *, Roland Benz

Lehrstuhl für Biotechnologie, Theodor-Boveri-Institut (Biozentrum) der Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

Received 11 October 1994; revised 15 December 1994; accepted 9 January 1995

Abstract

Voltage-clamp experiments were performed on lipid bilayer membranes to study the voltage dependence of the iodine-mediated halide transport. Under all experimental conditions only one exponential current relaxation, apart from the capacitive spike, could be resolved up to a clamp voltage of 200 mV. The current relaxation could be described by an initial conductance, G_0, the relaxation time constant, τ, and the relaxation amplitude, α, that is the difference between the initial current, I_0, and the steady state current, I_∞, divided by the steady state current. The occurrence of one single exponential relaxation suggested that one of the different transport steps involved in the carrier-mediated ion transport according to the Läuger-model is always in equilibrium. This is most probably the transport of the free carriers across the membrane. The voltage dependence of G_0, τ, and of α were used to determine the voltage dependence of the translocation rate constants of the complexed carriers, k_{AS}. In the case of the iodine-mediated iodide transport, G_0, τ and α were only mediate voltage-dependent, which means the voltage dependent translocation of the complex encounters a trapezoidal barrier shape. For the iodine-mediated bromide translocation G_0, τ and α exhibited no dependence on the applied clamp-voltage, which suggested that a square Nernst-Planck barrier limits the transport of the corresponding complex.

Keywords: Ion transport; Iodine; Potential energy barrier; Voltage-clamp; Lipid bilayer membrane

1. Introduction

Small ions such as sodium, potassium and chloride are extremely insoluble in lipid membranes because of its small relative dielectric constant. Responsible for the small solubility of ions and the insulator effect of membranes are the Born charging energy and the image force [1,2]. To overcome this considerable energy and to make ions soluble in membranes it is necessary to decrease the charge density of the ions. One possibility for establishing a smaller charge density is to enlarge the volume of the ions by adding bulky hydrocarbon groups. Two examples are dipicrylamine and tetraphenylborate which are widely prominent as so-called lipophilic ions. Both have a high permeability through artificial and natural membranes [3–9]. Another possibility to smear the charges over a large surface and thus to overcome the electrostatic energy within the membrane is the binding of small ions to large organic carrier molecules. Examples for this are the valinomycin- and macrotetrolide-mediated transport of potassium ions [10–13]. In these carrier-ion complexes, the positive charge is smeared across a large sphere, which again results in a decreased charge density of the entire complex.

The lipophilic ions and the ion-carrier complexes have to move across a potential energy barrier in the membranes. The shape of this barrier is dependent on the transport system. It can assume two extremes. A high voltage-dependence of the charged form of the carrier or the lipophilic ions is given for an Eyring-type of potential energy barrier [14,15], whereas a linear dependence of the translocation rate constant on membrane potential is obtained for a Nernst-Planck barrier [3,16,17]. In reality neither of the two barrier shapes is able to describe the voltage-dependence in a correct way. In particular, for voltages > 80–100 mV considerable discrepancies between the theoretical predicted and the experimental observed current voltage curves have been found. Only by a

---

Abbreviations: DPhPC, diphytanoylphosphatidylcholine (molecular mass 846 Da); DOPC, dioleoylphosphatidylcholine (molecular mass 786 Da).

* Corresponding author. E-mail: winter@vax.rz.uni-wuerzburg.d400.de. Fax: +49 931 8884529.

0005-2736/95/$09.50
SSDI 0005-2736(95)00019-4

modified Nernst-Planck barrier [18], which represents a trapezoid, it is possible to describe the current voltage characteristics of the carrier-mediated ion transport with more accuracy. This barrier model describes the transition of a square Nernst-Planck barrier with a linear voltage dependence to a triangular Eyring barrier with an exponential voltage dependence. Indeed, this formalism yielded a much better fit of the voltage dependence of lipophilic ions and carrier-mediated ion transport [4,19,20]. In all these studies the modified Nernst-Planck barrier gives the best fit of the voltage dependence. However, the observed current-voltage characteristics are still more exponential than linear.

In this study we present voltage-clamp experiments on the iodine-mediated halide transport. The data of the present study indicate a considerably different current-voltage dependence for these transport systems than reported for all the other systems mentioned above. This means that we observed in the case of the carrier-mediated halide transport a more linear rather than an exponential voltage dependence. This suggests that the shape of the central energy barrier can be better approximated by a square Nernst-Planck barrier than by triangular barrier of the Eyring-type. Another interesting outcome of our experiments is that the current-voltage characteristics varies with the transported halide. In the case of the iodine-mediated iodide transport the charged complex is only slightly dependent on the applied voltage, whereas for the iodine-mediated bromide transport the complex was virtually voltage-independent.

2. Materials and methods

Black lipid bilayer membranes were formed of a 1% solution of DPhPC and DOPC (Avanti Polar Lipids, Alabaster, AL) in n-decane or n-chlorodecane (Fluka, Buchs, Switzerland). The membranes were spread across circular holes with a diameter of about 1 mm in a wall separating two aqueous phases in a Teflon cell. Iodine (Merck, Darmstadt, Germany) was dissolved in ethanol to obtain concentrated stock solution. Small amounts of these stock solution were added to the aqueous phase containing 1 M NaCl (analytical grade, Merck, Darmstadt, Germany) and different concentrations of potassium iodide or potassium bromide (Aldrich Chemie, Steinheim, Germany). The final concentrations of I_2 in the aqueous phase was 3 μM. The aqueous solutions were prepared freshly each day with ultrapure water (18 MΩ) (MilliQ, Millipore, Bedford, MA), degassed and stored dark and cool to prevent oxidation of the I^- and Br^-. All experiments were carried out at least 5 min after the membranes had completely turned black to reach partition equilibrium for the adsorption of iodine to the lipid bilayer membranes.

