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Conductance Channels in Neutral Lipid Bilayers

I. The Conductance of a Model Channel

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Summary. A model channel for the conduction of ions (positive or negative but not both) through a lipid bilayer is presented. The transition-state theory is used to relate the current with voltage and ionic concentrations. Sites within the channel are considered to act cooperatively so that the ion is subjected to a ligand field in which it has complete freedom along the channel axis. The ions in the channel are treated as an ionic gas. Effects due to space-charges within the channel arising from the conducting ions are considered whereas surface-charge effects are neglected.

The ionic specificity of the channel is indicated and the theory compared to that in which equilibrium free energy changes are the dominant influence.

For two decades, the idea that ions penetrate membranes through specific channels (Hodgkin & Huxley, 1952) has been widely accepted although discrete channels have not been clearly identified in biological systems. This void between model and experiment has led to the investigation of channel-forming materials such as excitability-inducing material (EIM) (Ehrenstein, Lecar & Nossal, 1970), gramicidin A (Hladky & Haydon, 1970), and alamethicin (Gordon & Haydon, 1972) in bilayers where the number of channels can be made sufficiently small to allow their individual characteristics to be analyzed. From such studies, information has been obtained which when analyzed in conjunction with macroscopic systems, creates a clearer understanding of the mechanism of charge transport.

Presented here is a version of the "transition-state" theory (Eyring, Lumry & Woodbury, 1949) of membrane conductance extended to include space-charge effects. The conductance referred to is that of the unit channel which for simplicity will be considered to be unaffected by the presence of other channels within the membrane. Processes by which channels may open and close will be described elsewhere (Gordon,

1973) as this is an essential aspect of macroscopic conductances which necessarily requires extra postulates for their description even in the absence of a cooperative behavior between channels.

In the model presented here it is assumed that the sites within the channel are identical and sufficiently close together so that ions, having ion-site distances comparable to the intersite spacings, react with a composite ligand field which is approximately uniform throughout the total length of the channel. If channels are composed of proteins (or peptides) which take up conformations similar to those described for gramicidin channels (open helices) (Urry, 1971), then intersite spacings are probably about 1.5 Å. Ion-site distances could be calculated approximately if the field due to the individual site was known. The feasibility of the model is increased if the ion-site distances are increased by the ion retaining its solvation shell, or if the fields associated with the sites are very small. With the elimination of local effects within the channel the characteristic behavior of the ions can be likened to that of an ionic gas.

Mathematical Description of the Ion-Gas Model

The physical description given above necessitates two dominant potential energy barriers at the ends of the channel which represent the energy required for the transference of an ion from the aqueous phase to the channel and vice versa.

As shown in Fig. 1, the potential energy barriers within the channel (between *A* and *B*) have been smoothed out since ion-site vibrational amplitudes in this model are considered to be larger than intersite spacings. Thus, there is no resistance to ion flow between *A* and *B*.

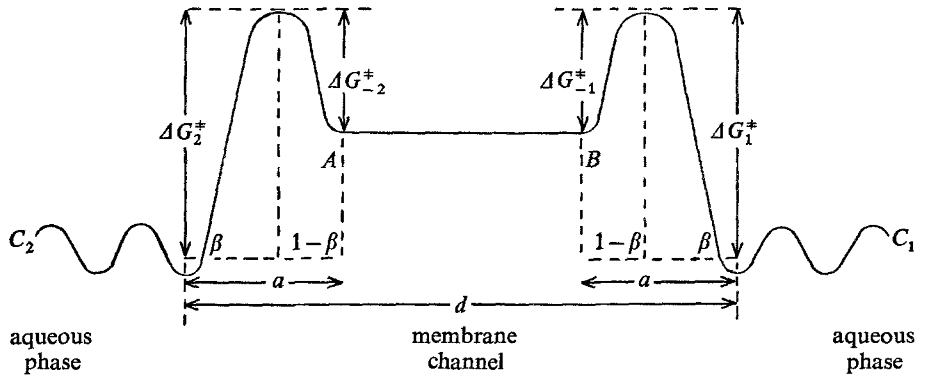

Fig. 1. Potential energy profile for transmembrane channel. The case illustrated is one in which the applied voltage gives rise to zero net current

