

AN ALLOSTERIC MODEL FOR ELECTRICALLY EXCITABLE SODIUM CHANNELS

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Abstract

An allosteric model of the Monod-Wyman-Changeux (1965) type for electrically excitable sodium channels is developed to give a physical counterpart to electrophysiological data. In this model it is assumed that the sodium conductance is regulated by the quaternary conformation of a multisubunit protein encompassing the sodium channel and that the gating current originates from the tertiary conformation change of the subunit carrying an electric dipole moment or electric charge; the accumulation of the tertiary conformation changes in one of the quaternary conformations induces the quaternary conformational transition and the sigmoidal dependence of the sodium conductance on the voltage is explained by this conformational transition. The model well reproduces the "crossover" interrelation that the normalized sodium conductance overtakes at an intermediate voltage and further exceeds the fraction of electric charges transferred by the gating current. The similar interrelation observed in kinetic measurement is also reproduced. Properties of the gating current, charge immobilization and difference between the ON and OFF kinetics at the same membrane potential, are explained by the tertiary conformation change occurring in the respective quaternary conformations. The voltage-dependent binding of the scorpion toxin to the sodium channel and the slowing of inactivation suggests that this toxin exhibits a preferential binding to one of the quaternary conformations. In this way, it is shown systematically that some properties of the channel arise from the tertiary conformation of the subunit and others from the quaternary conformation of the whole subunits, in contrast to the usual view of the correspondence of one function to one molecule.

1. Introduction

Since the well-known work by Hodgkin and Huxley (1952), many investigators have succeeded in interpreting their data of electrically excitable membranes in terms of the opening and closing of sodium and potassium channels. Although little is

known about the molecular structure of the channels, it is now generally considered that the rearrangement of the charged gating structure existing in a channel is responsible for the channel opening in response to a change of membrane potential. The motion of the charged structures in sodium channels generates a detectable current called the gating current or the asymmetrical displacement current (Keynes & Rojas, 1974; Armstrong & Bezanilla, 1974; Meves, 1974; Nonner *et al.*, 1975). Attempts to quantitatively correlate the voltage and time dependence of the gating current with the Hodgkin-Huxley activation parameter m have, however, met with unsatisfactory results (Armstrong & Bezanilla, 1974; Neumcke *et al.*, 1976; Armstrong & Bezanilla, 1977; Armstrong & Gilly, 1979). Although alternative kinetic schemes have been discussed to describe both the sodium conductance change and the gating current (Armstrong & Bezanilla, 1977; Armstrong & Gilly, 1979; Dubois & Schneider, 1982), the molecular mechanism leading to such kinetic schemes remains not to be inquired.

For chemical excitation, allosteric mechanisms have been proposed (Kijima *et al.*, 1978, 1980a,b; Heidmann & Changeux, 1978). In these models the binding of an agonist to a receptor induces conformational changes of the receptor molecule, by which an ion-conducting pore is formed. This mechanism is quite similar to a cooperative ligand binding to a protein consisting of subunits, where the ligand binding to a subunit affects the affinity of the other subunits through the induced conformational change (Monod *et al.*, 1965; Koshland *et al.*, 1966). In fact, it is suggested that the acetylcholine receptor consists of five subunits (Raferty *et al.*, 1980) and there is evidence of conformation changes (Heidmann & Changeux, 1978). For electrical excitation, such an allosteric mechanism would also be suggested from the studies of the effect of the scorpion (*L. quinquestriatus*) toxin on the sodium channel. This scorpion toxin slows the inactivation of the sodium channel in voltage clamp experiment without an apparent effect on the activation (Koppenhöfer & Schmidt, 1968; Narahashi *et al.*, 1972; Okamoto *et al.*, 1977). Its binding to the sodium channel is voltage-dependent, i.e., the binding is inhibited by depolarization, which indicates that some conformational change in the channel takes place resulting in reduced affinity for the toxin (Catterall, *et al.*, 1976; Catterall, 1977a, 1979). Furthermore, it seems unlikely that the scorpion toxin binds directly to the subunit or molecule responsible for inactivation, since this subunit is supposed to face the intracellular medium from the drug effect of pronase (e.g. Armstrong & Bezanilla, 1977) while the toxin binds reversibly to the sodium channel from the extracellular medium (Okamoto *et al.*, 1977; Catterall, 1979). Thus, the effect of the binding seems to be transmitted to the inactivation subunit which is spatially separated from the toxin binding site. Such transmission of binding effect is generally recognized in allosteric proteins.