The experimental setup for voltage clamp has been described previously [19–22]. In brief: the voltage clamp experiments were performed applying a single voltage pulse of an amplitude of 20–200 mV and a duration of 10 ms across the membrane with a fast pulse generator (Hewlett Packard 214B, Hewlett-Packard, Palo Alto, CA) connected to Ag/AgCl electrodes. The current flowing through the membrane was determined by measuring the voltage drop across a resistor (200 Ω) in series to the membrane. The voltage drop was measured with a Tektronix storage oscilloscope with a 7A22 differential amplifier (Tektronix, Beaverton, OR). The output signal of the oscilloscope was recorded with a digital storage oscilloscope (Nicolet 4094A, Nicolet Instruments, Madison, WI). The voltage relaxations were analyzed with a HP 98580AD computer (Hewlett-Packard) and a 486DX33 personal computer. After the capacitance spike, which was in good agreement with the calculated RC-time constant of the membrane, had subsided, the current transient in the voltage-clamp experiments could always be described by a single exponential. To analyze the transient, the stationary current was subtracted, the logarithm of the current plotted as a function of time, and a linear least-square fit program was used to determine the values of the relaxation time constant, τ, and the initial current, I_0.

The monolayer experiments were performed as reported previously [23]. After formation of the monolayer the surface potential of the pure lipid monolayer was used as reference. Following potassium iodide respectively bromide was added to the subphase in the concentrations mentioned above. After equilibration the surface potential was measured. Then the iodine was added to the subphase to reach a final concentration of 3 μM and again the surface potential was determined after equilibration.

3. Theoretical considerations

We have recent demonstrated that the so-called Läuger-model [24,25] provides an excellent description of the halogenide-mediated transport of halides across membranes [26]. This model has been described in detail in a number of publications [25,27,28]. Hence, we list only the basic assumptions of the kinetic model and the equations used to determine the translocation parameters from the experimental data. The basic equations which describe the iodine-mediated halide transport are given in the appendix (Eqs. (A1–A5)). The results of our previous investigation [25] support the assumption of the Läuger-model that a 1:1 complex is formed at the membrane-solution interface (IC-mechanism). The heterogeneous complexation reaction is described by overall rate constants k_R (association) and k_D (dissociation). The translocation of free and charged carriers through the membrane are treated as simple first order reactions with rate constants k_S and k_{AS}, respectively. Of all the rate constants only k_{AS} is assumed to be voltage-dependent. Its dependence is calculated on the basis of a single barrier of the Eyring or Nernst-Planck type [19]:

$$k'_{AS} = \frac{k_{AS}\frac{bu}{2}e^{u/2}}{\sinh\left(\frac{bu}{2}\right)} \tag{1}$$

$$k''_{AS} = \frac{k_{AS}\frac{bu}{2}e^{-u/2}}{\sinh\left(\frac{bu}{2}\right)} \tag{2}$$

$u = FV_m/RT$ is the reduced voltage. V_m is the membrane voltage, F is the Faraday constant, R is the gas constant and T is the temperature (the valency of the carrier-ion complex is -1). For $b = 0$ the carrier-ion complex encounters an Eyring barrier. For $b = 1$ (and also for small voltages in the case of an Eyring barrier) k'_{AS} and k''_{AS} are proportional to $(1 + u/2)$ and $(1 - u/2)$, respectively, as predicted by a Nernst-Planck model with a square barrier [19].

The concentrations of I^- and Br^- on both sides of the membrane are identical. Applying a potential across the membrane changes the interfacial concentrations of the free and complexed carrier as described previously [26]. The total concentration of carriers within the membrane (complexed and uncomplexed) is assumed to be constant at the time scale of an experiment [19,26,27].

In our previous investigation of the iodine-mediated halide transport using the charge-pulse technique we could detect only two out of three possible exponential relaxations within the time resolution of our experimental device [26]. These results are in agreement with the findings of the present study using the voltage-clamp technique, i.e., under all experimental conditions only one current relaxation (apart from the capacitive spike) could be recorded. These results indicate that one step of the iodine-mediated halide transport is always in equilibrium because it is much faster than the others [19,26]. We identified the translocation rate constant, k_S, of the free carrier to be much faster than all the other steps involved in the iodine-mediated halide transport [26]:

$$k_S \gg k_R c_A,\ k_D,\ k_{AS} \tag{3}$$

It is assumed that under voltage clamp conditions the system is at equilibrium for times < 0. At $t = 0$ a voltage V is applied. The subsequent flowing current, neglecting the capacitance transient, is given by the net flux of the charged complexes within the membrane multiplied by the valence, z, and F.

$$I = zF(k'_{AS}N'_{AS} - k''_{AS}N''_{AS}) \tag{4}$$

Analysis of the model shows that in general the time-course of the membrane current density is governed by two relaxation processes [19,21]. Due to the back translocation of the free carrier being too fast to be resolved, the time course of the current density, $I(t)$, reduces to just one exponential relaxation (see Appendix):

$$I(t) = I_\infty(I + \alpha e^{-\lambda t}) \tag{5}$$

with

$$\alpha = \frac{k'_{AS} + k''_{AS}}{k_D} \tag{6}$$

and

$$\lambda = \frac{1}{\tau} = k'_{AS} + k''_{AS} + k_D \tag{7}$$

The initial current I_0 is given by:

$$I_0 = zFN_{AS}\left(\tfrac{1}{2}(k'_{AS} + k''_{AS}) - k''_{AS}\right) \tag{8}$$

and the stationary current I_∞ (i.e., the current density after many relaxation times) by:

$$I_\infty = zFN_{AS}\frac{\frac{1}{2}k_D(k'_{AS} - k''_{AS})}{k'_{AS} + k''_{AS} + k_D} \tag{9}$$

4. Results

4.1. Kinetic voltage-clamp measurements

All voltage-clamp experiments presented in this study were performed on DOPC-membranes dissolved in n-chlorodecane. This system was used to increase the rate constant of the voltage dependent step, the translocation rate constant, k_{AS}, of the complexed carrier. Previous studies have shown that the translocation of charged molecules is higher by a factor of about 500 because of the increased dielectric constant of these membranes [19,26,29]. Fig. 1A illustrates a current record taken from a lipid bilayer membrane made of DOPC/n-chlorodecane immersed in a solution containing 1 M NaCl, 0.3 mM KI and 3 μM I_2 at a clamp voltage of 20 mV. The initial current decays with two clearly distinguishable relaxations. The fast relaxation with a time constant of about 2 μs reflects the capacitive spike (Fig. 1B). The second relaxation is caused by the movement of the complexes within the membrane. Fig. 1B illustrates the analysis data by the computer: the logarithm of $(I(t) - I_\infty)$, where $I(t)$ is the current at a time t and I_∞ is the steady state current. This means that we observed a single exponential current relaxation (despite the capacitive spike) with a time constant of 28 μs.