Consider the case where only cations of valency z can carry charge through the channel. If $E = \psi_2 - \psi_1$ where ψ_2 and ψ_1 are the potentials of the compartments 1 and 2, then the current from 2 into the channel is given by

$$i_2 = k'_2 C_2 zF \exp[-\Delta G_2^{\ddagger}/RT + a\beta zFE/dRT] \tag{1}$$

and from 1 into the channel by

$$i_1 = k'_1 C_1 zF \exp[-\Delta G_1^{\ddagger}/RT - a\beta zFE/dRT] \tag{2}$$

where β is the symmetry factor, the superscript $\ddagger$ indicates the energy of activation (*see* Fig. 1), $a\beta/d$ represents the fraction of the voltage affecting the rate process, F is the Faraday and C_1 and C_2 are the salt concentrations of the aqueous phases 1 and 2, respectively.

Let c_2 and c_1 be the concentrations in the ends of the channel on sides 2 and 1, respectively (at positions A and B in Fig. 1). The exit currents from the channel, i_{-2} and i_{-1}, to the aqueons solutions 2 and 1, respectively, are given by

$$i_{-2} = k'_{-2} c_2 zF \exp[-\Delta G^{\ddagger}_{-2}/RT - a(1-\beta) zFE/dRT] \tag{3}$$

and

$$i_{-1} = k'_{-1} c_1 zF \exp[-\Delta G^{\ddagger}_{-1}/RT + a(1-\beta) zFE/dRT]. \tag{4}$$

The net current flowing through the channel is

$$\begin{aligned} i &= (i_2 - i_{-2}) = -(i_1 - i_{-1}) \\ &= 1/2\,[i_2 - i_1 - (i_{-2} - i_{-1})]. \end{aligned} \tag{5}$$

A channel with a symmetrical conformation about a plane in the center of the membrane has the following relationships between its voltage-independent parameters

$$\begin{aligned} \Delta G_1^{\ddagger} &= \Delta G_2^{\ddagger} = \Delta G \\ \Delta G^{\ddagger}_{-1} &= \Delta G^{\ddagger}_{-2} = \Delta G^{\ddagger}_{-} \\ k'_1 &= k'_2 = k' \\ k'_{-1} &= k'_{-2} = k'_{-}. \end{aligned} \tag{6}$$

Thus,

$$\begin{aligned} i = 1/2\,\{&k' zF \exp(-\Delta G^{\ddagger}/RT)[C_2 \exp a\beta zFE/dRT \\ &- C_1 \exp - a\beta zFE/dRT] \\ &- k'_{-} zF \exp(-\Delta G^{\ddagger}_{-}/RT)[c_2 \exp - a(1-\beta) zFE/dRT \\ &- c_1 \exp a(1-\beta) zFE/dRT]\}. \end{aligned} \tag{7}$$

Let

$$\begin{aligned} k &= k' F \exp(-\Delta G^{\ddagger}/RT) \\ k_{-} &= k'_{-} F \exp(-\Delta G^{\ddagger}_{-}/RT) \end{aligned} \tag{8}$$

$$E_M = \frac{RT}{zF} \ln \frac{C_1}{C_2} \tag{9}$$

and

$$f = \frac{a z F}{d R T};$$

then

$$\begin{aligned} i = 1/2 \Big\{ & k z (C_1 C_2)^{1/2} \left[\left(\frac{C_2}{C_1} \right)^{1/2} \exp \beta f E \right. \\ & \left. - \left(\frac{C_1}{C_2} \right)^{1/2} \exp - \beta f E \right] - k_{-} z (c_1 c_2)^{1/2} \\ & \cdot \left[\left(\frac{c_2}{c_1} \right)^{1/2} \exp -(1-\beta) f E - \left(\frac{c_1}{c_2} \right)^{1/2} \exp (1-\beta) f E \right] \Big\}. \end{aligned} \tag{10}$$

