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Kinetics of the Iodine- and Bromine-mediated Transport of Halide Ions: Demonstration of an Interfacial Complexation Mechanism

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ABSTRACT Stationary and kinetic experiments were performed on lipid bilayer membranes to study the mechanism of iodine- and bromine-mediated halide transport in detail. The stationary conductance data suggested that four different 1:1 complexes between I_2 and Br_2 and the halides I^- and Br^- were responsible for the observed conductance increase by iodine and bromine (I_3^-, I_2Br^-, Br_2I^-, and Br_3^-). Charge pulse experiments allowed the further elucidation of the transport mechanism. Only two of three exponential voltage relaxations predicted by the Läuger model could be resolved under all experimental conditions. This means that either the heterogeneous complexation reactions k_R (association) and k_D (dissociation) were too fast to be resolved or that the neutral carriers were always in equilibrium within the membrane. Experiments at different carrier and halide concentrations suggested that the translocation of the neutral carrier is much faster than the other processes involved in carrier-mediated ion transport. The model was modified accordingly. From the charge pulse data at different halide concentrations, the translocation rate constant of the complexed carriers, k_{AS}, the dissociation constant, k_D, and the total surface concentration of charged carriers, N_{AS}, could be evaluated from one single charge pulse experiment. The association rate of the complex, k_R, could be obtained in some cases from the plot of the stationary conductance data as a function of the halide concentration in the aqueous phase. The translocation rate constant, k_{AS}, of the different complexes is a function of the image force and of the Born charging energy. It increases 5000-fold from Br_3^- to I_3^- because of an enlarged ion radius.

INTRODUCTION

Small ions such as sodium and potassium ions have an extremely small solubility in membranes, which means that they act like insulator in the absence of protein. Responsible for this small solubility is the Born charging energy and the image force (Neumke and Läuger, 1969; Parsegian, 1969). There exist several strategies for ions to overcome this energy. One is the increase of the volume of the ions by the addition of bulky hydrocarbon groups. Prominent examples for these so-called lipophilic ions are dipicrylamine and tetraphenylborate. Both lipophilic ions have a high permeability through artificial and natural membranes (Ketterer et al., 1971; Andersen and Fuchs, 1975; Bruner, 1975; Benz and Conti, 1981; Flewelling and Hubbell, 1986a, b; Benz, 1988).

Another possibility to overcome the Born charging energy is the binding of the small ions to a large organic carrier molecule. This acts like a cage for the ion and results in a smearing of the charge over a large surface. Examples for this are the valinomycin- and macrotetrolide-mediated transport of potassium ions (Andreoli et al., 1967; Mueller and Rudin, 1967; Stark and Benz, 1971; Benz and Stark, 1975). Valinomycin and the macrotetrolide actin antibiotics act according to the interfacial complexation (IC) mechanism proposed first by Läuger (Läuger and Stark, 1970; Stark et al., 1971; Benz and Läuger, 1976). The study of carrier-mediated ion transport using the voltage clamp or the charge pulse method yields detailed information on the carrier mechanism and on the structure and electrical properties of artificial lipid bilayer membranes (Stark et al., 1971; Knoll and Stark, 1975; Benz and Läuger, 1976; Benz et al., 1976).

The IC mechanism is very well established for a variety of carriers including valinomycin (Stark and Benz, 1971), the macrotetrolide actin antibiotics (Hladky, 1975a, b; Laprade et al., 1975), and the enniatins (Benz, 1978). However, it was not known whether the I_2-mediated I^- transport is an IC or a solution complexation mechanism (SC) (Läuger et al., 1967; Lesslauer et al., 1967). This transport system has occasionally been used for the study of electrical properties of membranes (Andreoli et al., 1967; McLaughlin et al., 1970) but it was never studied in detail. In this paper we show that the I_2-mediated I^- transport acts according to the IC mechanism. Furthermore, we demonstrate that this is not the only possible combination for the formation of membrane-soluble complexes between halogens and halides. Besides I_3^-, also I_2Br^-, I_2Br^-, and Br_3^- are able to increase the conductance of lipid bilayer membranes.

Charge pulse experiments were performed to study the transport mechanism in detail. Only two of three exponential voltage relaxations predicted by the Läuger model (Fig. 1) could be resolved for the different systems. This means that either the heterogeneous complexation reaction was too fast to be resolved or that the movements of the neutral carriers within the membrane are much faster than all the other steps involved in carrier-mediated ion transport. Experiments performed at different conditions revealed that the rate-limiting

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Abbreviations used: DPhPC, diphytanoyl phosphatidylcholine (molecular mass, 846 Da); DOPC, dioleoyl phosphatidylcholine (molecular mass, 786 Da).

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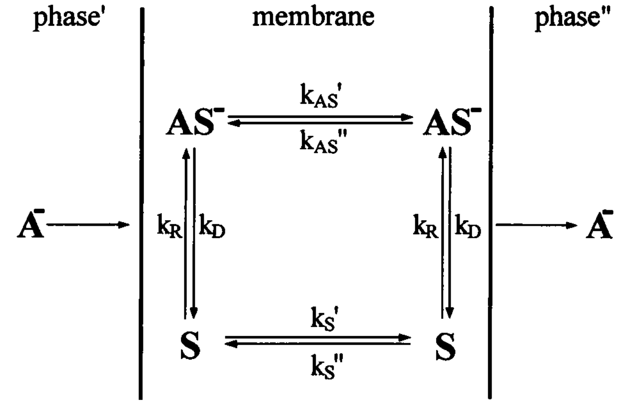

FIGURE 1 Model of the halogen-mediated transport of a halide ion A^-.

step is the interfacial complexation reaction. This suggests that the transport system exhibits some analogy to the macrotetrolide system but is different from the valinomycin or enniatin systems (Stark and Benz, 1971; Hladky, 1975a, b; Knoll and Stark, 1975; Laprade et al., 1975; Benz and Läuger, 1976; Benz, 1978; Laprade et al., 1982). The translocation rate constant, k_{AS}, of the different complexes is dependent on the image force and on the Born charging energy and thus is a function of the ion radius.

MATERIALS AND METHODS

Black lipid bilayer membranes were formed of a 1% solution of DPhPC and DOPC (Avanti Biochemicals, Alabaster, AL) in *n*-decane or *n*-chlorodecane (Fluka AG, 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) and bromine (Aldrich Chemie, Steinheim, Germany) were dissolved in ethanol to obtain concentrated stock solutions. Small amounts of these stock solutions were added to the aqueous phase containing 1 M NaCl (analytical grade, Merck) and different concentrations of potassium iodide or potassium bromide (Aldrich Chemie). The final concentrations of I_2 and Br_2 in the aqueous phase were between 10^{-7} M and 10^{-5} M. The aqueous solutions were prepared fresh each day with 18 MΩ extremely low conducting water (Millipore Super Q, Millipore Corp., Bedford, MA), degassed, and stored in the dark and cool to prevent oxidation of the I^-. 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 and bromine to the lipid bilayer membranes.

The charge pulse experiments were performed with current pulses with a length of 10–100 ns as described previously (Benz and Läuger, 1976). In brief, one Ag/AgCl electrode was connected to a fast-pulse generator (Philips PM 5712) through a transistor used as a fast diode (reverse resistance $\gg 10^{11}$ Ω) and the other electrode was grounded. The voltage between these two electrodes was measured with a high-input resistance fast-voltage amplifier based on a Burr Brown operational amplifier (amplification 20-fold) and a digital storage oscilloscope (Nicolet 4094A). The time resolution of the detection system was about 200 ns. The voltage relaxations were analyzed with a HP 98580AD computer and a 486DX/33 personal computer. In all experiments, the voltage decay following the charge pulses could be fitted to exponential relaxations.

The concentration of the I_3^- and the I_2Br^- complex in the aqueous phase was measured with a UV/VIS spectrophotometer (Perkin-Elmer Lambda 2).

Theoretical considerations

Fig. 1 illustrates the kinetic model proposed here for the description of iodine- and bromine-mediated halide transport through lipid bilayer membranes. This model is the Läuger model and has been described previously (Läuger and Stark, 1970; Benz and Läuger, 1976; Läuger et al., 1981). Here, we list only the basic assumptions of the kinetic model and the equations which permit the calculation of the transport parameters from the experimental data. The model assumes a 1:1 carrier-ion complex which 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 stability constant of the carrier-anion complex in the membrane-water interface is given by $K_m = k_R/k_D$ (the corresponding stability constant for complex formation in the aqueous phase is K_a). 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 (Benz and McLaughlin, 1983):

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

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

$u = FV_m/RT$ is the reduced voltage. V_m is the membrane voltage, F, R and T are standard symbols (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 (Benz and McLaughlin, 1983).