This situation was comparable to that for charge-pulse experiments: in these experiments two voltage relaxations have been observed, since the membrane voltage represents an additional variable in these measurements [26]. One single exponential current relaxation out of two predicted by the 'Läuger'-model means that one of the different reactions involved in iodine-mediated iodide transport is always in equilibrium. We have demonstrated in a

Table 1
Dependence of voltage-clamp data on the complex type and halide concentration for small voltages

Salt	τ/(μs)	α	I_0 (mA/cm^2)	I_∞ (mA/cm^2)	N_{AS} (pmol/cm^2)	k_D (1/s)	k_{AS} (1/s)
0.1 mM KI	24 ± 1	7 ± 1	0.87 ± 0.33	0.11 ± 0.04	1.48 ± 0.33	5200 ± 500	18100
0.3 mM KI	24 ± 3	6 ± 2	1.39 ± 0.54	0.19 ± 0.05	1.90 ± 0.42	6000 ± 600	17500
0.1 M KBr	146 ± 9	0.9 ± 0.1	0.17 ± 0.02	0.10 ± 0.02	2.89 ± 0.48	3600 ± 200	1600
0.3 M KBr	163 ± 8	1.8 ± 0.4	0.19 ± 0.05	0.07 ± 0.02	2.70 ± 0.67	2200 ± 300	2000
1.0 M KBr	206 ± 34	3.9 ± 0.3	0.26 ± 0.04	0.05 ± 0.01	3.74 ± 0.96	1000 ± 300	2000

Kinetic parameters of the iodine-mediated halide translocation across DOPC/n-chlorodecane membranes at $V_m = 20$ mV. The data are given as means ± S.D. of at least eight experiments. For all systems 1 M NaCl was used as electrolyte. The temperature was 20° C. The aqueous phase contained 3 μM I_2. The voltage dependent translocation constant, k_{AS}, was obtained by linearizing Eqs. (1) and (2) (see text). The rate constant of the dissociation process, k_D, and the total surface concentration, N_{AS}, of the complexed carriers were calculated from the experimental data according to Eqs. (11) and (12).

previous study that this step is the movement of the free carriers in the membrane [26]. This means that also under voltage-clamp conditions only the translocation rate, k_{AS}, the dissociation rate, k_D, and the surface concentration of the complexed carrier, N_{AS}, can be evaluated from one single voltage clamp experiment (Eqs. (8), (11) and (12)).

Table 1 shows the experimental data obtained from voltage-clamp measurements performed on DOPC/n-chlorodecane membranes at two different halide concentrations (100 and 300 μM) and a fixed iodine concentration (3 μM). Each data set represents the mean value of at least eight membranes. The data were derived at a clamp voltage of 20 mV. The increase of the carrier concentration by a factor of three had neither any significant influence on the time constant nor on the amplitude of the current relaxation. Similarly the kinetic parameters of the system were independent of the carrier concentration. This is expected from the theory since the relaxation parameters are not dependent on the carrier concentration in a voltage-clamp experiment (in contrast to charge-pulse relaxation). The increase of the iodide concentration had some influence on the relaxation parameters since the total surface concentration of the complexes changed. Similar results were obtained for voltage-clamp experiment with the iodine/bromide system (see Table 1). However, the scatter of the experimental data was for this system somewhat higher probably caused by the smaller amplitude of the current relaxation. It is noteworthy that the kinetic parameters of both systems, the iodine/iodide- and the iodine/bromide-system, as determined here with the voltage-clamp technique are in good agreement with those obtained previously from charge-pulse measurements [26].

Table 2
Results of voltage-clamp experiments performed with different complexes as a function of the applied clamp potential

V_m (mV)	τ/(s)	α	I_0 (mA/cm^2)	I_∞ (mA/cm^2)	N_{AS} (pmol/cm^2)	k_D/(1/s)	$k'_{AS} + k''_{AS}$ (1/s)
(A) 0.1 mM KI							
20	24 ± 1	7 ± 1	0.87 ± 0.33	0.11 ± 0.04	1.48 ± 0.33	5200 ± 500	36200
30	23 ± 2	7 ± 1	1.29 ± 0.52	0.16 ± 0.06	1.51 ± 0.39	5100 ± 400	37200
50	21 ± 1	8 ± 1	2.08 ± 1.07	0.23 ± 0.09	1.57 ± 0.40	5100 ± 400	43800
70	17 ± 1	10 ± 2	3.27 ± 1.48	0.28 ± 0.10	1.58 ± 0.35	5200 ± 600	55800
100	13 ± 1	14 ± 1	5.82 ± 1.46	0.38 ± 0.08	1.72 ± 0.40	5100 ± 200	73800
120	10 ± 1	18 ± 2	7.84 ± 2.02	0.40 ± 0.08	1.79 ± 0.47	5100 ± 600	92200
140	8 ± 1	22 ± 4	10.39 ± 2.89	0.45 ± 0.08	1.88 ± 0.51	5400 ± 900	118700
160	6 ± 1	31 ± 11	15.03 ± 3.71	0.48 ± 0.09	2.09 ± 0.44	5200 ± 500	157200
180	5 ± 1	45 ± 8	24.13 ± 1.93	0.54 ± 0.12	2.48 ± 0.44	4600 ± 300	206700
200	4 ± 1	57 ± 15	34.54 ± 3.02	0.62 ± 0.14	2.92 ± 1.54	4500 ± 400	250600
(B) 0.3 M KBr							
20	163 ± 8	1.8 ± 0.4	0.19 ± 0.05	0.07 ± 0.02	2.70 ± 0.67	2200 ± 300	4000
30	159 ± 11	1.9 ± 0.3	0.28 ± 0.07	0.10 ± 0.02	2.70 ± 0.64	2200 ± 200	3700
50	145 ± 10	1.9 ± 0.3	0.47 ± 0.12	0.16 ± 0.04	2.84 ± 0.71	2300 ± 300	4400
70	128 ± 9	2.0 ± 0.4	0.68 ± 0.18	0.23 ± 0.06	3.10 ± 0.81	2600 ± 300	5300
100	102 ± 10	2.1 ± 0.4	1.06 ± 0.26	0.32 ± 0.11	3.44 ± 0.90	3200 ± 400	6600
120	91 ± 11	2.1 ± 0.3	1.32 ± 0.32	0.42 ± 0.10	3.71 ± 0.95	3600 ± 500	7700
140	77 ± 7	2.0 ± 0.3	1.58 ± 0.45	0.54 ± 0.12	3.93 ± 0.81	4300 ± 500	8900