Since there is no resistance to ion flow between A and B (Fig. 1), c_2 and c_1 are related to c, the concentration at the midpoint of the channel by the Boltzmann distribution; that is,

$$c_2 = c \exp - \frac{n F (d-2a) E}{2 d R T}$$

and (11)

$$c_1 = c \exp \frac{n F (d-2a) E}{2 d R T}.$$

From Eq. (11)

$$(c_2 c_1)^{1/2} = c.$$

Thus, Eq. (10) becomes

$$\begin{aligned} i = 1/2 \Big\{ & k z (C_1 C_2)^{1/2} \left[\exp \left(\beta f E - \frac{d f}{2a} E_M \right) - \exp \left(- \beta f E + \frac{d f}{2a} E_M \right) \right] \\ & + k_{-} z c \left[\exp \left((1-\beta) f E + \left(\frac{d-2a}{2a} \right) f E \right) \right. \\ & \left. - \exp \left(-(1-\beta) f E - \left(\frac{d-2a}{2a} \right) f E \right) \right] \Big\} \\ = {} & k z (C_1 C_2)^{1/2} \sinh f \left(\beta E - \frac{d}{2a} E_M \right) + k_{-} z c \sinh f \left(\frac{d}{2a} - \beta \right) E. \end{aligned} \tag{12}$$

As E approaches E_M, i approaches 0. Therefore, at $E = E_M$,

$$k z (C_1 C_2)^{1/2} \sinh f \left(\beta - \frac{d}{2a} \right) E_M = k_{-} z c \sinh f \left(\beta - \frac{d}{2a} \right) E_M;$$

that is,

$$k (C_1 C_2)^{1/2} = k_{-} c = k_{-} (c_1 c_2)^{1/2}. \tag{13}$$

Substituting Eq. (13) in Eq. (12),

$$\begin{aligned} i &= kz(C_1C_2)^{1/2}\left[\sinh f\left(\beta E-\frac{d}{2a}\right)E_M+\sinh f\left(\frac{d}{2a}-\beta\right)E\right] \\ &= 2kz(C_1C_2)^{1/2}\left[\sinh\frac{fd}{4a}(E-E_M)\cosh f\left(\left(\beta-\frac{d}{4a}\right)E-\frac{d}{4a}E_M\right)\right] \qquad (14) \\ &= 2kz(C_1C_2)^{1/2}\sinh\frac{zF}{4RT}(E-E_M)\cosh\left[\left(\frac{\beta azF}{dRT}-\frac{zF}{4RT}\right)E-\frac{zF}{4RT}\right]. \end{aligned}$$

It is interesting to notice that the $i-E$ relationship is not symmetrical about the value $E=E_M$ unless $E_M=0$. Concerning the concentration of ions within the channel it should be noted that from Eq. (13), c is independent of the applied voltage and that c_1 and c_2 are influenced by the voltage and the product C_1C_2. Thus, at $E=0$, $i_{-2}=i_{-1}$ since $c_2=c_1$ [*see* Eqs. (3) and (4)]; i.e., the rate of flow of ions out of the channel is identical for each end and independent of the distribution of ions between the two aqueous phases. This implies that somewhere between the two aqueous phases the ion momentarily suffers a total exchange of momentum due to an interaction with its surroundings and on regaining energy from this environment the sense of direction is lost. The localization of the ion at some point between A and B is contrary to the notion of ions having complete freedom along the axis of the channel. Thus, for consistency, the ion must be capable of exchanging energy with its surroundings while ascending and descending the potential energy barriers at the ends of the channel as it attempts to exit and enter, respectively.

