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Conductance Channels in Neutral Lipid Bilayers

II. High-Level Conductances of Multi-Channel Systems

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Summary. The macroscopic conductances of membranes containing model channels are discussed in thermodynamic terms. Channels are considered to be either neutral or ionic and the energetics of the opening processes are analyzed in terms of solvation energies, the change of dielectric and the displacement of charge within the systems. The ionic nature of the channel is due to either the complexing of free solution ions with the channel-forming material or fixed charges on this material. The dependence of the macroscopic conductances on the unit channel conductance, voltage, salt concentration, unit channel concentration, pH, temperature, membrane thickness and dielectric constant are considered.

Although there may be many ways of constructing molecular models to represent ionic conductance channels, it seems most reasonable to suppose that the channels will be composed of molecules having hydrophobic and hydrophilic fractions. The former fraction being necessary for favorable interactions with the lipid bilayer so that lysis does not occur when the membrane is bridged and the other fraction being essential for the production of a central pore of high dielectric constant which would allow the penetration of ions. It is possible that the channel-forming material (CFM) changes its conformation as it passes from the aqueous phase (or membrane surface phase) into the membrane so that it is accommodated more favorably in its new environment. The model put forward by Lenard and Singer (1966) and Wallach and Zahler (1966) for structural proteins of membranes may also be useful in describing channel structure. The structural protein molecule was considered to have both random-coil and helical conformation but the latter only occurred within the membrane. The section of CFM within the membrane may even exhibit a uniformity throughout its entire length (Urry, 1971).

The thermodynamics of the opening and closing of model channels can be discussed in quite simple terms if the following assumptions are made: (*i*) The dielectric constants of the membrane (ε_1) and CFM (ε_2) are invariable under all conditions. (*ii*) Channels of any particular type have the same uniform cross-section. (*iii*) The movement of the conduction-ions is far more rapid than that of the CFM.

In this paper three types of channels are analyzed and these are differentiated according to their ionic nature. The "neutral CFM model" is one in which no ions (whether free or part of the CFM) cross the membrane-aqueous phase interfaces until the channel is opened and although the CFM may have ionic groups, they cannot leave the aqueous phase. The second type, the "ion-CFM model", has the same characteristics as the previous one except that a free solution ion must complex with the CFM and pass through the membrane during the channel-opening process. If the free solution ion of the latter model is replaced by an ion of the CFM then the channel can have significantly different characteristics and will be named the "ionic CFM model."

Neutral CFM Model

If the hydrocarbon region of the membrane has a thickness δ and if A and l are the uniform cross-sectional area and length of penetration, respectively, of the entering CFM (*see* Fig. 1) then the change in the mem-

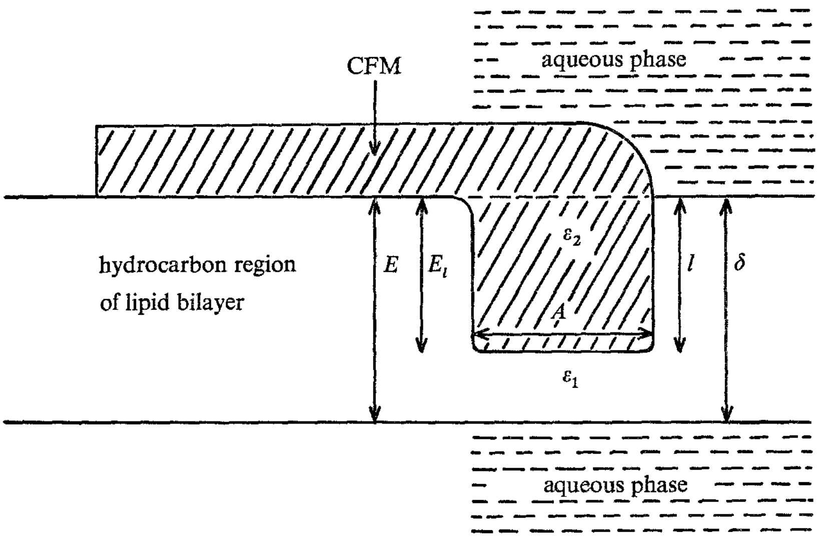

Fig. 1. The neutral CFM model. Polar head groups of the lipid are omitted for simplicity. The part of the CFM molecule penetrating the bilayer need not necessarily be an end portion

brane capacitance due to this entry is given by

$$\Delta C = \left[\frac{l}{A\varepsilon_2} + \frac{\delta - l}{A\varepsilon_1}\right]^{-1} - \frac{A\varepsilon_1}{\delta}. \tag{1}$$