Results of voltage-clamp experiments in the presence of two different halide ions in the aqueous phase. The data are given as means ± S.D. of at least eight experiments performed on DOPC membranes. For both systems 1 M NaCl was used as electrolyte. The temperature was 20° C. The aqueous phase contained 3 μM I_2. The rate constants, k'_{AS}, k''_{AS} and k_D, and the total surface concentration, N_{AS}, of the complexed carriers were calculated from the experimental data according to Eqs. (1), (2), (8), (11) and (12).

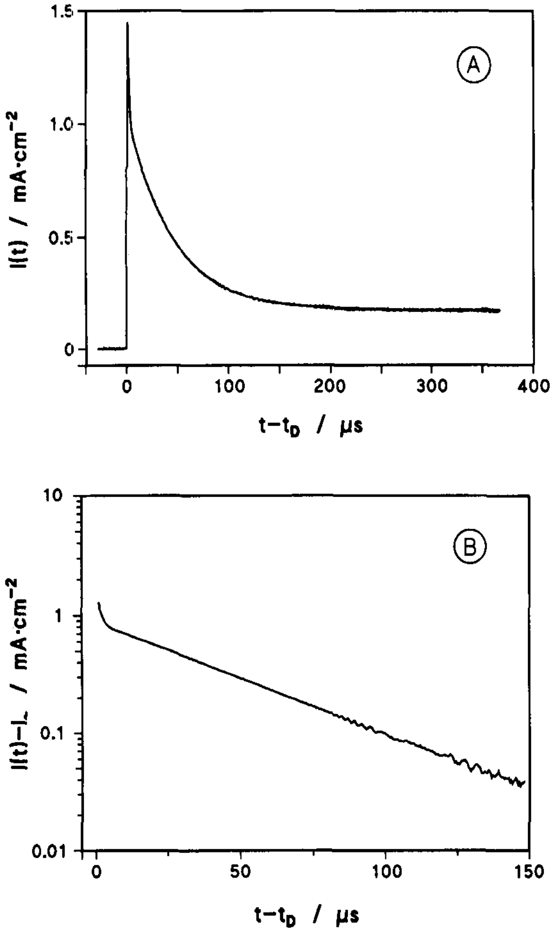

Fig. 1. (A) Voltage-clamp experiment on application of 20 mV on a DOPC/n-chlorodecane membrane, bathed in an aqueous solution containing 1 M NaCl, 3 μM I_2 and 0.3 mM KI. The temperature was 20° C. (B) Semilogarithmic plot of the current versus time curve. The current decay was fitted to only one single exponential relaxation with the following relaxation parameters: $I_0 = 1.03$ mA/cm^2, $I_\infty = 0.17$ mA/cm^2, $\tau = 28$ μs. The rate constants were calculated according to Eqs. (8), (11) and (12): $k_{AS} = 14000$ s^{-1}, $k_D = 5800$ s^{-1} and $N_{AS} = 1.89$ pmol/cm^2.

4.2. Shape of the internal membrane potential barrier

In further experiments we increased the clamp voltage from 20 mV up to 200 mV. The experimental data are summarized in Table 2. The analysis of the experimental data was performed using Eqs. (8), (11) and (12). Table 2 shows the sum of the translocation rate constants in the both directions, $k'_{AS} + k''_{AS}$ the dissociation rate constant, k_D, and the surface concentration of the complexed carrier, N_{AS}. The dissociation rate constant, k_D, remained nearly unchanged for increasing clamp voltage. Only the surface concentration of the complexed carrier, N_{AS}, increased slightly with rising clamp potential. This may be caused by a small contribution of the solution complexation mechanism (SC-mechanism) to the current although most of the current was due to the interfacial complexation mechanism (IC-mechanism) as has been shown previously [26]. The data of Table 2 support our assumption that from all the steps involved in the iodine-mediated halide transport only the translocation of the complexed carrier across the membrane is assumed to be voltage dependent (see Eqs. (1) and (2)).

We used the voltage dependent parameters, i.e., the initial conductance, G_0, the relaxation time constant, τ, and the current relaxation, α, for the evaluation of the internal energy barrier which is encountered by a complex AS^- crossing a low dielectric membrane. For a given membrane the voltage-clamp data were highly reproducible. However, for different membranes we observed some variations. Hence for analysis the initial conductance, $G_0(V)$, plotted as a function of the applied reduced voltage, was normalized by division by the corresponding conductivity at 20 mV, $G_0(20)$.

From the Eqs. (1), (2) and (A11) its apparent that $G_0(V)/G_0(20)$ is a function of the applied reduced volt-