If in the complete traverse of the channel the ion does not gain or lose energy to its environment then

$$i=C_2kz\exp\frac{\beta' zFE}{RT}-C_1kz\exp-\frac{(1-\beta')zFE}{RT} \qquad (15)$$

where

$$\begin{aligned} \beta' &= \frac{\beta a}{d} \quad \text{for } E>E_M \\ &= 1-\frac{\beta a}{d} \quad \text{for } E<E_M; \end{aligned} \qquad (16)$$

that is,

$$\begin{aligned} i=kz(C_1C_2)^{1/2}&\left[\exp\left(\frac{\beta' zFE}{RT}-\frac{zFE_M}{2RT}\right)\right. \\ &\left.-\exp\left(-\frac{(1-\beta')zFE}{RT}+\frac{zFE_M}{2RT}\right)\right]. \end{aligned} \qquad (17)$$

This case is identical with the one in which there is only one activation energy barrier within the channel framework but its position is dependent on whether $E > E_M$ or $E < E_M$.

Because of the complexity of Eqs. (14) and (17) an analysis is best undertaken by choosing the experimental conditions which allow simplification of the expressions and evaluation of the parameters. Confirmation or rejection of the theory is then obtained by substituting the obtained values back into the more general Eqs. (14) and (17).

In the case in which ions exchange energy "thermally" as they attempt to enter and exit from the channel, and where E_M and E are small ($E+E_M<$ 50 mV),

$$\cosh f\left(\left(\beta-\frac{d}{4a}\right)E-\frac{d}{4a}E_M\right)$$

is approximately unity [*see* Eq. (14)] and thus

$$i \doteqdot \frac{kz^2F(C_1C_2)^{1/2}}{2RT}(E-E_M); \tag{18}$$

i.e., i is independent of β and linearly dependent on E.

The derivation of Eq. (14) is only valid if the concentration of ions (or number of ions per unit time) within the channel is negligibly small. With this restriction lifted, the linear pressure P (one-dimensional analogue of a real gas pressure) of the ionic gas in the conduction channel is given by

$$P=RTc+hc^2 \tag{19}$$

where c is an average linear concentration and the second term represents the interaction between the ions. The coefficient h is a constant dependent on the co-ordination number (i.e., $N=2$) and the dielectric constant ε.

$$h=\frac{\frac{1}{2}NF^2}{\varepsilon}. \tag{20}$$

The intrinsic free energy of the ions in the channel $\bar{G}_{\mathrm{ch}}^{\mathrm{in}}$ is given by the thermodynamic relationship

$$d\bar{G}_{\mathrm{ch}}^{\mathrm{in}}=L\,dP \tag{21}$$

where L is the length of channel per unit number of ions, i.e. $L=c^{-1}$.

Thus,

$$d\bar{G}_{\mathrm{ch}}^{\mathrm{in}}=\frac{dP}{c} \tag{22}$$

but from Eq. (19),

$$dP=(RT+2hc)\,dc.$$

Therefore,

$$d\bar{G}_{\text{ch}}^{\text{in}} = \left(\frac{RT}{c} + 2h\right) dc$$
$$\bar{G}_{\text{ch}}^{\text{in}} = \bar{G}_{\text{ch}}^{0} + RT \ln c + 2hc \tag{23}$$

where $\bar{G}_{\text{ch}}^{0}$ is the standard free energy of the ions in the channel.

Also the free energy of the ions in the aqueous solutions is similarly given by

$$(\bar{G}_{\text{aq}})_1 = \bar{G}_{\text{aq}}^{0} + RT \ln C_1 + zF\psi_1$$
$$(\bar{G}_{\text{aq}})_2 = \bar{G}_{\text{aq}}^{0} + RT \ln C_2 + zF\psi_2.$$

At equilibrium,

$$\bar{G}_{\text{aq}} = (\bar{G}_{\text{aq}})_1 = (\bar{G}_{\text{aq}})_2 = \tfrac{1}{2}[2\bar{G}_{\text{aq}}^{0} + RT \ln C_1 C_2 + zF(\psi_1 + \psi_2)]. \tag{24}$$