In the allosteric model a multisubunit protein system can exhibit two kinds of physical properties; one originating from the tertiary conformation of the subunit and the other from the quaternary conformation of the entire protein. It is a purpose of the present paper to show that the interrelation between the macroscopic sodium conductance change and gating current can be quantitatively explained, if these physical quantities are assumed to originate from the quaternary and tertiary

conformations, respectively. Although information about the molecular organization of the sodium channel is limited (for the recent review see Catterall, 1982), we imagine that the sodium channel is encompassed by a protein composed of subunits. We develop an allosteric model of the Monod-Wyman-Changeux (1965) type for the sodium channel, assuming the following features of the channel protein. The clamp voltage tends to change the charge distribution in the channel protein, for example, by the reorientation of the electric dipole moments carried by each of the subunits (tertiary conformation change) and the change of subunit-configuration is induced during this dipole reorientation owing to the conformational interaction between the subunits. An ion-conducting pore is formed by this rearrangement of the subunits (quaternary conformation change), for example, at the central position of the subunits arranged in a ring form. The steep rise in the sodium conductance change is attributed to the conformational interaction between the subunits.

2. The Model

(a) Molecular assumption

In our allosteric model, for the purpose of reducing the number of parameters we simply assume that the sodium channel protein consists of N identical subunits each of which carries an electric dipole moment. The gating current is considered to originate from the direction change of these dipole moments (tertiary conformation change) which are confined to either an inward or outward direction perpendicular to the membrane surface.

We regard the opening and closing kinetics of a single channel observed in the patch clamp experiment (e.g. Fukushima, 1981) as a result of the subunit-configuration change (quaternary conformation change) in the channel protein and assume three distinct subunit-configurations, i.e. resting, active and inactive quaternary conformations. Only the active conformation has an ion-conducting pore and a non-zero voltage-independent single channel conductance. Thus the activation of sodium channel is attributed in the present paper to the quaternary conformation transition from the resting to active conformation and the inactivation to the other transition from the active to inactive conformation.

We have thus a total of $3 \times 2 \times N$ states, from among which distinguishable states are selected and schematically shown in Fig. 1. In this figure, R_i , for example, stands for the channel protein in the resting quaternary conformation with i outward dipole moments. The gating current is produced by the tertiary conformation changes (vertical transitions in Fig. 1) and the sodium conductance is regulated by the quaternary conformation changes (horizontal transitions in Fig. 1).

(b) Normalized sodium conductance and intramembrane charge transfer

It is unlikely that the dipole reorientation occurs with the same energy changes in the three quaternary conformations, since the channel protein exists in an oligomeric

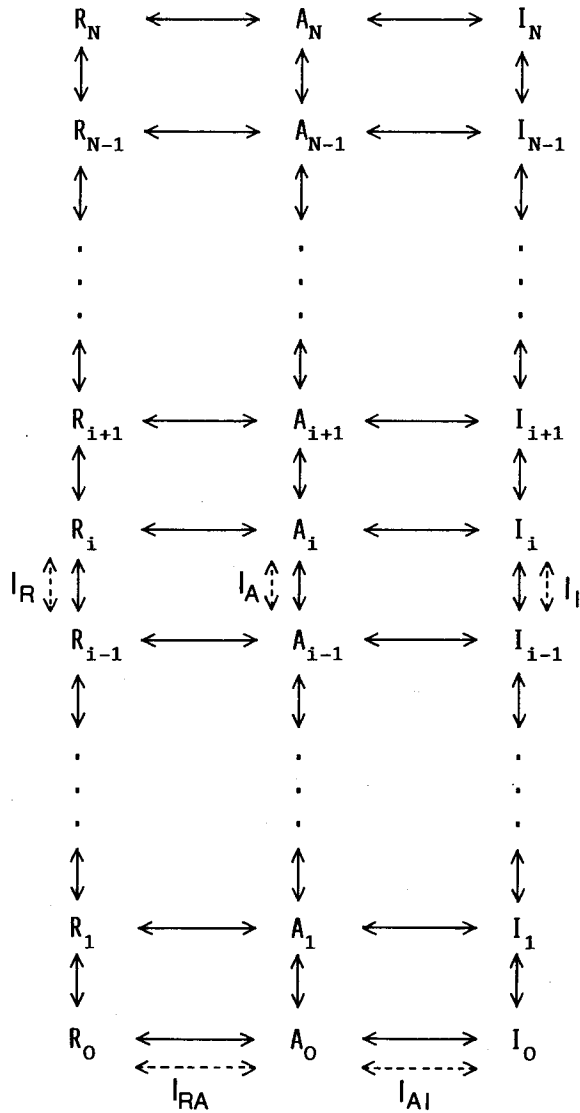


Fig. 1 Distinguishable states of the channel protein and assumed transitions between them. Three quaternary conformations are accessible to the channel protein, and R_i , A_i and I_i stand for the protein in the resting, active and inactive quaternary conformations with i outward dipoles, respectively. Horizontal transitions show the quaternary conformation change and produce the single channel conductance change. Vertical transitions show the orientation change of dipole and produce the gating current. Either the quaternary conformation transition or dipole reorientation is allowed in a unit time. The multiplying factors l_R , l_A , l_I , l_{RA} and l_{AI} of the transition probability are also shown (see text).