The concentrations of I^- and Br^- on both sides of the membrane are identical. The interfacial concentrations of free and complexed carriers on the two sides of the membrane change with time as given by the Eqs. A1 to A4 (see Appendix and Benz and Läuger, 1976). The total concentration of carriers within the membrane (complexed and uncomplexed) is assumed to be constant at the time scale of an experiment (Eq. A5). The current density, I_m, within a membrane is given by

$$I_m = F\cdot(-k'_{AS}\cdot N'_{AS} + k''_{AS}\cdot N''_{AS}) \tag{3}$$

Equation 3 allows the calculation of the specific stationary conductance, G_m, in the limit of small voltages ($V_m \ll 25$ mV; see Appendix):

$$G_m = \frac{I_m}{V_m} \tag{4}$$

The rate of decay of the membrane voltage V_m following a brief charge pulse duration is determined by the specific membrane capacity C_m and by the current density I_m in the membrane given by the movement of the charged carrier molecules within the membrane:

$$\frac{dV_m}{dt} = \frac{I_m}{C_m} = F\cdot\frac{-k'_{AS}\cdot N'_{AS} + k''_{AS}\cdot N''_{AS}}{C_m} \tag{5}$$

Equation 5 represents together with Eqs. A1 to A5 a system of three coupled differential equations. For small voltages ($u \ll 1$; $V_m \ll 25$ mV), this can be solved to give the time course of the membrane voltage:

$$V_m(t) = V_0\cdot(a_1\cdot e^{-t/\tau_1} + a_2\cdot e^{-t/\tau_2} + a_3\cdot e^{-t/\tau_3}) \tag{6}$$

$$a_1 + a_2 + a_3 = 1 \tag{7}$$

The relaxation times τ_i and the relative amplitudes a_i (i = 1, 2, 3) are known functions of the four rate constants, the total number of carrier molecules, N_0 and the specific membrane capacity, C_m (Benz and Läuger, 1976).

In this investigation only two relaxation processes could be resolved within the limitations of our experimental device. Furthermore, preliminary voltage clamp experiments with the same system show only one current relaxation (apart from the capacitive spike; Klotz and Benz, unpublished results). These results suggest that one of the different reactions involved in carrier-mediated anion transport (see Fig. 1) is always in equilibrium because it is much faster than the others (Benz and Läuger, 1976; Benz and McLaughlin, 1983). This means either that $k_S \gg k_R\cdot c_A$, k_D, k_{AS} or that $k_R\cdot c_A$, $k_D \gg k_{AS}$, k_S (the case $k_R\cdot c_A$, k_D, $k_S \gg k_{AS}$ is unlikely, as it would mean that the voltage-dependent step is rate-limiting and only one relaxation is obtained under charge pulse conditions (Benz and Läuger, 1976)). The first case was found to be consistent with the experimental results (see Discussion). In this case, the differential Eqs. A1 to A4 in the Appendix and Eq. 5 reduce to a system of two differential equations (see Appendix). The solution for the membrane voltage following a charge pulse of short duration has the form:

$$V_m(t) = V_0 \cdot (a_1 \cdot e^{-t/\tau_1} + a_2 \cdot e^{-t/\tau_2}) \tag{8}$$

$$a_1 + a_2 = 1 \tag{9}$$

Defining the quantities P_1, P_2, and P_3 (see Appendix) as

$$P_1 = \frac{1}{\tau_1} + \frac{1}{\tau_2} \tag{10}$$

$$P_2 = \frac{1}{\tau_1 \cdot \tau_2} \tag{11}$$

$$P_3 = \frac{a_1}{\tau_1} + \frac{a_2}{\tau_2} \tag{12}$$

the rate constants k_D and k_{AS} and the total number of complexed carriers in the membrane, N_{AS}, are given by:

$$k_D = \frac{P_2}{P_3} \tag{13}$$

$$k_{AS} = \frac{P_1 - P_3 - k_D}{2} \tag{14}$$

$$N_{AS} = \frac{P_3}{2 \cdot B \cdot k_{AS}} \tag{15}$$

with

$$B = \frac{F^2 \cdot z^2}{4 \cdot R \cdot T \cdot C_m} \tag{16}$$

The voltage relaxation following a brief charge pulse could be fitted to just two exponential relaxation processes, whereas the general case of the carrier model requires the knowledge of three relaxations for complete description (Benz and Läuger, 1976; Benz and McLaughlin, 1983). Thus, information on the rate constants k_R and k_S cannot be obtained from one single experiment. However, the stability constant, K_m, for the binding of the halides to the carrier may be obtained by plotting the specific membrane conductance, G_m, as a function of the halide concentration by assuming a value for $K_m = k_R/k_D$ that gives the best fit to the data.

RESULTS

Stationary conductance data

Stationary conductance experiments were performed on DOPC/*n*-decane membranes to get some insight into the molecular mechanism of iodine-mediated iodide transport. Fig. 2 *A* shows the effect of an increase of the I_2-concentration on the stationary conductance, G_m. For an increase of the iodine concentration by more than two orders of magnitude, we observed a linear dependence of G_m on the I_2-concentration. This result suggested that I_2 is probably the carrier (and not I_4 as has been suggested previously (McLaughlin et al., 1970)). Similarly, I_2 increased also the membrane conductance in the presence of bromide in a linear manner (Fig. 2 *A*), although the specific membrane conductance was considerably smaller than in the presence of iodide. Interestingly, we observed also an effect of bromine on G_m in the presence of iodide (Fig. 2 *A*) and G_m was also in this case linearly dependent on the bromine concentration. Furthermore, we noticed that bromine was also able to increase the membrane conductance in the presence of bromide. However, the influence of 10^{-5} M Br_2 on the membrane conductance in 0.1 M KBr was rather small, and G_m increased only 10-fold as compared with the absence of bromine (data not shown). Both iodine and bromine were not able to increase the membrane conductance in the presence of chloride.

These results suggested that also mixed complexes between bromine and iodide and vice versa are formed and permeate the DOPC/*n*-decane membranes. In another set of experimental conditions we varied the iodide and bromide concentrations in the aqueous phase and kept the iodine con-

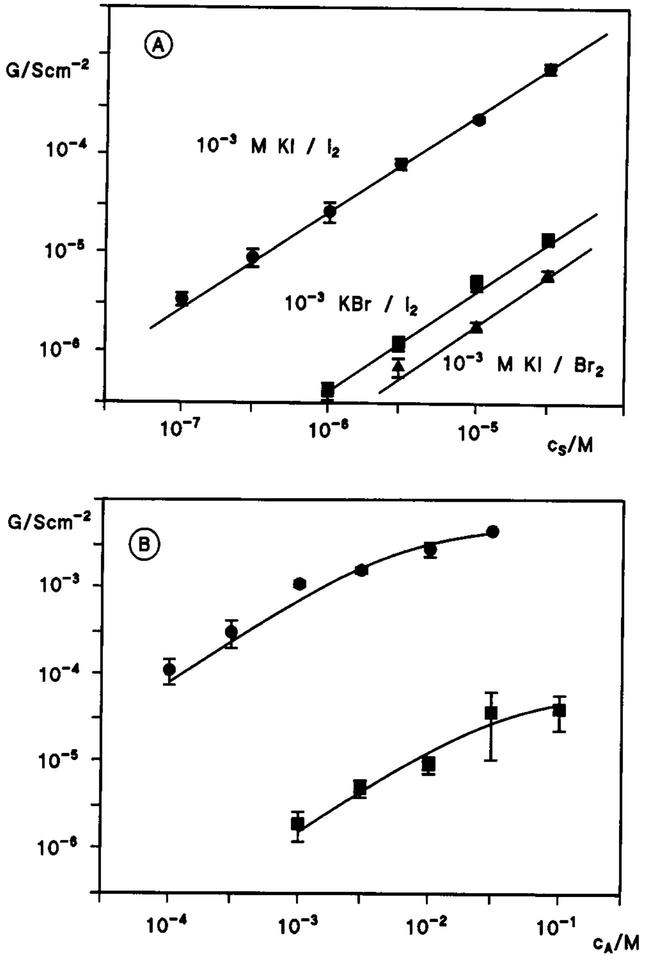

FIGURE 2 (*A*) Specific conductance, *G*, of DOPC/*n*-decane membranes given as functions of the I_2^- or Br_2^- concentration in the aqueous phase (indicated as c_S on the *abscissa*). Besides the halogens, the aqueous phase contained either 1 mM KI or KBr. The data points represent means of at least five membranes. The applied membrane voltage was 20 mV; T = 20°C. The lines represent least squares fits of the data. (*B*) Specific conductance, *G*, of DOPC/*n*-chlorodecane membranes given as functions of the KI^- or KBr^-concentration, c_A, in the aqueous phase. Besides the halides, the aqueous phase contained either 1 or 10 μM I_2. The applied membrane voltage was 20 mV; T = 25°C. The data points represent means of at least five membranes. The solid lines represent least squares fits of the experimental data using Eq. 17, and K_m = 146 1/M (I_3^-), and K_m = 25 1/M (I_2Br^-).