However, the free energy of ions in the channel is also affected by the equilibrium potentials ψ_1 and ψ_2 in the bathing aqueous solutions. It is readily seen that

$$\bar{G}_{\text{ch}} = \bar{G}_{\text{ch}}^{\text{in}} + \tfrac{1}{2}(\psi_1 + \psi_2) zF. \tag{25}$$

Thus, the difference in free energy between the aqueous and channel states is

$$\begin{aligned} \Delta\bar{G} &= \bar{G}_{\text{ch}} - \bar{G}_{\text{aq}} \\ &= \Delta\bar{G}^{0} + RT \ln c + 2hc - RT \ln (C_1 C_2)^{1/2} \\ &= 0 \text{ at equilibrium;} \end{aligned} \tag{26}$$

that is,

$$\Delta\bar{G}^{0} = \tfrac{1}{2} RT \ln C_1 C_2 - RT \ln c - 2hc. \tag{27}$$

From Eq. (27) it can be seen that the ionic interactions have increased the potential energy profile within the channel region (*AB*, Fig. 1) by the amount $2hc$ and this results in a further increase of $\beta\ 2hc$ in the activation energy of the terminal barriers,

and

$$G_s^{\ddagger} = G^{\ddagger} + \beta 2hc$$
$$G_{s-}^{\ddagger} = G_{-}^{\ddagger} - (1-\beta) 2hc \tag{28}$$

where the suffix s denotes the space charge.

Thus, Eq. (12) becomes

$$\begin{aligned} i = {} & kz(C_1 C_2)^{1/2} \exp -\frac{2\beta hc}{RT} \sinh \left(\beta E - \frac{d}{2a} E_M\right) \\ & + k_- zc \exp \frac{2(1-\beta) hc}{RT} \sinh \left(\frac{d}{2a} - \beta\right) E \end{aligned}$$

where E_M and E are very small and at $E = E_M$,

$$k(C_1 C_2)^{1/2} \exp -\frac{2\beta hc}{RT} = k_- c \exp \frac{2(1-\beta) hc}{RT}$$

$$\therefore\ i=2kz(C_1\,C_2)^{1/2}\exp-\frac{2\beta hc}{RT}$$
$$\cdot\left[\sinh\frac{fd}{4a}(E-E_M)\cdot\cosh f\left(\left(\beta-\frac{d}{4a}\right)E-\frac{d}{4a}E_M\right)\right]$$

at low values of E and E_M; that is,

$$i=\frac{kz^2F(C_1\,C_2)^{1/2}}{2RT}\left(\exp-\frac{2hc\beta}{RT}\right)\cdot(E-E_M). \tag{29}$$

It is assumed in Eqs. (28) and (29) that the field due to the space charge falls off linearly across the activation energy barriers so that the positions of the latter do not alter with the additional field.

From Eq. (27) and with the substitution $K=\exp-\Delta\bar{G}^0/RT$ the following derivation can be made for the condition where c is small:

$$\begin{aligned} c&=(C_1\,C_2)^{1/2}K\exp\left[-\frac{2hc}{RT}\right]\\ &\doteqdot(C_1\,C_2)^{1/2}K\left[1-\frac{2hc}{RT}\right]. \end{aligned} \tag{30}$$

Collecting like terms

$$c\left(1+\frac{2h(C_1\,C_2)^{1/2}K}{RT}\right)=(C_1\,C_2)^{1/2}K. \tag{31}$$

On substituting Eq. (31) into Eq. (30)

$$\exp\left[-\frac{2hc}{RT}\right]=\left[1+\frac{2h(C_1\,C_2)^{1/2}K}{RT}\right]^{-1}$$
$$\therefore\ \exp\left[-\frac{2\beta hc}{RT}\right]=\left[1+\frac{2h(C_1\,C_2)^{1/2}K}{RT}\right]^{-\beta}.$$

By the binomial expansion and the termination of the series after the second term,

$$\exp\left[-\frac{2\beta hc}{RT}\right]=1-\frac{2\beta h(C_1\,C_2)^{1/2}K}{RT}. \tag{32}$$