form and the quaternary conformation would reflect the structural hindrance for the dipole reorientation. Thus we write down the "equilibrium constants", or more precisely the statistical weights, the logarithms of which are proportional to the energy changes for the dipole reorientation from the inward to outward direction in the resting, active and inactive quaternary conformations as

$$\begin{aligned}
 G_R &= \exp\{2q(V - V_R)\} \\
 G_A &= \exp\{2q(V - V_A)\} \\
 G_I &= \exp\{2q(V - V_I)\},
 \end{aligned}
 \tag{1}$$

respectively. The derivation of these equations from the free energy assumed in the present model is given in Appendix A. Here, V stands for the membrane potential and q is proportional to the magnitude of the dipole moment. The equilibrium constants are thus modulated by the "quaternary conformational constraints" V_R , V_A and V_I . Owing to the difference between the quaternary conformational constraints, transitions between the resting, active and inactive states can occur in response to a change of membrane potential, as will be described.

We consider that the dipole moment is small and originates from a local region of the subunit, as will be discussed. The local small dipoles would change their directions independently of each other. Then, the populations of R_i , A_i and I_i states in Fig. 1 are given by

$$\begin{aligned}
 R_i &\propto {}_N C_i L G_R^i \\
 A_i &\propto {}_N C_i G_A^i \\
 I_i &\propto {}_N C_i M G_I^i,
 \end{aligned}$$

where L and M are voltage-independent constants defined as population ratios of R_0 and I_0 states relative to A_0 state, respectively and ${}_N C_i$ represents the number of ways to choose i outward dipoles from the total N dipoles. Therefore, the fractional populations in the resting, active and inactive quaternary conformations at a given membrane potential are calculated as

$$\begin{aligned}
 f_R &= L(1 + G_R)^N / Z \\
 f_A &= (1 + G_A)^N / Z \\
 f_I &= M(1 + G_I)^N / Z,
 \end{aligned}
 \tag{2}$$

where Z is the partition function, proportional to the total population and given by

$$Z = L(1 + G_R)^N + (1 + G_A)^N + M(1 + G_I)^N.
 \tag{3}$$

The fraction f_A represents that of open channels and is proportional to the macroscopic steady-state sodium conductance obtained in the usual voltage clamp experiment.

On the application of a step voltage V from a holding potential V_H , an intramembrane charge transfer is produced by the dipole reorientation. The normalized fraction f_Q of the transferred charges is calculated from the partition function (3) as

$$(4) \quad f_Q = f_R \frac{G_R}{1+G_R} + f_A \frac{G_A}{1+G_A} + f_I \frac{G_I}{1+G_I}$$

where we have assumed that the holding potential is very low. A detailed derivation of this equation is given in Appendix B. The meaning of equation (4) may be readily understood, because the charge transfer occurring in one of the quaternary conformations is given by $G_X/1+G_X$ ($X=R, A$ or I) and the fraction of that conformation is given by f_X .

It is to be noted that our system can exhibit transitions between the quaternary conformations when a clamp voltage is applied. At a large negative membrane potential the channel protein takes the R_O state in the case where $L \gg 1$ and $M \ll 1$. At a large positive potential, however, a quaternary conformational transition from the resting to active state occurs and the protein takes the A_N state, if the condition

$$(5) \quad LG_R^N < G_A^N, \text{ i.e., } \ln L < 2qN(V_R - V_A)$$

is satisfied. The sigmoidal change of sodium conductance is attributed to this quaternary conformational transition, as can be seen from equation (2). In the following section we will numerically analyze the normalized steady-state conductance obtained by compensating the inactivation. The transition from the active to inactive conformation can occur in a similar way. This feature of the conformational transition induced by the dipole reorientation is essentially equivalent to the allosteric transition model (MWC model) of Monod *et al.* (1965), in which the ligand binding induces the quaternary conformational transition. The electric potential corresponds to the chemical potential of the free ligand and the ratios G_A/G_R and G_I/G_R correspond to the allosteric constant, which is defined as the ratio of two affinities for the ligand in the original two-state MWC model.