centration at 10^{-6} M (KI) and 10^{-5} M (KBr). The results of these measurements are summarized in Fig. 2 *B*. At small iodide and bromide concentrations, we observed a linear increase of conductance versus concentration curve, which is consistent with the assumption that I_3^- and I_2Br^- are probably the complexed carriers. However, at high iodide and bromide concentrations, we found a strong saturation of the stationary conductance which suggested saturation of the carrier molecules (i.e., the majority of carriers within the membrane were complexed). This can also be demonstrated by the analysis of the data of Fig. 2 *B* using Eq. 17 (Stark and Benz, 1971):

$$G_m = \frac{F^2 \cdot \beta_S \cdot k_R \cdot c_A \cdot c_0}{4RT \cdot (K_m \cdot c_A + 1) \cdot (1 + 2z + \nu \cdot c_A)} \tag{17}$$

d is the membrane thickness, $z = k_{AS}/k_D$, and $\nu = k_{AS} \cdot k_R/(k_S \cdot k_D)$ are combinations of the four rate constants. The partition coefficient of the free carrier, β_S, is given by:

$$\beta_S = \frac{N_S}{2 \cdot c_S} \tag{18}$$

The saturation of the conductance versus iodide (and bromide) may be explained by a non-negligible value for ν. However, current-voltage curves suggested that ν is very small for both systems and independent from c_A (data not shown), which means that the saturation is probably caused by complex formation. On the basis of Eq. 17 a value of K_m = 146 l/mol and K_m = 25 l/mol may be calculated for the formation of the I_3^- and the I_2Br^- complexes by a fit of the experimental data of Fig. 2 *B* using Eq. 17. These results are in reasonable agreement with those of the charge pulse experiments (see below). Experiments with the bromine-iodide and the bromine-bromide systems suggested also a linear increase at small anion concentration and saturation at very high concentrations. However, the data were by far not accurate enough to calculate a precise stability constant for the heterogeneous surface reaction as in the case of I_3^- and I_2Br^-.

Analysis of the charge pulse experiments

With the DOPC/*n*-decane system we did not resolve more than one relaxation in charge pulse experiments. This means that the rate-limiting step is in this case k_{AS} (i.e., k_S, $k_R \cdot c_A$, $k_D \gg k_{AS}$) if we are dealing here with an IC mechanism. To increase the rate constant k_{AS} we performed charge pulse experiments on membranes from DOPC/*n*-chlorodecane since it has been shown previously that the *n*-chlorodecane-induced change of the dielectric constant of the membrane interior increases the translocation constant of charged molecules (and also the stationary conductance at small ion concentration) by a factor of about 500 (Benz and McLaughlin, 1983; Dilger and Benz, 1985). These effects were also observed in this study. Fig. 3 shows oscillographic records of four single charge pulse experiments taken at the same I_2 (1 μM) but different KI concentrations (30 μM (curve 1), 100 μM (curve 2), 300 μM (curve 3) and 1 mM (curve 4)) on DOPC/*n*-chlorodecane membranes. For analysis the voltage versus time curves were plotted semilogarithmic which allowed us to discriminate two distinct exponential relaxations of the initial voltage V_0. The parameters of these voltage decays were determined by two successive least-squares fits as described in detail (Benz, 1988). The analysis of curve 4 of Fig. 3 yielded the following relaxation times and amplitudes: $V_0 \cdot a_2$ = 2.52 mV, τ_2 = 326.0 μs; $V_0 \cdot a_1$ = 6.20 mV, τ_1 = 10.8 μs. From these data the values for N_{AS}, k_{AS}, and k_D were calculated according to Eqs. 10 to 16 by using C_m = 560 nF/cm^2; N_{AS} = 1.56 pmol/cm^2, k_{AS} = 12,500 s^{-1}, k_D = 4,300 s^{-1}.

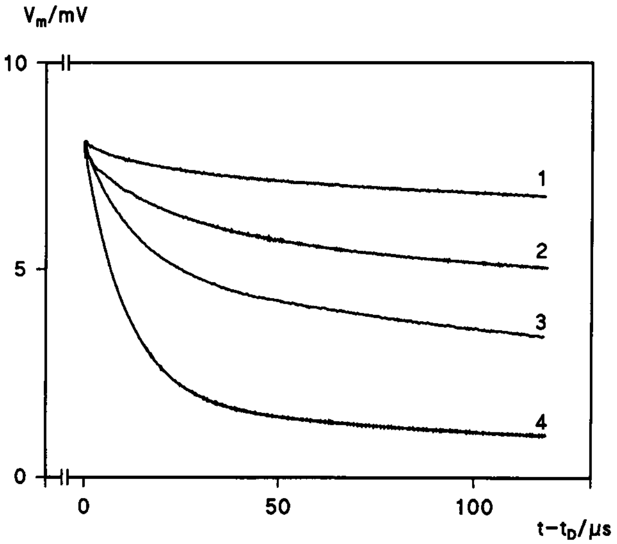

FIGURE 3 Charge pulse experiments with the iodine-iodide system. The decay of the membrane voltage, V_m, after a charge pulse was recorded for different iodide concentrations. The four single experiments were performed on DOPC/*n*-chlorodecane membranes bathed in an aqueous phase containing 1 μM I_2 and 1 M NaCl. The different curves were recorded at the following KI concentrations: curve 1, 0.03 mM; curve 2, 0.1 mM KI; curve 3, 0.3 mM KI; curve 4, 1 mM KI; T = 20°C.

The iodine-mediated iodide transport

Under all experimental conditions used here only two exponential relaxations could be resolved. This means that one of the different steps involved in I_2-mediated iodide transport is always in equilibrium. This is probably the movement of the free carrier, i.e., $k_S \gg k_R \cdot c_A, k_D, k_{AS}$ (see Discussion). This means also that only the translocation rate of the complexed carrier, k_{AS}, the surface concentration of the complexed carrier, N_{AS}, and the dissociation rate of the complexed carrier, k_D, could be calculated according to Eqs. 10–15 (C_m = 560 nF/cm^2) from one single charge pulse experiment. Table 1 shows the experimental data obtained from charge pulse experiments performed on DOPC/*n*-chlorodecane membranes at different iodine concentrations and a fixed iodide concentration of 1 mM. The data of Table 1 represent the mean of at least 10 membranes. The increase of the carrier concentration in the aqueous phase by a factor of 100 resulted in a strong decrease of both time constants and in an increase of the relative amplitude of the fast relaxation process. This

TABLE 1 Dependence of charge pulse data on the iodine concentration in the presence of iodide-forming I_3^- complexes

I_2/[M]	τ_1/(μs)	τ_2/(μs)	a_1	N_{AS}/(pmol/cm^2)	k_{AS}/(l/s)	k_D/(l/s)
1 × 10^{-7}	22 ± 1	2,410 ± 690	0.06 ± 0.01	0.05 ± 0.01	18,500 ± 1,000	6,200 ± 700
3 × 10^{-7}	18 ± 2	730 ± 210	0.29 ± 0.07	0.30 ± 0.10	17,100 ± 900	4,900 ± 400
6 × 10^{-7}	16 ± 2	520 ± 130	0.38 ± 0.05	0.43 ± 0.10	17,400 ± 800	5,100 ± 700
1 × 10^{-6}	10 ± 2	300 ± 30	0.70 ± 0.08	1.70 ± 0.70	13,700 ± 911	4,800 ± 800
3 × 10^{-6}	6 ± 3	190 ± 90	0.81 ± 0.13	4.28 ± 2.55	12,900 ± 2,300	7,800 ± 2,400
6 × 10^{-6}	3 ± 1	110 ± 20	0.93 ± 0.01	8.04 ± 1.28	13,300 ± 1,400	10,100 ± 1,800
1 × 10^{-5}	2 ± 1	80 ± 20	0.94 ± 0.02	12.52 ± 5.60	13,200 ± 1,400	13,700 ± 3,700

Results of charge pulse experiments in the presence of different I_2 concentrations in the aqueous phase. The data are given as means ± SD of at least 10 experiments performed on DOPC membranes. For all systems, 1 M NaCl was used as electrolyte. The temperature was 20°C. The aqueous phase contained 1 × 10^{-3} M potassium iodide. The rate constants 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 10–16.

is obviously caused by the increase of the surface concentration of the carriers in the membrane. The rate constant k_{AS}, as calculated from the relaxation data, was more or less independent on the iodine concentration. Similar results were found for the dissociation constant k_D, which was approximately constant for iodine concentrations between 0.3 and 3 μM and increased then by a factor of about 3, indicating saturation effects at high carrier concentrations in the membrane.