The energy gained by the system due to a change in the dielectric is

$$u_\varepsilon = E^2 \Delta C/2 \tag{2}$$

where E is the membrane potential.

$$u_\varepsilon = \frac{A l \varepsilon_1 (\varepsilon_2 - \varepsilon_1) E^2}{\delta(\delta\varepsilon_2 - l(\varepsilon_2 - \varepsilon_1))}. \tag{3}$$

When the channel is finally formed

$$l = \delta \tag{4}$$

and

$$u_{c,\varepsilon} = \frac{A(\varepsilon_2 - \varepsilon_1) E^2}{\delta}. \tag{5}$$

The overall energetics of the system include other important factors which occur simultaneously with the dielectric change. The solvation and intramolecular bond energies will vary with the distance of penetration and the conformational changes will certainly affect the entropy of the system. These voltage-independent variables can be grouped together as a free energy change

$$(\Delta G)_l = \Delta G_\varepsilon \cdot l \tag{6}$$

where ΔG_ε is the free energy change of one mole of a unit length of CFM and the subscript l indicates the dependence of this energy on the length of penetration.

What happens to the lipid molecules that once occupied the position of the open channel? If the membrane is made larger by an area equal to A then no net change in energy of the system results from this cause, but if they are displaced from the bilayer region then an extra energy term involving the surface tension of the bilayer and A would have to be taken into account. This effect will be considered to be negligible in the following argument.

With N independent CFM molecules in contact with the membrane, the number n_ε penetrating a distance l is given by the Boltzmann distribution

$$\frac{n_\varepsilon}{N - n_\varepsilon} = \exp \frac{U_\varepsilon}{RT} \tag{7}$$

where

$$U_\varepsilon = L u_\varepsilon - \Delta G_\varepsilon \cdot l \tag{8}$$

and L is Avogadro's number; that is,

$$n_{\varepsilon}=\frac{N \exp U_{\varepsilon}/RT}{1+\exp U_{\varepsilon}/RT}. \tag{9}$$

The number of open channels $n_{c,\varepsilon}$ is given by

$$n_{c,\varepsilon}=\frac{N \exp U_{c,\varepsilon}/RT}{1+\exp U_{c,\varepsilon}/RT} \tag{10}$$

where

$$U_{c,\varepsilon}=\frac{LA(\varepsilon_2-\varepsilon_1)E^2}{\delta}-\Delta G_{\varepsilon}\cdot\delta. \tag{11}$$

The fraction $n_{c,\varepsilon}/N$ obtained from Eq. (10) can, in the case of $N=1$, represent the fraction of the time the channel remains open.

For a large number of channels, $n_{c,\varepsilon}$ is proportional to the observed macroscopic conductance G.

If the single channels are ohmic and have conductance g, and if $n_{c,\varepsilon} \ll N$ then

$$\ln G=\frac{LA(\varepsilon_2-\varepsilon_1)}{\delta}E^2-\Delta G_{\varepsilon}\cdot\delta+\ln N g \tag{12}$$

where the only variables are G and E.

If the CFM molecules are too small to bridge the hydrocarbon layer then it may be necessary for two or more to join to form a channel. For a channel constructed of x CFM molecules

$$\frac{n_{c,\varepsilon}}{(N-x n_{c,\varepsilon})^x}=K \exp U_{c,\varepsilon}/RT \tag{13}$$

where K is the equilibrium constant for the x-mer-monomer equilibrium.

This result is independent of whether one side of the membrane supplies all or some of the x molecules. However, the kinetics of the opening and closing of the channel will depend on the location of the CFM molecules if more than one is necessary for channel formation.

Finally, as $n_{c,\varepsilon}$ approaches N the last term of Eq. (12) becomes a poor substitute for $\ln(N-n_{c,\varepsilon})g$ and so the simple relationship between $\ln G$ and E^2 is lost.