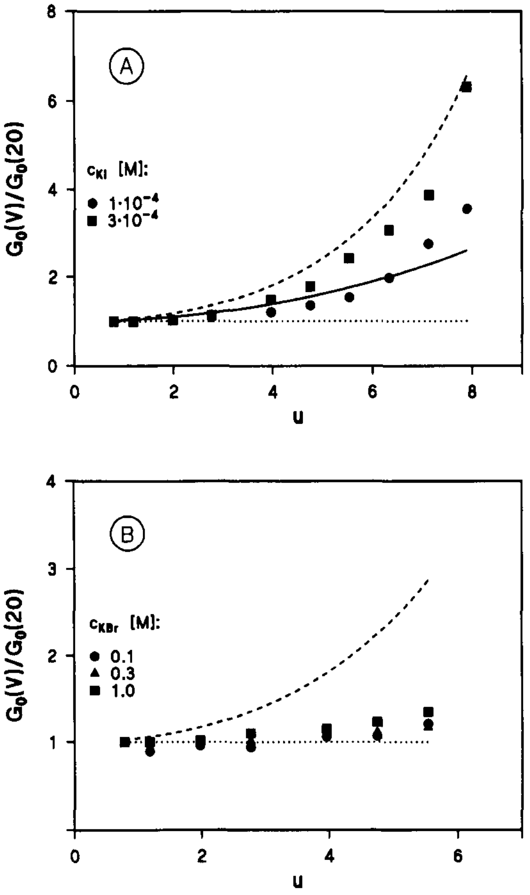

Fig. 2. Conductance versus voltage curves for the iodine-mediated transport of iodide (A) and bromide (B), respectively through diphytanoylphosphatidylcholine/n-decane membranes. The initial conductance, $G_0(V)$, was divided by the initial conductance, $G_0(20)$, measured on application of 20 mV and plotted against the reduced voltage. Experiments performed on different membranes are shown by different symbols. The dotted line represents the voltage dependence obtained by a Nernst-Planck type of barrier ($b = 1$; Eq. (10)), and the dashed line is the prediction of Eq. (10) in the limit that b approaches 0, a single-barrier Eyring model. The solid in (A) was drawn according to Eq. (10) with $b = 0.7$.

age, u, and the parameter b, reflecting the steepness of the energy barrier.

$$\frac{G_0(V)}{G_0(20)} = \frac{b\sinh\left(\frac{u}{2}\right)}{\sinh\left(\frac{bu}{2}\right)} \tag{10}$$

Fig. 2 shows the voltage dependence of the experimentally determined initial conductance $G_0(V)/G_0(20)$ of the iodine/iodide- (A) and the iodine/bromide-system (B). It demonstrates also the predictions of two theoretical approaches. The dotted lines reflect the situation if an ion encounters an Nernst-Planck barrier with a square barrier shape, equivalent to $b = 1$. For such a barrier $G_0(V)/G_0(20)$ is constant and independent of the applied voltage. The dashed lines show the voltage dependence when the carrier-ion complex has to cross a single-jump Eyring barrier with a triangular barrier shape (i.e., $b = 0$). In this case $G_0(V)/G_0(20)$ has an exponential dependence on the applied voltage. Comparison of these two extreme cases with the experimental data presented in Fig. 2A and B, respectively, clearly indicated different barrier shapes for both types of complex. In the case of the iodine-mediated iodide transport the fit of the data according to Eq. (10) yielded an value of $b = 0.7$ (solid line in Fig. 2A). The comparison between experimental data and the solid line shows that the fit was not exceptional but provided a good description for the data up to 120 to 150 mV. Above these membrane potentials we obtained some variation between experimental data and theoretical predictions. However, the use of smaller values for b corresponding to steeper potential energy barriers lead to worse fits for membrane potentials up to 100 mV. Interestingly, the iodine/bromide complex was virtually voltage-independent, which means that it encounters a square potential barrier of the Nernst-Planck type (Fig. 2B).

Almost identical results were obtained plotting the inverse relaxation time constants, λ, against the applied voltage for both iodine/halide systems. According to Eqs. (1), (2) and (7) the voltage dependence of the inverse relaxation time constant, λ, could be described by:

$$\lambda = \frac{1}{\tau} = 2k_{\mathrm{AS}}\left(\frac{bu}{2}\right)\frac{\cosh\left(\frac{u}{2}\right)}{\sinh\left(\frac{bu}{2}\right)} + k_{\mathrm{D}} \tag{11}$$

In Fig. 3 the data of the iodine/iodide (A) and the iodine/bromide system (B) are compared to the predictions of an Eyring (dashed line) and a Nernst-Planck barrier (dotted line), respectively. The values obtained for b by fitting the data with Eq. (11) were consistent with the results of the plots of the membrane conductance (Fig. 2). This means that we obtained a value of $b = 0.7$ for the fit of the I_3^--system. On the other hand, the inverse relaxation time constant of the I_2Br^--system showed no dependence on the applied voltage (i.e., $b = 1$).

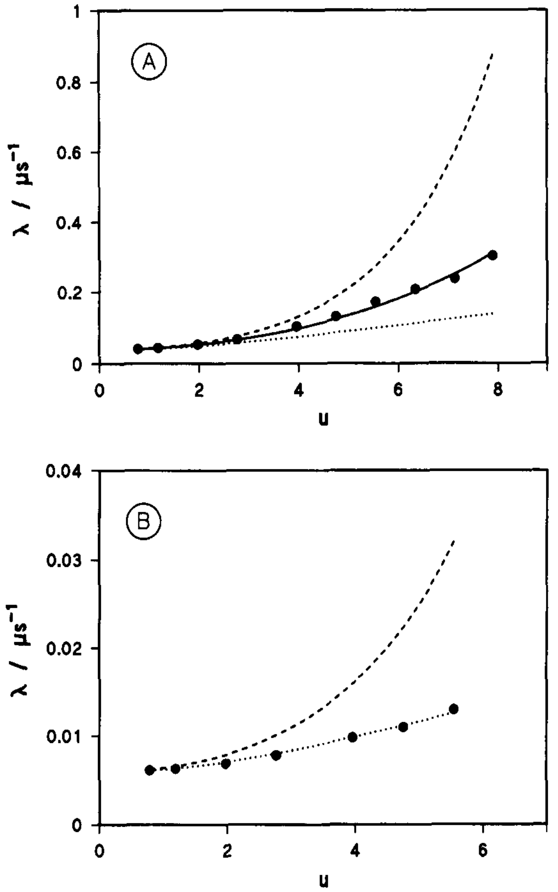

Fig. 3. Voltage dependence of the inverse relaxation time constant, λ, for the iodine-mediated transport of iodide (A) and bromide (B), respectively. The lines through the data were drawn using Eq. (11) when (A): $k_{\mathrm{AS}} = 16500\ \mathrm{s}^{-1}$, $k_{\mathrm{D}} = 5700\ \mathrm{s}^{-1}$ and $b = 0.7$ and (B): $k_{\mathrm{AS}} = 1900\ \mathrm{s}^{-1}$, $k_{\mathrm{D}} = 2200\ \mathrm{s}^{-1}$ and $b = 1$. The aqueous halide concentration was 0.1 mM KI (A) and 0.3 M KBr (B), respectively, at a fixed iodine concentration of 3 μM; diphytanoylphosphatidylcholine/n-decane membranes.