In a second set of experimental conditions we varied the iodide concentration at constant iodine concentration. These experiments were performed on membranes made of two different lecithins, DOPC and DPhPC. The results are summarized in Table 2. Again the relaxation parameters were found to be largely dependent on the KI concentrations, but the rate constants k_{AS} and k_D were independent on the iodide concentration within the limits of experimental variations. Only the total concentration of complexed carriers, N_{AS}, increased about 50-fold between 1 μM and 1 mM KI and saturated for even higher iodide concentrations. This saturation is easy to understand on the basis of Eq. 23 and suggests that the complex formation in the aqueous phase is not negligible. With DPhPC membranes the relaxation parameters showed the same behavior, but the translocation rate constant, k_{AS}, had only half of the value of those derived from DOPC membranes (and reflected also a slower first relaxation, τ_1). This may be caused by a somewhat smaller dielectric constant of the membrane interior of DPhPC membranes as compared with those made of DOPC.

Iodine-bromide, bromine-iodide, and bromine-bromide systems

During the investigation of the iodine-iodide system we noticed that it is also possible to obtain voltage relaxations with the addition of iodine to bromide. Furthermore, voltage relaxations were also observed with the addition of bromine to iodide and bromide, respectively. These results suggested the formation of mixed I_2Br^-, Br_2I^-, and Br_3^- complexes. Subsequently, charge pulse experiments were performed with DOPC/*n*-chlorodecane membranes at constant bromide concentration and increasing concentrations of iodine. We then kept the iodine concentration constant and increased the bromide concentration. The dependence of the relaxation parameters on the experimental conditions was very similar to the results obtained from the I_3^- system. However, the time constant of the fast (first) relaxation process was approximately a factor of 7 larger, indicating a slower translocation

TABLE 2 Results of charge pulse experiments on iodine-endowed PC/*n*-chlorodecane membranes with different iodide concentrations forming I_3^- complexes

KI/[M]	τ_1/(μs)	τ_2/(μs)	a_1	N_{AS}/(pmol/cm^2)	k_{AS}/(l/s)	k_D/(l/s)
DOPC						
1 × 10^{-5}	20 ± 2	3,640 ± 870	0.04 ± 0.01	0.03 ± 0.01	20,400 ± 2,600	6,900 ± 800
3 × 10^{-5}	23 ± 2	1,870 ± 280	0.08 ± 0.01	0.07 ± 0.01	17,500 ± 2,000	6,200 ± 600
1 × 10^{-4}	20 ± 0	1,160 ± 320	0.16 ± 0.05	0.15 ± 0.06	18,100 ± 1,100	5,400 ± 400
3 × 10^{-4}	14 ± 2	350 ± 60	0.34 ± 0.03	0.39 ± 0.06	20,100 ± 2,600	7,800 ± 1,000
1 × 10^{-3}	10 ± 2	300 ± 30	0.70 ± 0.08	1.70 ± 0.70	13,700 ± 900	4,800 ± 800
3 × 10^{-3}	9 ± 3	280 ± 50	0.73 ± 0.09	1.97 ± 0.62	12,300 ± 1,000	5,000 ± 200
1 × 10^{-2}	6 ± 0	310 ± 30	0.86 ± 0.01	3.75 ± 0.34	12,500 ± 1,100	1,900 ± 200
3 × 10^{-2}	6 ± 1	550 ± 60	0.87 ± 0.02	4.04 ± 0.72	11,800 ± 700	2,100 ± 200
1 × 10^{-1}	5 ± 1	250 ± 100	0.91 ± 0.02	6.43 ± 1.61	9,000 ± 1,000	4,600 ± 1,400
DPhPC						
6 × 10^{-5}	35 ± 5	1,410 ± 350	0.11 ± 0.03	0.11 ± 0.03	10,200 ± 1,800	5,600 ± 800
1 × 10^{-4}	29 ± 4	800 ± 260	0.29 ± 0.09	0.31 ± 0.11	10,600 ± 1,000	4,100 ± 1,200
3 × 10^{-4}	27 ± 5	533 ± 260	0.35 ± 0.04	0.45 ± 0.09	9,700 ± 1,800	6,100 ± 2,700
1 × 10^{-3}	20 ± 1	238 ± 20	0.48 ± 0.08	0.78 ± 0.10	9,800 ± 500	8,100 ± 200

Results of charge pulse experiments in the presence of different KI concentrations in the aqueous phase. The data are given as means ± SD of at least 10 experiments. For all systems, 1 M NaCl was used as electrolyte. The membranes were formed of DOPC and DPhPC dissolved in *n*-chlorodecane. The temperature was 20°C. 1 × 10^{-6} M I_2 was added to the aqueous phase. The rate constants 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. 10–16.

rate constant, k_{AS}, of the I_2Br^- system. The experimental data obtained from the two bromine systems showed a similar tendency with respect to variations of iodide and bromide, respectively. With all three systems we were able to observe only two exponential relaxations. This means that again only the rate constants k_{AS} and k_D and the total concentration of complexes within the membrane, N_{AS}, could be calculated. Similarly as with the iodine-iodide system, we noticed saturation effects. In particular, the total surface concentration of charged carriers did not exceed 3 pmol/cm^2 in the presence of bromide or bromine.

The experimental data of all systems are summarized in Table 3. The time constants τ_1 and τ_2 showed a considerable dependence on the type of complex. The variations of τ_1 were caused by the variations of k_{AS}, which increased approximately 5000-fold from Br_3^- through mixed complexes to I_3^-. This indicates probably an influence of the effective radii of the complexes on their mobility in membranes (see Discussion). The data in Table 3 indicate some variations of N_{AS}, k_{AS}, and k_D. It is noteworthy that these variations were not caused by the analysis of the experimental data of one single experiment, because in all cases the time constants of the two relaxation processes were separated by at least one order of magnitude. This means that the deviations of the parameters represented the variations of individual membranes.

Temperature dependence

In another set of experimental conditions, we studied the temperature dependence of the iodine-mediated iodide transport (see Table 4). The translocation rate constant, k_{AS}, increased fourfold for a temperature rise of 30°K. The partition coefficient of the complex, β_{AS}, decreased a factor of four for the same temperature range; β_{AS} was calculated using Eq. 19:

$$\beta_{AS} = \frac{N_{AS}}{2 \cdot c_{AS}} \qquad (19)$$

The temperature dependence of k_{AS} was used to calculate the activation energy, E_a, for transmembrane movement of I_3^-. Fig. 6 shows an Arrhenius plot of the translocation rate constant, k_{AS}, of I_3^- systems in DOPC/*n*-chlorodecane membranes. The value calculated for E_a was approximately 26 kJ/mol.

Influence of ionic strength on transport parameters

In the next set of experimental conditions we investigated the influence of the ionic strength on the iodine-mediated iodide transport. Variations in the ionic strength by a factor of 10 (0.1–1 M) had a negligible influence on the kinetic parameters (data not shown). Only k_D slightly increased with decreasing NaCl concentration reflected in the experimental data by a small increase of τ_1.

Stability constants for the formation of I_3^- and I_2Br^- complexes in the aqueous phase

Iodine shows in the aqueous phase a maximum adsorption at a wavelength of 450 nm. Interestingly, the maximum adsorptions of I_3^- and I_2Br^- are at a different wavelength (350 nm, the corresponding molar extinction coefficient is 36,000 (mol·cm)$^{-1}$). This result allowed the calculation of the stability constant for the formation of the two complexes in the aqueous phase. The stability constant, K_a, was 140 l/mol for the formation of I_3^- and 26 l/mol for that of I_2Br^-.