The Ion-CFM Model

The opening of a neutral channel for the passage of ions can arise, if before or during its formation an ion "complexes" with the CFM; i.e., an ion moves into the unopened channel (where $0 \leqq l < \delta$) and assists the process of its opening. If it is assumed that the channel is uniform throughout its length (they may have discrete sites or continuous ligand field as in the ion-gas model), the applied electric field will cause the ion to spend most of its time at the point of lowest energy which is to be found at the farthest distance from the aqueous phase from which it came. If the potential difference between the two ends of the unopened channel is E_l then

$$E_l = E - E_{\delta - l}. \tag{14}$$

However,

$$E_{\delta - l} = E_l \frac{(\delta - l)\varepsilon_2}{l\varepsilon_1}.$$

Therefore

$$E_l = E \bigg/ \left(\frac{[\delta - l]\varepsilon_2}{l\varepsilon_1} + 1 \right). \tag{15}$$

The energy gained by the system due to the transference of the ion is

$$\begin{aligned} u_q &= E_l\, q \\ &= E \cdot q \bigg/ \left(\frac{[\delta - l]\varepsilon_2}{l\varepsilon_1} + 1 \right) \end{aligned} \tag{16}$$

where q is the charge on the ion.

The associated free energy change due to solvation and entropy effects are independent of l and are therefore unlike the corresponding term of the previous model.

Thus, the total molar energy change for the system is

$$U_q = Lu_\varepsilon + Lu_q - \Delta G_\varepsilon \cdot l - \Delta G_q \tag{17}$$

and the energy necessary for the opening of a channel is given by

$$U_{q,c} = \frac{LA(\varepsilon_2 - \varepsilon_1)E^2}{\delta} + EzF - \Delta G_\varepsilon \cdot \delta - \Delta G_q \tag{18}$$

where z is the valency of the ion.

Eq. (18) holds only when the ionic movement is much faster than the molecular movement of the CFM so that each conformational change of the latter results in an instantaneous redistribution of ions to retain equilibrium conditions throughout the opening and closing processes.

The number of open channels is given by $n_{c,q}$ where

$$\frac{n_{c,q}}{\theta(N - n_{c,q})} = \exp U_{q,c}/RT$$

and θ is a function of the aqueous phase concentration of ions which participate in the gating of the channel. θ is the fraction of CFM molecules complexed with ions and at a first approximation can be related to the aqueous phase concentration C_0 by a Langmuir isotherm.

$$\theta = \frac{K_1 C_0}{1 + K_1 C_0}. \tag{19}$$

As C_0 increases, θ tends to 1 whereas at low values of C_0, θ is proportional to C_0.

Two features distinguish the case where $U_{c,\varepsilon} \ll U_{c,q}$ (i.e., where ion participation in the opening of the channel is essential) and these are best illustrated when $n_{c,q} \ll N$, which is a condition most readily obtainable at low C_0 and low E. Under these conditions

$$\ln G = \ln \theta + \ln N g + (zFE - \Delta G_q + U_{c,\varepsilon})/RT. \tag{20}$$

Thus, $\ln G$ is proportional to E (and not E^2 as in the previous model) and secondly assuming that g and θ are proportional to C_0 then G is proportional to C_0^2 (not C_0 as in the previous model).

The neutral CFM model arises when $U_{c,\varepsilon} = U_{c,q}$. A complicated analysis results for the intermediate cases where neither of the energies associated with the transferred charge or the change in dielectric, dominate. Even under the condition of $n_{c,q} \ll N$ the indices of the voltage and concentration in Eq. (20) vary from 1 to 2 and 2 to 1, respectively, as the voltage is increased. Further difficulties will arise in the analysis as $n_{c,q}$ approaches N.

The Ionic CFM Model

If the ion taking part in the gating process is not a free solution ion but a part of the CFM then the energy required for opening the channel will be identical to $U_{c,q}$. However, important differences between this model and the previous one could arise. First, the ionic CFM model is not dependent on the concentration of ions in the aqueous phase, so that θ, of Eq. (19), will be unity. Exceptions are possible if the ionic groups of the CFM are dissociated, weak acids or bases, in which case, the total conductance will be dependent on the pH.