A third possibility for the identification of the shape of the central membrane potential barrier consists in the plot of the relaxation amplitude, α, as a function of the applied voltage. From Eqs. (1), (2) and (6) one obtains:

$$\alpha = k_{\mathrm{AS}}bu\frac{\cosh\left(\frac{u}{2}\right)}{\sinh\left(\frac{bu}{2}\right)} + \frac{1}{k_{\mathrm{D}}} \tag{12}$$

From Fig. 4A its clearly visible that the data obtained from the iodine-mediated iodide transport could be fitted according to Eq. (12) with sufficient accuracy. Again a value of $b = 0.7$ (solid line), could be used as fit parameter. Examination of the data of the iodine/bromide system exhibited a totally different situation. The lines were drawn assuming $b = 1$. Accurate fitting of the data would give even a value of $b > 1$, which did not make much sense. This means that once again the translocation rate constant of the iodine-bromide complex appeared to be voltage independent.

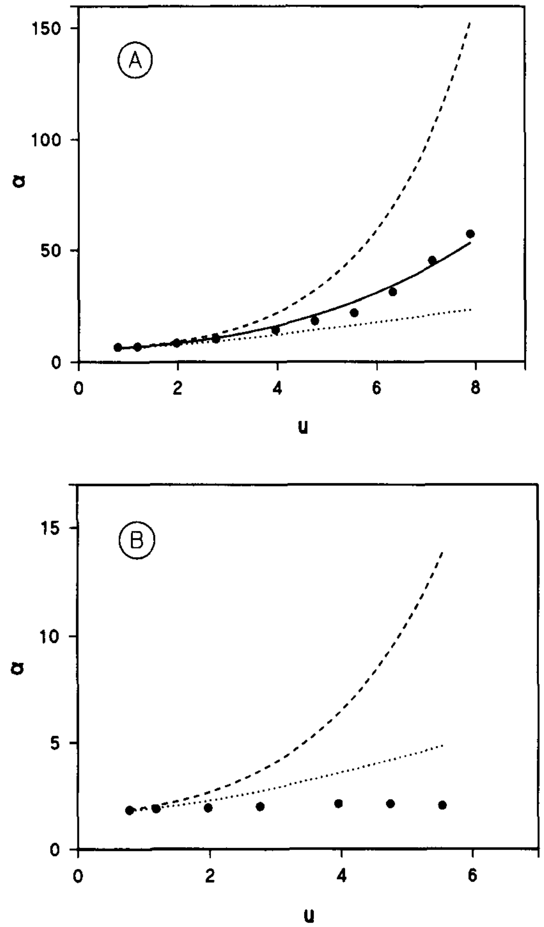

Fig. 4. Voltage dependence of the current relaxation amplitude, α, for the iodine-mediated transport of iodide (A) and bromide (B), respectively. The lines through the data were drawn using Eq. (12) when (A): $k_{AS} = 16500\ s^{-1}$, $k_D = 5700\ s^{-1}$ and $b = 0.7$ and (B): $k_{AS} = 1900\ s^{-1}$, $k_D = 2200\ s^{-1}$ and $b = 1$. The aqueous halide concentration was 0.1 mM KI (A) and 0.3 M KBr (B) respectively at a fixed iodine concentration of 3 μM; diphytanoylphosphatidylcholine/n-decane membranes.

4.3. Voltage dependence of the stationary current

In our previous investigation [26] we identified the heterogeneous interfacial reaction with the rate constants k_R (association) and k_D (dissociation) to be the rate-limiting step of the entire carrier-mediated halide transport. Therefore the stationary current, I_∞, after subsiding of the initial current, I_0, is limited by this step and should saturate for increasing clamp potential according to Eq. (9). In both cases, for I_3^- and I_2Br^-, saturation of the stationary current could be observed (data not shown). The experimental data were in good agreement with the theoretical prediction (see Eq. (9)) up to 150 mV. Rising the clamp voltage a considerable deviation from the theory was found. Inspection of Fig. 2A shows that also the initial conductance data of the iodine/iodide-system and the theoretical predictions differ for potentials higher than 150 mV. These results are in contrast to those recently reported for a chloride transport system in a marine alga [21]. In this publication the discrepancy between the theoretical prediction and the experimental observed dependence of the stationary current on the applied voltage was explained by the assumption of another transport system in the plasmalemma.

5. Discussion

5.1. The Läuger-model provides a good description of the voltage-clamp results

In our previous study [26] we have investigated the iodine- and bromine-mediated halide transport in terms of the kinetic parameters of ion transport, the description of the complex formation and the magnitude of the central potential energy barrier. In the present work we focused our interest on the profile of the central energy barrier inside the membrane, which must be overcome by the halogen-halide complexes to cross the membrane. Therefore we performed voltage-clamp experiments on membranes in the presence of the iodine/iodide and the iodine/bromide complexes. This was done since the treatment of carrier transport under charge-pulse conditions leads to non-linear differential equations that are difficult to handle [6]. This means that both methods have their advantages: the charge-pulse method has a superior time resolution but it is difficult to elucidate the voltage-dependence of the transport systems. The voltage-clamp allows a simple description of the barrier shape but has a limited time resolution. In particular it was impossible to determine the current relaxations for the bromine-iodide and the bromine-bromide systems, which could easily be measured under charge-pulse conditions. It is noteworthy that charge-pulse and voltage-clamp experiments yielded nearly identical results for the two systems investigated here.

The Läuger-model provided the most adequate description of the iodine-mediated halide transport. As reported elsewhere for similar carrier systems [26,31] only one out of two theoretical predicted current relaxations could be resolved. There are two possible explanations for the existence of only one single current exponential within such a model system under voltage-clamp conditions. One is that the time resolution of the detection system is too small to resolve the two current relaxations predicted by the Läuger-model as has been argued previously [21]. However, it seems to be more likely that the amplitude of the second current relaxation is too small to be resolved [31]. The findings of our charge-pulse measurements performed on the iodine/halide systems [26] confirm this suggestion and provide evidence that one of the steps involved in the carrier-mediated ion transport is always in equilibrium [19,27,30], i.e., $k_S \gg k_R c_A$, k_D, k_{AS}. That means the translocation of the free carrier is much faster than all the other steps involved in the halide transport. Here, we gave the first theoretical treatment of this case under voltage-clamp conditions.