(c) Kinetic behavior

The master equation describing the kinetic behavior of the system is written in the form:

$$(6) \quad \frac{\partial f}{\partial t}(X, t) = \sum_{X'} \{w(X' \rightarrow X)f(X', t) - w(X \rightarrow X')f(X, t)\},$$

where $f(X, t)$ is the probability of finding the channel protein in a given state X at time t and $w(X' \rightarrow X)$ represents the transition probability per unit time from X' to X state. For the kinetics of the gating current and macroscopic sodium conductance change, we only consider transitions indicated in Fig. 1. The transition probabilities for the

dipole reorientation can be related to the free energies of each state in Fig. 1 as,

$$(7) \quad \begin{aligned} F(R_i) &= -kT \ln(LG_R^i) \\ F(A_i) &= -kT \ln(G_A^i) \\ F(I_i) &= -kT \ln(MG_I^i), \end{aligned}$$

where k is the Boltzmann constant and T is the absolute temperature. They are written in the following form from the condition of detailed balance (e.g. Glauber, 1963):

$$(8) \quad \begin{aligned} w(S_i \rightarrow S_{i+1}) &= l_S(V) \exp\{- (F(S_{i+1}) - F(S_i)) / 2kT\} \\ w(S_{i+1} \rightarrow S_i) &= l_S(V) \exp\{- (F(S_i) - F(S_{i+1})) / 2kT\}, \end{aligned}$$

where S stands for the quaternary conformation R, A or I , and $i=0, 1, 2, \dots, N-1$. Similarly, we can write the probability for the quaternary conformation transition as

$$(9) \quad w(S_i \rightarrow S'_i) = l_{SS'}(V) \exp\{- (F(S'_i) - F(S_i)) / 2kT\},$$

where S and S' represent two different quaternary conformations and $i=0, 1, 2, \dots, N$. The dipole reorientation produces an intramembrane charge displacement flow j_{dis} , which is proportional to the gating current I_g :

$$(10) \quad I_g \propto j_{dis} = 2q \sum_S \sum_{i=0}^N \{w(S_i \rightarrow S_{i+1})f(S_i, t) - w(S_{i+1} \rightarrow S_i)f(S_{i+1}, t)\}.$$

Here, the summation of S is taken over R, A and I states. The quaternary conformation transitions do not contribute to the gating current since we assume for simplicity no change in the magnitude of dipole moment upon the transition.

Instead of the gating current the following normalized fraction of the charge transfer is presented by some authors for the purpose of reducing experimental errors (e.g. Neumcke *et al.*, 1976). Electric charges are transferred by the intramembrane current flow upon an application of step voltage from the holding potential. This amount is also proportional to

$$(11) \quad Q(t) = \int_0^t j_{dis}(t) dt.$$

The normalized fraction f_Q of the charge transfer is given as

$$(12) \quad f_Q(t) = Q(t) / Q_{max},$$

where

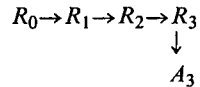
$$(13) \quad Q_{max} = Q(t \rightarrow \infty).$$

The macroscopic sodium conductance g_{Na} is proportional to the fraction $f_A(t)$ in the active conformation:

$$(14) \quad g_{Na} \propto f_A(t) = \sum_{i=0}^N f(A_i, t).$$

The basic quantity $f(S_i, t)$ describing the kinetic behavior can be determined by the integration of the master equation corresponding to the reaction scheme shown in Fig. 1.

As far as the activation of sodium channel is concerned, the present model for $N=3$ is equivalent to the Hodgkin-Huxley description in a special case where the channel protein follows the sequence



on the application of a large positive clamp voltage to the resting potential. In this sequence it is noted that the charge transfer accompanied by the dipole reorientation saturates before the sodium conductance does. However, experiments show the reversed relation that the charge transfer saturates after the sodium conductance (Neumcke *et al.*, 1976; Armstrong & Gilly, 1979). Therefore, it is expected that the channel protein makes a quaternary conformational transition at some intermediate stage during the course of full dipole reorientation, as will be described. While the Hodgkin-Huxley model describes the inactivation as an independent process of the activation, the inactivation proceeds after the activation in the present model. This will also be discussed.