DISCUSSION

SC or IC mechanism?

The halogen-mediated halide transport could be explained by two different carrier mechanisms, the SC or the IC mechanism. There exist two reasons, which make us think that the second possibility is valid in the case of charge transfer associated with the complexes I_3^-, I_2Br^-, Br_2I^-, and Br_3^-. First, the excellent fit of the experimental data to the modified Läuger model argues in favor of the IC mechanism. On the other hand, two voltage relaxations have also been observed in charge pulse experiments with carriers, which act according to the SC mechanism (Benz et al., 1976). Therefore, we present here also another argument, which follows the treatment of Stark and Benz (Stark and Benz, 1971). The maximum current density, I_d^{max}, of charged molecules through the unstirred layers on both sides of the membranes is given in the absence of a chemical reaction by (Stark and Benz, 1971):

$$I_d^{max} = \frac{F \cdot D_{AS} \cdot c_{AS}}{\delta} \qquad (20)$$

TABLE 3 Dependence of charge pulse data on the complex type

Complex	$\tau_1/(\mu s)$	$\tau_2/(\mu s)$	a_1	N_{AS}/(pmol/cm^2)	k_{AS}/(l/s)	k_D/(l/s)
I_3^-	14 ± 2	350 ± 60	0.34 ± 0.03	0.39 ± 0.06	20,100 ± 2,600	7,800 ± 1,000
I_2Br^-	100 ± 6	560 ± 90	0.32 ± 0.06	0.80 ± 0.10	1,600 ± 100	4,200 ± 700
Br_2I^-	230 ± 20	17,000 ± 3,000	0.33 ± 0.07	0.32 ± 0.10	1,400 ± 300	180 ± 20
Br_3^-	47,800 ± 1,400	290,000 ± 87,000	0.17 ± 0.06	0.74 ± 0.44	3 ± 1	12 ± 1

Dependence of the relaxation parameters on the complex type. The data are given as means ± SD of at least 10 experiments. For all systems, 1 M NaCl was used as electrolyte. The membranes were formed of DOPC dissolved in *n*-chlorodecane. The temperature was 20°C. In the case of the I_3^- system 1×10^{-6} M I_2 was added to the aqueous phase, whereas 3×10^{-6} M I_2 and Br_2, respectively was used for the other systems. The halide concentration ranged from 300 μM (I_3^-) to 1 mM (Br_2I^-) and 3 mM (I_2Br^-) to 200 mM (Br_3^-). The rate constants 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. 10–16.

TABLE 4 Temperature dependence of the charge pulse data

T/(°C)	$\tau_1/(\mu s)$	$\tau_2/(\mu s)$	a_1	$N_{AS}/(\mathrm{pmol/cm^2})$	$k_{AS}/(1/s)$	$k_D/(1/s)$	$\beta_{AS}/(10^{-3}\ \mathrm{cm})$
10	21 ± 2	940 ± 230	0.51 ± 0.08	0.67 ± 0.24	11,300 ± 1,700	2,200 ± 500	8.6
20	14 ± 2	350 ± 60	0.34 ± 0.03	0.39 ± 0.06	20,100 ± 2,600	7,800 ± 1,000	4.8
30	14 ± 1	390 ± 100	0.26 ± 0.08	0.29 ± 0.10	22,900 ± 2,600	10,000 ± 2,300	3.7
40	10 ± 1	310 ± 80	0.13 ± 0.04	0.15 ± 0.05	34,800 ± 3,000	21,700 ± 2,300	1.9

Temperature dependence of relaxation parameters and rate constants of the I_3^- system. The data are given as means ± SD of at least 10 experiments performed on DOPC/*n*-chlorodecane membranes. For all systems, 1 M NaCl was used as electrolyte. The aqueous phase contained 1×10^{-6} M I_2 and 3×10^{-4} M KI. The rate constants 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. 10–16. β_{AS} was calculated according to Eq. 19.

D_{AS} is the diffusion coefficient of the complexes in the aqueous phase (concentration c_{AS}) and $\delta = 0.03$ cm is the thickness of the unstirred layers. I_d^{max} is very small in the absence of a chemical reaction and does not exceed 10^{-9} A/cm^2 at a carrier concentration of 10^{-6} M and an ion concentration, c_A, of 10^{-3} M ($D_{AS} = 10^{-6}$ cm^2/s; $K_a = 140$ l/mol; $c_{AS} = 3 \times 10^{-7}$ M). The current density, I_c^{max}, in the case of a chemical reaction between carriers and ion in the unstirred layers is much larger and given by (Stark and Benz, 1971):

$$I_c^{max} = F \cdot c_A \cdot c_S \cdot \sqrt{2 \cdot K_a \cdot k_R^a \cdot D_{AS}} \qquad (21)$$

k_R^a is the association rate constant for complex formation in the aqueous phase, which we do not know for the complexes considered here. However, assuming that this rate constant is close to diffusion control and as large as 10^8 1/(Ms), the current density I_c^{max} does not exceed 1.6×10^{-5} A/cm^2 under the experimental conditions mentioned above ($c_A = 10^{-3}$ M; $c_S = 10^{-6}$ M). In DOPC/*n*-chlorodecane membranes we measured at 10^{-3} M KI and 10^{-6} M I_2 a stationary conductance of 1.0×10^{-3} S/cm^2. This specific conductance corresponds to a current density of 1.0×10^{-4} A/cm^2 at 100 mV, which is considerably above the limiting current density I_c^{max} calculated according to Eq. 21. This result means that halogen-mediated halide transport across lipid bilayer membranes occurs at least for I_3^- (and probably also for the other systems) according to the IC mechanism.

In this study we presented evidence that the transport systems associated with the anion transport in the presence of I_2 and Br_2 are consistent with the formerly proposed model for the carrier-mediated ion transport (Läuger and Stark, 1970; Benz and Läuger, 1976). Under all experimental conditions, only two of three possible relaxations predicted by the Läuger model could be resolved. This means that one reaction involved in carrier-mediated ion transport shown in Fig. 1 is always in equilibrium (Benz and Läuger, 1976; Dilger and Benz, 1985). We assume that this is the translocation of the free carriers, i.e., $k_S \gg k_R \cdot c_A, k_D, k_{AS}$. We will show in the following that this assumption is consistent with the experimental data.

Which is limiting: k_S or the heterogeneous reaction rate constants?

Two possible restrictions of the Läuger model could influence the model shown in Fig. 1 in such a way that only two voltage relaxations can be observed in charge pulse experiments. One possibility is $k_S \gg k_R \cdot c_A, k_D, k_{AS}$, which means that the exchange of free carriers across the membrane is much faster than all the other reactions involved in carrier-mediated halide transport. The other is that the heterogeneous reaction is always in equilibrium, i.e., $k_R \cdot c_A, k_D \gg k_{AS}, k_S$. Other possibilities do not exist, because in the case $k_R \cdot c_A, k_D, k_S \gg k_{AS}$ (i.e., the translocation of the complexes is rate-limiting), only one voltage relaxation can be observed (Benz and Läuger, 1976).

The first case (i.e., $k_S \gg k_R \cdot c_A, k_D, k_{AS}$) allows the calculation of k_{AS}, k_D and N_{AS} from one single charge pulse experiment. From the formalism of the second case (i.e., $k_R \cdot c_A, k_D \gg k_{AS}, k_S$) the rate constants k_{AS} and k_S can be calculated as functions of the halide concentrations (Wang et al., 1991). Furthermore, it is possible to estimate N_0 from the charge pulse data. The analysis of the experimental data in terms of the two possibilities shows that it is impossible to explain the concentration dependence of the experimental data in the case $k_R \cdot c_A, k_D \gg k_{AS}, k_S$. This is because the total surface concentration, N_0, of carriers should be independent or only little dependent on the halide concentration in the aqueous phase (Fig. 4), which we did not observe. Furthermore, K_{AS} $(= k_{AS} \cdot K_m \cdot c_A/(K_m \cdot c_A + 1))$ and K_S $(= k_S/(K_m \cdot c_A + 1))$ are functions of the halide concentration, which is also not consistent with the experimental data (Fig.4). This means that indeed the heterogeneous surface reaction is the rate-limiting step. It is noteworthy that the alkali ion transport mediated by the macrotetrolide actin antibiotics (of the nonactin, monactin, dinactin, trinactin, and tetranactin series) represents similar systems. Also in these cases the translocation of the free carriers is always in equilibrium (Benz and Stark, 1975; Hladky, 1974; Hladky, 1975b; Lapointe and Laprade, 1982).