5.2. The voltage dependence of the charge translocation step varies with the ion radius

Two different formalisms have been developed to explain the voltage dependence of the charge translocation through low dielectric membranes. One is based on the assumption of a steep barrier in the center of the membrane. A charged molecule crossing the membrane overcomes this type of barrier in one single step. Hence the ion transport can be described by a simple first-order kinetics [14,15]. The voltage dependence of the so-called Eyring barrier is given according to the theory of absolute reaction rates [15]. Following Eqs. (1) and (2) it is very steep and follows an exponential function of the applied voltage.

The other formalism is derived from the integrated form of the Nernst-Planck equation. This means that it has to do with a diffusion process, which implies a very shallow voltage dependence of the charge translocation, i.e., the charge transport occurs in many steps [1]. Evidence has been presented that neither the Eyring barrier nor the Nernst-Planck formalism describes the barrier shape best [18]. Indeed, a trapezoidal barrier (i.e., the use of Eqs. (1) and (2)) provided the best explanation for the voltage-dependence of charge transfer across membranes [4,19,20,22]. The use of the initial conductance, $G_0(V)$, is a well-established method to evaluate the voltage dependence of the charge translocation through membranes [4,19,20,22]. In most of these studies the smaller base of the trapezoid, b was found to be between 0.2 and 0.5, which means that the voltage dependence of the charge translocation step was still more exponential than linear. There exists only one exception. In a recent study of the chloride carrier in *V. utricularis* [31] evidence has been given that a square barrier ($b = 1$) limits the charge transfer within a biological membrane.

Our data show a substantial influence of the structure of the halogen-halide complexes on the translocation rate constant, k_{AS}. This may be explained by an increase of the effective radii (i.e., the smearage of the charge across larger spheres) of these complexes from I_2Br^- to I_3^-. The increase of the effective radii apparently results in a decrease of the central barrier in the membrane. The change of the barrier height could be calculated from the change of the free energy difference, ΔG^0, between the aqueous phase and the center of the membrane which is given by [3,9]:

$$\Delta G^0 = -RT\ln\left(\beta_{AS} k_{AS} \frac{d}{D}\right) \tag{12}$$

ΔG^0 has for I_3^- and for I_2Br^- values of -10.1 kJ/mol and 3.0 kJ/mol, respectively ($\beta_{AS} = 7.2 \cdot 10^{-3}$ cm and $0.3 \cdot 10^{-3}$ cm, respectively [26]). For the calculation of ΔG^0 a value for the diffusion coefficient, D, of 10^{-6} cm^2/s was assumed. The absolute height of the barrier in the middle of the membrane (with respect to the depths of the interfacial wells) is given by the difference between ΔG^0 and ΔG. ΔG is obtained from the partition coefficient β_{AS}, as has been shown previously [2,19,20,32] and has values of -28.9 and -21.2 kJ/mol, respectively for the two complexes.

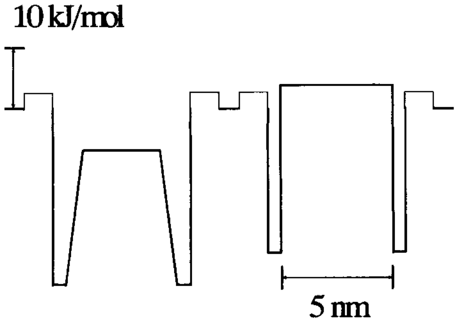

Fig. 5. Profile of the free energy, ΔG, of the complexes I_3^-, and I_2Br^- in a DOPC/n-chlorodecane membrane. See text for details.

Fig. 5 shows the free energy profiles for I_3^- and I_2Br^- in DOPC membranes. The profiles are only approximate values because a value for the diffusion coefficient of the complexes within the membranes had to be assumed for the calculation of ΔG^0 ($D = 10^{-6}$ cm^2/s). It is obvious that both the absolute barrier height and the shape of the potential energy barrier are different for the two complexes. It is noteworthy that I_3^- is more soluble in the membrane interior than it is in the aqueous phase. Similar results have been found for the anionic forms of the uncouplers FCCP (carbonylcyanide *p*-trifluoromethoxyphenylhydrazone) [19] and S-13 (5-chloro-3-*tert*-butyl-2′-chloro-4′nitrosalicylanilide) [20] in phosphatidylcholine/chlorodecane membranes. The shape of the barrier for I_3^- can be adequately modelled as a trapezoid with a shorter side which has a length about 0.7 of the base. I_2Br^- encounters a higher square barrier (~ 8 kJ/mol) and its adsorption wells are somewhat shallower.

Appendix 1

The halogen-mediated halide transport is described according to the Läuger-model by the following differential equations:

$$\frac{dN'_{AS}}{dt} = (-k'_{AS}N'_{AS} + k''_{AS}N''_{AS}) + (-k'_D N'_{AS} + k'_R c'_A N'_S) \tag{A1}$$

$$\frac{dN''_{AS}}{dt} = (k'_{AS}N'_{AS} - k''_{AS}N''_{AS}) + (-k''_D N''_{AS} + k''_R c''_A N''_S) \tag{A2}$$

$$\frac{dN'_s}{dt} = (-k'_S N'_S + k''_S N''_S) + (k'_D N'_{AS} - k'_R N'_S) \tag{A3}$$

$$\frac{dN''_S}{dt} = (k'_S N'_S - k''_S N''_S) + (k''_D N''_{AS} - k''_R c''_A N''_S) \tag{A4}$$

N'_{AS}, N''_{AS} and N'_S and N''_S are the surface concentrations of the complexed and the free carriers on the left and on the right side of the membrane. We assume that the total concentration of carriers does not vary during the voltage-clamp experiment.

$$N_0 = N_{AS} + N_S = N'_{AS} + N''_{AS} + N'_S + N''_S \tag{A5}$$

The current density within the membrane is given by the flux of charges due to the carrier system (Eq. (4)).