Substituting Eq. (32) into Eq. (29) yields

$$i_s=\frac{kz^2F(C_1\,C_2)^{1/2}}{2RT}\left(1-\frac{2\beta h(C_1\,C_2)^{1/2}K}{RT}\right)(E-E_M). \tag{33}$$

The channel conductance is given by

$$g=\frac{i_s}{E-E_M} \tag{34}$$

and therefore

$$g/(C_1\,C_2)^{1/2}=kz^2F/2RT-\beta hKkz^2F(C_1\,C_2)^{1/2}/(RT)^2. \tag{35}$$

The linearity of the plot $g/(C_1C_2)^{\frac{1}{2}}$ *vs.* $(C_1C_2)^{\frac{1}{2}}$ allows the evaluation of k and βK. The second term of Eq. (35) represents the effect of the space-charge [compare Eq. (18)].

Another approach to the problem is to lift the restriction on E and to make $E_M=0$; i.e., $C_1=C_2=C$.

Rewriting Eq. (5)

$$\begin{aligned} i &= i_2 - i_{-2} \\ &= kCz\exp\beta fE - k_- c_2 z\exp -(1-\beta)fE \\ &= kCz\exp\beta fE - k_- zc\exp -\frac{nF(d-2a)E}{2dRT}\exp(\beta-1)fE \\ &= kCz\left(\exp\beta fE - \exp\left[\left(-\frac{d}{2a}\right)+(\beta-1)\right]fE\right) \\ &= kCz\left(\exp\beta fE - \exp\left(\beta-\frac{d}{2a}\right)fE\right) \\ &= kCz\exp\beta fE\left[1-\exp\frac{d}{2a}fE\right] \\ &= kCz\exp\beta fE\left[1-\exp\frac{nF}{2RT}E\right] \end{aligned}$$

or

$$\ln\left[\frac{i}{1-\exp\frac{nFE}{2RT}}\right] = \ln kCz + \frac{\beta azFE}{dRT}. \tag{36}$$

From the plot of $\ln i\Big/\left(1-\exp\frac{nFE}{2RT}\right)$ *vs.* E, k and $\beta a/d$ can be evaluated. For low values of E it can be shown that

$$i_s = \left(1-\frac{2\beta hCK}{RT}\right)i.$$

Therefore, for channels where K is large and space-charge effects become important

$$\ln\left[\frac{i_s}{C\left(1-\exp\frac{nFE}{2RT}\right)}\right] = \ln kz\left(1-\frac{2\beta hCK}{RT}\right)+\frac{\beta azFE}{dRT}. \tag{37}$$

Utilizing the two independent variables C and E the values of k, βK and $\beta a/d$ can be obtained.

In conclusion it should be pointed out that the selectivity of the channels described above depends on the parameters k and β (or β') [*see* Eqs. (14) and (17)]. To correlate the free energy change $\Delta\bar{G}^0$ with the selectivity

(Eisenman, 1961) of the channel is to imply that β is constant and independent of the nature of the ions and that

$$d(\ln k)/d(\Delta \bar{G}^0)=\beta; \tag{38}$$

i.e., the principle of linear free energy is implied. This assumption may not be easily justified in the models described above unless the wide range of solvation entropies of the ions in the bathing solution is closely paralleled by the corresponding channel entropies (Frost & Pearson, 1961). However, as space charges effects become more important, i.e. $K(=\exp(-\Delta \bar{G}^0))$ increases, there is a tendency for this equilibrium property to participate in a selectivity mechanism [*see* Eqs. (35) and (37)].

Although concentrations of electrolytes have been considered, it is perhaps more appropriate to use electrolyte activities in the derivations. However, in the case of either cation or anion fluxes the important entity is the activity of the penetrating ions which can not be determined (MacInnes, 1961).

Eqs. (14) and (17) represent total exchange of energy and no exchange of energy, respectively, between the ion and its surroundings as it crosses the membrane and although intermediate cases exist they have not been discussed.

I would like to thank Dr. S. B. Hladky for many valuable discussions.

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