How large is the on rate of the heterogeneous surface reaction?

The analysis of the experimental data in terms of the Läuger model and its simplifications yields data on the translocation rate constant of the complexed carriers, k_{AS}, the dissociation rate constant, k_D, and the total surface concentration of complexes, N_{AS}, within the membrane. Information on the heterogeneous complexation rate, k_R, cannot be derived from a single charge pulse experiment. However, the stability constant of the complexes within the membrane, K_m, may be obtained from the stationary data (see above). The aqueous

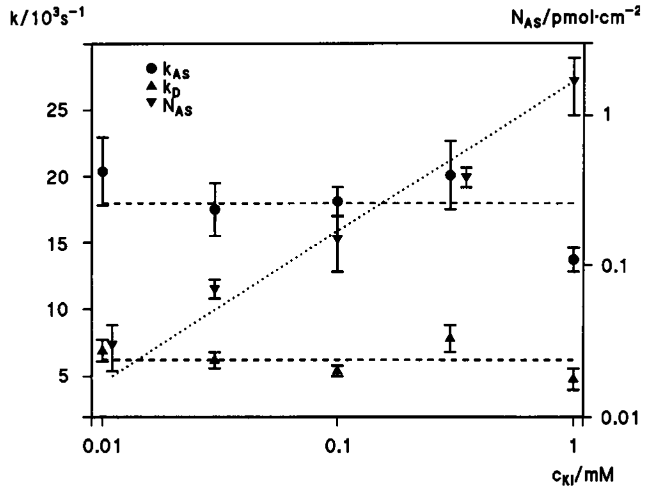

FIGURE 4 Dependence of the total surface concentration of I_3^- complexes, N_{AS}, in the membrane, and of the rate constants k_{AS}, and k_D, on the aqueous KI concentration, c_{KI}. Please note that the data points representing the values for N_{AS} are related to the right y axis, whereas the left y axis is valid for the rate constants, k_{AS}, and k_D. The data were derived from charge pulse experiments on DOPC/*n*-chlorodecane membranes. The dashed lines reflect the theoretical predictions according to Eqs. 10 to 16 (see text). The data are given as means ± SD of at least 10 experiments. The aqueous phase contained 1 μM I_2, and 1 M NaCl; T = 20°C. Note that N_{AS}, k_{AS}, and k_D correspond in the case $k_R \cdot c_A$, $k_D \gg k_{AS}$, k_S to N_0, K_{AS}, and K_S. For further explanation, see text.

stability constant K_a may be obtained by plotting N_{AS} as a function of the aqueous halide concentration and fitting the data to:

$$N_{AS} = N_0 \cdot \frac{K_m \cdot c_A}{K_m \cdot c_A + 1} \tag{22}$$

$$N_{AS} = 2 \cdot \beta_S \cdot c_0 \cdot \frac{K_m \cdot c_A}{K_a \cdot c_A + 1} \tag{23}$$

Fig. 5 shows the plot of the data for N_{AS} for I_3^- (*A*) and I_2Br^- (*B*) as a functions of the I^- and Br^- concentrations in the aqueous phase and the fit of the experimental data with Eq. 23, using $\beta_S = N_{AS}/(2 \cdot K_m \cdot c_A \cdot c_S)$. A linear range for N_{AS} as a function of c_A was followed by saturation at high iodide and bromide concentration. The aqueous stability constants for I_3^- and I_2Br^- derived from the fits of N_{AS} with Eq. 23 were similar to those derived from the photometric determination of the complex concentrations in the aqueous phase (Table 5).

The evaluation of K_m from the stationary data allowed the calculation of k_R according to $k_R = K_m \cdot k_D$. The results for K_m and k_R of these systems are given in Table 5. The values for K_m are dependent on the type of the carrier and on the type of complexed ion. The partition coefficients for the free carriers and the complexes between membrane and the aqueous phase (i.e., β_S and β_{AS}) and the corresponding stability constants for complex formation (K_a and K_m) are related according to the following equation (Stark and Benz, 1971):

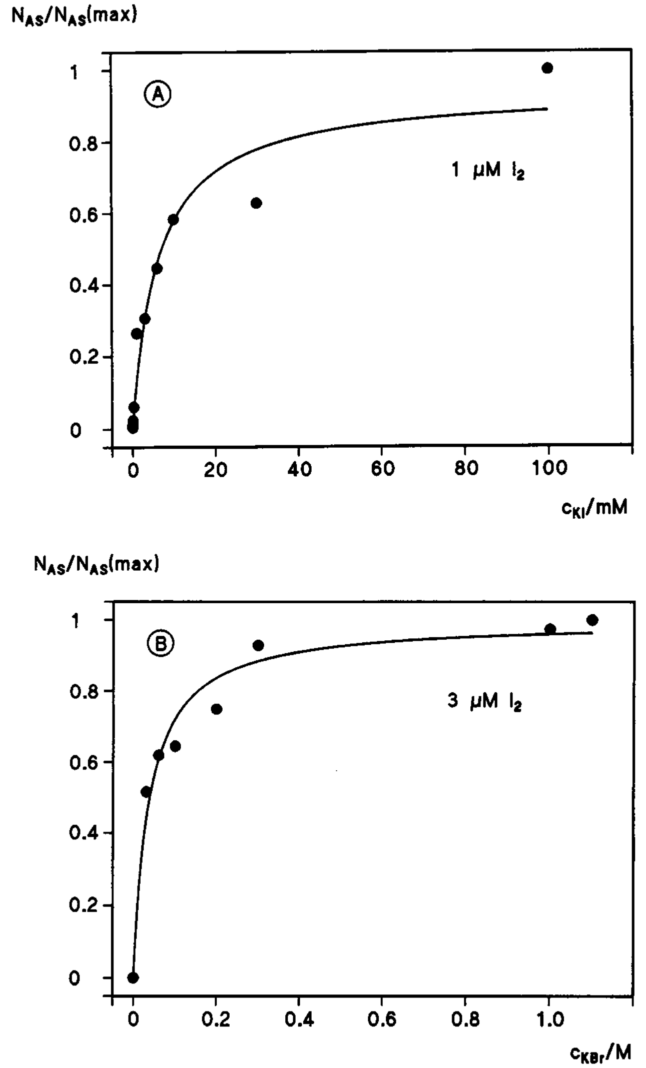

FIGURE 5 Dependence of the total surface concentration, N_{AS}, of I_3^- complexes (*A*) or I_2Br^- complexes (*B*) on the aqueous concentration of KI (*A*) or KBr (*B*). The concentrations of KI and KBr are indicated on the abscissa as c_{KI} and c_{KBr}, respectively. The solid lines were drawn according to Eq. 23 by assuming K_a = 159 l/M (KI) and K_a = 26 l/M (KBr). The data were taken from Table 5. For further explanation, see text.

$$K_m = \frac{\beta_{AS} \cdot K_a}{\beta_S} \tag{24}$$

The partition coefficients were strongly dependent on the type of carrier or complex (Table 5). In particular, β_{AS} of I_3^- was 15 times larger than the partition coefficient of the I_2Br^- system.

How large are the translocation rate constants of the free carriers?

The analysis of the experimental results shows that the translocation rate constants of the free carriers, k_S, (i.e., the movement of I_2 and Br_2 within the membrane) are too fast to be resolved. However, since we know the other three

TABLE 5 Equilibrium constants of the complexes in the membrane, K_m, and the bulk phase, K_a, as well as the partition coefficient of the complexed, β_{AS}, and the free form, β_S, of the halogen

Complex	K_m/(l/mol)*	K_a/(l/mol)‡	K_a/(l/mol)§	k_R/(l/mol·s)	$\beta_{AS}/(10^{-3}$ cm)	$\beta_S/(10^{-3}$ cm)
I_3^-	146	140	159	6×10^5	7.2	7.6
I_2Br^-	25	26	26	1×10^5	0.2	0.3

The values for K_m were obtained from the stationary data (Fig. 2*B*). For the Br_2 systems β_{AS} was evaluated assuming that all bromine was complexed at high halide concentrations in the aqueous phase. β_S was calculated according to Eq. 18.

* Derived from stationary conductance data.

‡ Derived from photometric measurements of I_3^- and I_2Br^-.