In a previous study we have demonstrated that the translocation rate constant, k_S of the free carriers is much faster than the other rate constants involved in iodine-mediated halide transport. This means that the free carriers are always in equilibrium within the membrane:

$$N'_S = N''_S \tag{A6}$$

Furthermore, the surface concentration of the free carriers is independent of time [26]:

$$N_S(t) = N_S(\infty) = N_0 \frac{k_S}{k_D + k_R c_A} \tag{A7}$$

The differential equations (A1)–(A4) together with the conditions (A5) and (A7) reduce to one single differential equation using the following substitution [30].

$$\nu = \frac{N'_{AS}}{N_{AS}}; \tag{A8}$$

$$\frac{d\nu}{dt} = -(k'_{AS} + k''_{AS} + k_D)\nu + \left(k''_{AS} + \frac{k_D}{2}\right) \tag{A9}$$

The combination of Eqs. (4) and (A8) yields the following expression for the current density:

$$I = zFN_{AS}(\nu(k'_{AS} + k''_{AS}) - k''_{AS}) \tag{A10}$$

For times $t < 0$ the entire system is in equilibrium, i.e., according to Eqs. (A6) and (A8):

$$\nu = \frac{N'_{AS}}{N_{AS}} = \frac{1}{2} \tag{A11}$$

Together with the Eqs. (1) and (2) the description of the initial current, I_0, can be given as shown in Eq. (8). Using the substitutions:

$$a = -(k'_{AS} + k''_{AS} + k_D);\ b = k''_{AS} + \frac{k_D}{2} \tag{A12}$$

yields the solution for the differential equation (A8):

$$\nu(t) = \left(\frac{1}{2} + \frac{b}{a}\right)e^{at} - \frac{b}{a} \tag{A13}$$

Hence for times $t \to \infty$ Eq. (A13) reduces to

$$\nu(\infty) = -\frac{b}{a} \tag{A14}$$

Replacement of a and b in Eq. (A14) leads to the expression for the steady state current, I_∞, Eq. (9).

In the limit of small voltages, $V_m \ll 25$ mV, the specific conductivities, G_0 and G_∞, can be calculated from Eqs. (8) and (9) by division of the corresponding current densities by the membrane voltage, V_m.

$$G_0 = \frac{I_0}{V_m} = \frac{z^2F^2N_{AS}}{RTzu}\left(\tfrac{1}{2}(k'_{AS} + k''_{AS}) - k''_{AS}\right) \tag{A15}$$

and the stationary current I_∞ (i.e., the current density after many relaxation times) by:

$$G_\infty = \frac{z^2F^2N_{AS}}{RTzu}\,\frac{\tfrac{1}{2}k_D(k'_{AS} - k''_{AS})}{k'_{AS} + k''_{AS} + k_D} \tag{A16}$$

References

[1] Neumke, B. and Läuger, P. (1969) Biophys. J. 9, 1160–1170.

[2] Parsegian, A. (1969) Nature (London) 221, 844–846.

[3] Ketterer, B., Neumke, B. and Läuger, P. (1971) J. Membr. Biol. 5, 225–245.

[4] Andersen, O. and Fuchs, M. (1975) Biophys. J. 15, 795–830.

[5] Bruner, L. (1975) J. Membr. Biol. 22, 125–141.

[6] Benz, R. and Conti, F. (1981) J. Membr. Biol. 59, 91–104.

[7] Flewelling, R. and Hubbell, W. (1986a) Biophys. J. 49, 531–540.

[8] Flewelling, R. and Hubbell, W. (1986b) Biophys. J. 49, 541–552.

[9] Benz, R. (1988) Biophys. J. 54, 25–33.

[10] Andreoli, T., Tiefenberg, M. and Tosteson, D. (1967) J. Gen. Physiol. 50, 2527–2545.

[11] Mueller, P. and Rudin, D. (1967) Biochem. Biophys. Res. Commun. 26, 398–404.

[12] Stark, G. and Benz, R. (1971) J. Membr. Biol. 5, 133–153.

[13] Benz, R. and G. Stark, (1975) Biochim. Biophys. Acta 382, 27–40.

[14] Zwolinsky, B., Eyring, H. and Riese, C. (1949) J. Phys. Chem. 53, 1426–1453.

[15] Johnson, F., Eyring, H. and Stover, B. (1974) The theory of rate processes in biology and medicine, John Wiley & Sons, New York.

[16] Knoll, W. and Stark, G. (1975) J. Membr. Biol. 25, 249–270.

[17] Benz, R., Läuger, P. and Janko, K. (1976) Biochim. Biophys. Acta 455, 701–720.

[18] Hladky, S. (1974) Biochim. Biophys. Acta 352, 71–85.

[19] Benz, R. and McLaughlin, S. (1983) Biophys. J. 41, 381–398.

[20] Kasianowicz, J., Benz, R. and McLaughlin, S. (1987) J. Membr. Biol. 95, 73–89.

[21] Stark, G., Benz, R., Ketterer, B. and Läuger, P. (1971) Biophys. J. 11, 981–994.

[22] Dilger, J.P. and McLaughlin, S. (1979) J. Membr. Biol. 46, 359–384.

[23] Bürner, H., Benz, R., Gimmler, H., Hartung, W. and Stillwell, W. (1993) Biochim. Biophys. Acta 1150, 165–172.

[24] Läuger, P. and Stark, G. (1970) Biochim. Biophys. Acta 211, 458–466.

[25] Läuger, P. (1972) Science 178, 24–30.

[26] Klotz, K.-H. and Benz, R. (1993) Biophys. J. 65, 2661–2672.

[27] Benz, R. and Läuger, P. (1976) J. Membr. Biol. 27, 171–191.

[28] Läuger, P., Benz, R., Stark, G., Bamberg, E., Jordan, P.C., Fahr, A. and Brock, W. (1981) Q. Rev. Biophys. 14, 513–598.

[29] Dilger, J.P. and Benz, R. (1985) J. Membr. Biol. 85, 181–189.

[30] Wang, J., Wehner, G., Benz, R. and Zimmermann, U. (1991) Biophys. J. 59, 235–248.

[31] Wang, J., Zimmermann, U. and Benz, R. (1993) Biophys. J. 64, 1004–1016.

[32] Pickar, A. and Benz, R. (1978) J. Membr. Biol. 44, 353–376.