Second, the ionic CFM can bear a charge of the same or of the opposite sign to the conduction ions whereas in the second model the ions referred to are the same in sign. If the ions are opposite in sign, negative resistance characteristics can result in one quadrant of the $i-E$ curve. This arises when there are many open channels at $E=0$ and increasing E results in an increase of the barrier to the transfer of the ion of the CFM; i.e., a minus

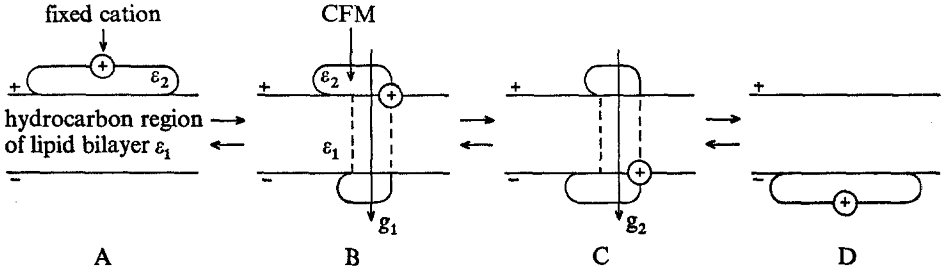

Fig. 2. A special case of the ionic CFM model

sign replaces the plus sign in Eq. (18) and corresponding signs in later equations. Under these conditions an increase in voltage can lead to an accompanying decrease in the conductance.

However, at high voltages where the term containing the factor E^2 dominates, there is an increase in the macroscopic conductance, for the dielectric energy term always increases favorably with the voltage. Increasing E negatively (i.e., in the opposite direction to that taken above) increases $n_{c,q}$ because both voltage-dependent terms favor the process.

Another variation of this model is one in which the position of attachment of the ion to the CFM is not at the leading edge of the penetrating molecule. This occurs when the open unit channel can have two conducting states which depend on whether the attached ion is at the entrance or exit of the channel and also on the relationship between the sign of the fixed ion and that of the conduction ions. The simple model of Fig. 2 indicates that transitions between the four states can arise: those between the two open-channel states and those between the two open-channel states and their corresponding closed-channel states. The latter two equilibria rely entirely on $U_{c,\varepsilon}$ whereas the former depends on $(U_{c,q}-U_{c,\varepsilon})$ which is only associated with the transference of charge. The total conductance

$$G=G_1+G_2 \tag{21}$$

where the subscripts represent the two open states

$$\ln\frac{n_1}{N-n_1-n_2}=U_{c,\varepsilon}/RT \tag{22}$$

and

$$\ln\frac{n_2}{N-n_1-n_2}=U_{c,q}/RT \tag{23}$$

where

$$n_1 g_1=G_1 \tag{24}$$

and

$$n_2 g_2 = G_2. \tag{25}$$

The sign of the term in $U_{c,q}$, having a linear dependence in E, will depend on the signs of the ionic species. Simple solutions relating G with E can be obtained if simplifying assumptions, such as $n_1 + n_2 \ll N$ and $U_{c,\varepsilon} \ll U_{c,q}$ or $U_{c,\varepsilon} \doteqdot U_{c,q}$ are used; i.e., the model reverts to the simple ionic CFM model or the neutral CFM model, respectively.

Discussion

An understanding of the mechanisms of membrane conductances relies basically on a knowledge of relationships between the current, membrane voltage, and concentration of conduction-ions. However, the description of mechanisms using only thermodynamic data is rather limited and thus kinetic properties of systems should be investigated simultaneously. This paper presents a consideration of simple systems and an analysis of them in thermodynamic terms. The dependence of the conductance with time on instantaneous changes of voltages, etc. has not been discussed. The unit channel conductance has been treated in some of the arguments as being independent of voltage and linearly related to concentration, but, from the ion-gas model (Gordon, 1973) it can be seen that this treatment is an oversimplification.

It was stated that unit channels with two discrete conductance levels could arise in the ionic CFM model. This can be seen most readily by taking an example. In the ion-gas model, g depends on the concentration of conducting ions at both the entrance and exit of the channel. However, this balance of concentrations of conducting species is upset by the introduction of a fixed charge at either the entrance or exit and the new concentrations that arise will be given to a first approximation by the Debye-Huckel formula. These two concentrations are not independent of the location of the fixed charge and thus the two values of g may differ considerably (causing rectification) and their difference is most likely to be dependent on the salt concentration, becoming less pronounced as the latter increases.

The inequality $\varepsilon_2 \gg \varepsilon_1$ has been assumed throughout the paper, for the reversal of this inequality was thought to be highly improbable. In the neutral CFM model it leads to a decrease in conductance with increasing voltage.

Where diffusion polarization of macroscopic systems occurs it should be noted that channels remain discrete and that planar-diffusion models may not be acceptable solutions to the problem. On the other hand, spherical diffusion layers around entrances and exits will become less acceptable as the concentration of channels increases. Therefore, in the case where diffusion of ions to or from the channel is rate limiting, values of g may become dependent on the proximity of neighboring channels.

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