§ Derived from the fit of N_{AS} as a function of c_A according to Eq. 23.

rate constants of the I_2^-- or Br_2^--mediated halide transport, it is possible to assume a value for k_S and to calculate the parameters of the three voltage relaxations from our model. We find that $k_S > 10^5$ 1/s in the case of I_2 and $k_S > 10^3$ 1/s in the case of Br_2. Smaller translocation rate constants would lead to amplitudes for the third voltage relaxation, which are large enough to be resolved in our experiments. Translocation rate constants for uncharged solutes through membranes between 10^3 and 10^5 1/s are not unreasonable, since k_S for valinomycin-mediated cation transport is on the order of 10^5 1/s (Stark et al., 1971; Knoll and Stark, 1975; Benz and Läuger, 1976; Lapointe and Laprade, 1982) and those for macrotetrolide-mediated K^+ transport are similar (Benz and Stark, 1975; Laprade et al., 1982). Furthermore, assuming a diffusion coefficient of 10^{-7} cm^2/s for I_2 within the membrane (which is not unreasonable), the translocation rate constant of the free carrier is approximately 10^6 1/s.

Energy of adsorption of free carriers and the carrier-ion complexes to lipid bilayer membranes

The data of Tables 3 and 5 indicate that the complex with the highest translocation rate, I_3^-, has also the largest partition coefficient. This result is in contrast to a recent study of the membrane transport of tetraphenylborate analogues (Benz, 1988). In this study, the adsorption of the analogues decreases with increasing membrane permeability. Table 6 shows the energy of adsorption of free carriers and the carrier-ion complexes to lipid bilayer membranes. The partition coefficients, β, between the aqueous phase and the adsorption site were used to calculate the binding constants, K, and the free energies, ΔG, of adsorption according to:

$$K = \frac{\beta}{l_a} \tag{25}$$

$$\Delta G = -R \cdot T \cdot \ln K \tag{26}$$

where l_a is equal to the thickness of the adsorption plane in the membrane. It is assumed that l_a is temperature-independent and approximately equal to 0.5 nm (corresponding to the thickness of the polar head group layer). ΔG was calculated from the data of the partition coefficients given in Table 5. Since the partition coefficient for Br_2 could not be evaluated, it was impossible to calculate its free energy of adsorption to the membrane.

TABLE 6 Thermodynamic parameters of the adsorption of halogen-halogenide complexes to DOPC membranes at T = 20°C

Complex	$K(AS)$ (10^3)	$K(S)$ (10^3)	ΔG (kJ/M)	ΔH (kJ/M)	$T\Delta S$ (kJ/M)
I_3^-	144	152	-28.9	-9.8	13.5
I_2Br^-	4	6	-21.2		
Br_2I^-	2		-18.5		
Br_3^-	2		-18.5		

The values for the binding constants for the complexed and the free carrier, *K(AS)* and *K(S)*, were calculated from the corresponding partition coefficients, β_{AS} and β_S, presented in Table 5 according to Eq. 25. The free energy of adsorption, ΔG, was evaluated using Eq. 26. The enthalpy of adsorption, ΔH, and the entropy, $T\Delta S$, were calculated according to Eq. 28.

There existed some difference between the free energy of adsorption between the neutral I_2 (given by ΔG^*) and those of the complexes I_3^- and I_2Br^- (i.e., ΔG). This result is easy to understand, since ΔG contains, besides ΔG^*, two additional energy contributions, that of the image force (which tends to increase ΔG) and that of the large positive dipole potential of the membranes (which tends to decrease ΔG, see also below). For DOPC membranes, the dipole potential is about 224 mV (Pickar and Benz, 1978). Its energy contribution is:

$$\Delta W_D = -\phi \cdot F \tag{27}$$

The enthalpy of adsorption, ΔH, was determined from the temperature dependence of the adsorption of the complexes:

$$\Delta H = \Delta G + T \cdot \Delta S = R \cdot T^2 \cdot \frac{d \ln K}{dT} \tag{28}$$

The activation energy, E_a, for the I_3^- complex was obtained from an Arrhenius plot (Fig. 6). The thermodynamic parameters of the adsorption of free carrier and complexes to the membrane are summarized in Table 6. ΔH was negative for the I_3^- complexes, whereas $T\Delta S$ was positive, which means that the adsorption of the negatively charged complexes is entropy-driven. Similar conclusions were already drawn for dipicrylamine (Bruner, 1975; Benz et al., 1976), for tetraphenylphosphonium, the positively charged analogue of tetraphenylborate (Flewelling and Hubbell, 1986a) and for some tetraphenylborate analogues (Benz, 1988).

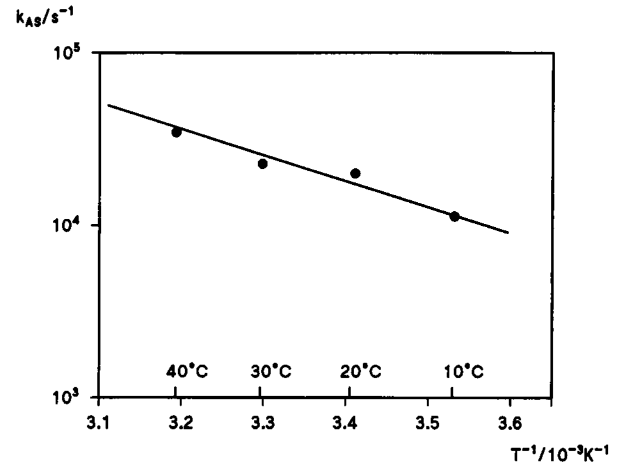

FIGURE 6 Arrhenius plot of the translocation rate constant, k_{AS}, of I_3^- in DOPC/*n*-chlorodecane membranes. The straight line corresponds to an activation energy of 26 kJ/mol.

Energy profile for the movement of the complexes across DOPC membranes

Table 3 demonstrates 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 smear of the charge across larger spheres) of these complexes in the series Br_3^- over mixed complexes 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, given by (Ketterer et al., 1971; Benz, 1988):

$$\Delta G^0 = -R \cdot T \cdot \ln\left(\beta_{AS} \cdot k_{AS} \cdot \frac{d}{D}\right) \qquad (29)$$

Table 7 shows the values for ΔG^0 and for the difference between ΔG^0 and ΔG, i.e., the absolute height of the barrier in the middle of the membrane (with respect to the depths of the interfacial wells). For the calculation of ΔG^0 a value for the diffusion coefficient, D, of 10^{-6} cm²/s was assumed. In the series from Br_3^- to I_3^- the free energy difference ΔG^0 decreased by 26.3 kJ/mol. The absolute height of the central barrier ($\Delta G^0 - \Delta G$) decreased simultaneously by 22.2 kJ/mol.

TABLE 7 Free energy differences and activation energy for the translocation of halogen-halogenide complexes across DOPC membranes and the corresponding effective radii

Complex	ΔG^0/(kJ/M)	ΔG^0/(kJ/M)	E_a/(kJ/M)	r/(nm)
I_3^-	−10.1	18.8	25.4	0.374
I_2Br^-	3.0	24.2		0.309
Br_2I^-	7.1	25.6		0.293
Br_3^-	21.8	40.3		0.247

The free energy, ΔG^0, was determined by using Eq. 29. The value for the activation energy, E_a, was obtained from an Arrhenius plot (Fig. 6). The effective radii were calculated according to Eq. 32 (see text).

Fig. 7 shows the free energy profiles for Br_3^-, Br_2I^-, I_2Br^-, and I_3^- 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 ($D = 10^{-6}$ cm²/s). Consider first the free energy profile for I_3^-. The drop of the free energy, ΔG, as referred to the aqueous phase is about −24 kJ/mol (about −6 kcal/mol). The free energy difference between the aqueous phase and the center of the membrane is about −5 kJ/mol (−1 kcal/mol), which means that the central barrier (with respect to the depth of the adsorption wells) is 19 kJ/mol (5 kcal/mol) for I_3^-. Furthermore, it means that this complex 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*-trifluoromethoxy-phenylhydrazone) (Dilger and Benz, 1985) and S-13 (5-chloro-3-tert-butyl-2′-chloro-4′-nitrosalicylanilide) (Kasianowicz et al., 1987) in phosphatidylcholine/chlorodecane membranes. Preliminary voltage-clamp experiments suggested that the shape of the barrier can be adequately modelled as a trapezoid with a shorter side which has a length about 0.65 of the base (Klotz and Benz, unpublished results) and that the other complexes experience a similar interfacial barrier. A comparison between the free energy profiles for the other three complexes shows that their adsorption wells are somewhat shallower. Simultaneously their central barriers increased by 8.3 (I_2Br^-), 10.8 (Br_2I^-), and by 26.3 kJ/mol (Br_3^-).

Effective radii of the halogen-halide complexes within membranes

As discussed above, the dramatic decrease of the height of the central barrier (i.e., the decrease of ΔG^0 from Br_3^- to I_3^-) is most likely caused by the smearing of the negative charge across a larger sphere, i.e., the effective diameter of

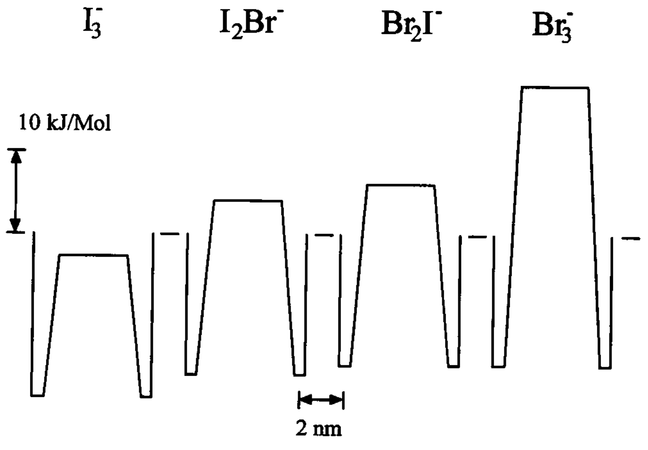

FIGURE 7 Profile of the free energy, ΔG, of the complexes I_3^-, I_2Br^-, Br_2I^-, and Br_3^- in DOPC/*n*-chlorodecane membranes. See text for details.

the complexes increases, which leads to decrease in the charge density. A quantitative description of this effect is somewhat difficult to obtain. The reason for this is that ΔG^0 contains several different contributions:

$$\Delta G^0 = \Delta W_E + \Delta W_D + \Delta G^* \tag{30}$$

where ΔW_E is the contribution of the Born energy and of the image force and ΔW_D is the contribution of the dipole potential (Parsegian, 1969). ΔG^* is the neutral contribution. ΔW_E is given by :

$$\begin{aligned}\Delta W_{\#} &= \frac{F \cdot e}{8\pi \cdot \epsilon_0 \cdot r} \cdot \left(\frac{1}{\epsilon_m} - \frac{1}{\epsilon_2}\right) \\ &\quad - \frac{e^2}{4\pi \cdot \epsilon_0 \cdot \epsilon_w \cdot d} \cdot \ln\left(\frac{2\epsilon_w}{\epsilon_w + \epsilon_m}\right)\end{aligned} \tag{31}$$

ΔW_E is the electrostatic energy (in units of kT), which must be overcome by an ion of radius r and charge e to go from the aqueous phase (relative dielectric constant ϵ_w) into the center of the bilayer membrane (thickness d and relative dielectric constant ϵ_m). Only the first term is dependent on the ion radius, whereas the second term is dependent on the membrane thickness and so has the same value for all complexes.

In the following, we assume that the change of ΔG^* is negligible for the different complexes in comparison with the change of the electrostatic energy, and that all the other contributions to ΔG^0 are independent on the structure of the different complexes. Using these assumptions the effective radii may be calculated from the difference of the free energies ΔG^0 according to:

$$\Delta G^0 = A \cdot \left(\frac{1}{r^*} - \frac{1}{r}\right) \tag{32}$$

The radius r of the I_3^- complex was estimated from its three-dimensional atomic model. The values for r^* are calculated according to Eq. 29 ($A = 2.32 \times 10^{-3}$ Jcm/mol) and are given in Table 7. Interestingly, the effective radii did not vary much from the I_3^- system through the I_2Br^- to the Br_2I^- system. A major change occurred from I_2Br^- to the Br_3^- complex. This may be caused by the fact that the radius of the I^- ion is 0.220 nm, whereas that of the Br^- ion is 0.196 nm. That means the step from the I_2Br^- to the Br_3^- represents a more substantial change of the radius of the membrane-permeant ion than the others.

APPENDIX

The equations below describe the halogen-mediated halide transport according to the Läuger model. Applying a potential across the membrane changes the interfacial concentrations of the free and complexed carrier as follows:

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

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

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

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

During the experiment, the total concentration of carriers does not vary.

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

It is assumed that the translocation of the free carriers is much faster than the other steps involved in the IC mechanism.

$$k_S \gg k'_{AS}, \quad k''_{AS}, \quad k_R \cdot c_A, \quad k_D \tag{A6}$$

Therefore the differential Eqs. A1 to A4 can be reduced to a system of three independent differential equations (Benz and Läuger, 1976). Straightforward calculations similarly done by Wang et al. (1991) lead to the following nonlinear differential equations (Benz and Läuger, 1976; Benz and McLaughlin, 1983) with the substitutions

$$\nu = \frac{N'_{AS}}{N_{AS}}; \quad u = \frac{F \cdot V_m}{R \cdot T} \tag{A7}$$

$$\frac{d\nu}{dt} = -(k'_{AS} + k''_{AS} + k_D) \cdot \nu + \left(k''_{AS} + \frac{k_D}{2}\right) \tag{A8}$$

$$\frac{du}{dt} = \frac{z \cdot F^2}{R \cdot T \cdot C_m} \cdot ((k'_{AS} + k''_{AS}) \cdot \nu - k''_{AS}) \tag{A9}$$

Restriction to small voltages, $V_m \ll 25$ mV, yields the linearized Eqs. A12 and A13 together with the voltage dependence of the translocation rate constants (Lesslauer et al., 1967),

$$k'_{AS} = k_{AS} \cdot \left(1 + \frac{z \cdot u}{2}\right); \quad k''_{AS} = k_{AS} \cdot \left(1 - \frac{z \cdot u}{2}\right) \tag{A10}$$

where k_{AS} is the translocation rate constant at zero voltage

$$\frac{d\nu}{dt} = -(2 \cdot k_{AS} = k_D) \cdot \nu - \frac{z \cdot k_{AS}}{2 \cdot u} + \frac{k_{AS} + k_D}{2} \tag{A11}$$

$$\begin{aligned}\frac{du}{dt} &= \frac{8 \cdot B \cdot N_{AS} \cdot k_{AS}}{z} \cdot \nu - (2 \cdot B \cdot N_{AS} \cdot k_{AS}) \\ &\quad \cdot u + \frac{4 \cdot B \cdot N_{AS} \cdot k_{AS}}{z}\end{aligned} \tag{A12}$$

The characteristic equation of the linear system appears as

$$\lambda^2 - P_1 \cdot \lambda + P_2 = 0 \tag{A13}$$

with λ being the reciprocal value of the relaxation time, τ, and

$$P_1 = 2 \cdot k_{AS} + k_D + 2 \cdot B \cdot N_{AS} \cdot k_{AS} \tag{A14}$$

$$P_2 = (2 \cdot k_{AS} + k_D) \cdot (2 \cdot B \cdot N_{AS} \cdot k_{AS}) - 4 \cdot B \cdot N_{AS} \cdot k_{AS}^2 \tag{A15}$$

Together with the initial conditions $V_m(0) = V_m^0$ and $N'_{AS} = N''_{AS} = (N_{AS})$ a solution for $V(t)$ can be given.

$$V(t) = V_0 \cdot (a_1 \cdot e^{-t \cdot \lambda_1} + a_2 \cdot e^{-t \cdot \lambda_2}) \tag{A16}$$

The inverse relaxation times, λ_1 and λ_2, and amplitudes, a_1 and a_2, can simply be described as

$$\lambda_1 + \lambda_2 = P_1 = 2 \cdot k_{AS} + k_D + 2 \cdot B \cdot N_{AS} \cdot k_{AS} \tag{A17}$$

$$\begin{aligned}\lambda_1 \cdot \lambda_2 &= P_2 \\ &= (2 \cdot k_{AS} + k_D) \cdot (2 \cdot B \cdot N_{AS} \cdot k_{AS}) - 4 \cdot B \cdot N_{AS} \cdot k_{AS}^2\end{aligned} \tag{A18}$$

$$a_1 \cdot \lambda_1 + a_2 \cdot \lambda_2 = P_3 = 2 \cdot B \cdot N_{AS} \cdot k_{AS} \tag{A19}$$

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