3. Correlation between sodium conductance and gating current

(a) Steady-state relationship

Fig. 2 illustrates the steady-state relationship between the normalized charge transfer and macroscopic sodium conductance at various clamp voltages measured on frog nodes of Ranvier by Neumcke *et al.* (1976). They obtained the steady-state conductance by compensating the inactivation of sodium channels. Since the inactivation proceeds much more slowly than the activation, information about the activation can be obtained separately. The charge transfer data, which correspond to f_Q in our model, were shown by them to be approximately on a curve given by $e^{aV+b}/1 + e^{aV+b}$ with appropriate values of a and b . On the other hand, the normalized sodium conductance, which is proportional to the fraction f_A in the present model, appears to demonstrate a strong sigmoidal change. Their data, furthermore, show a characteristic relationship that the conductance change saturates at a lower voltage than the charge transfer. They have pointed out that this relation cannot be reproduced by the Hodgkin-Huxley (1952) model. The model describes the

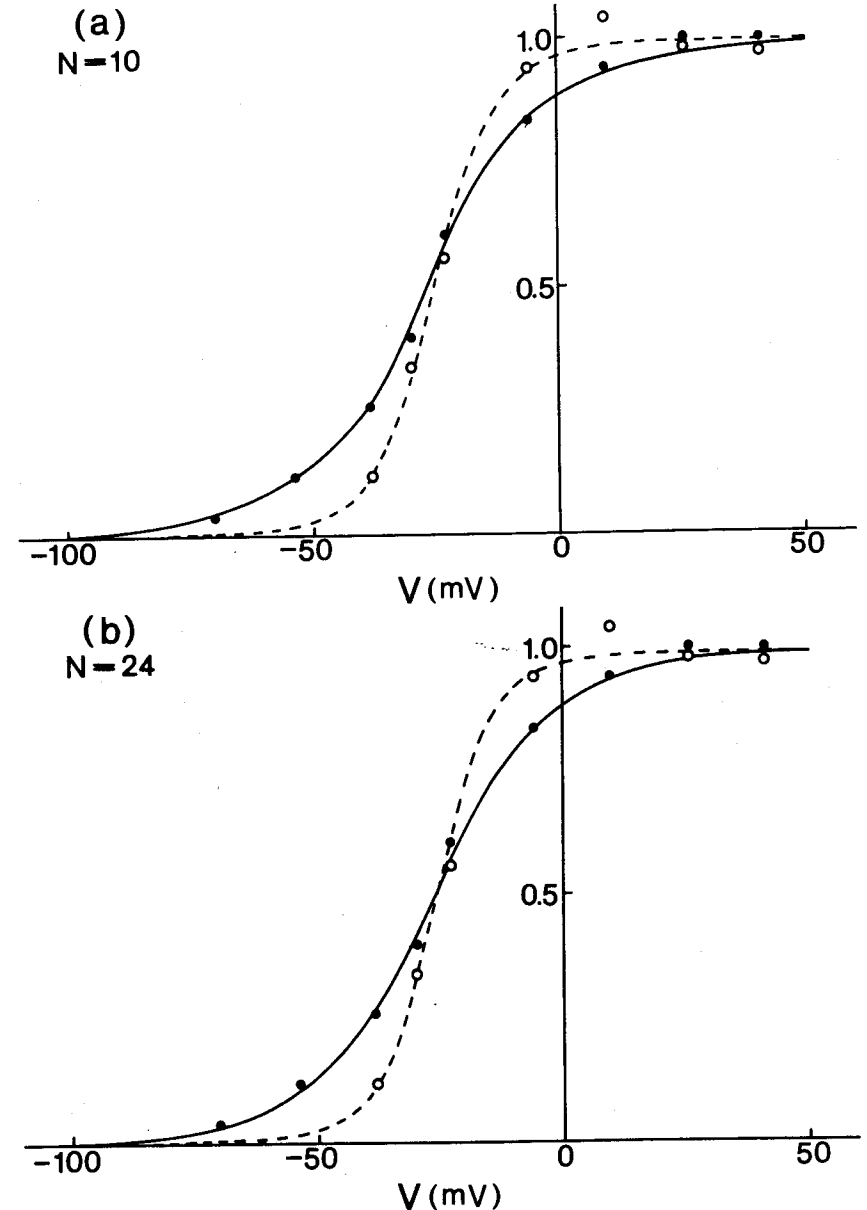


Fig. 2 Steady-state charge transfer and sodium conductance as functions of clamp voltage. The normalized data of the charge transfer (●) and macroscopic conductance (○) are Neumcke *et al.* (1976) on frog node of Ranvier. The f_Q and f_A curves calculated from equations (2) and (4) are shown by the solid and broken lines, respectively. (a) $N=10$, $q=0.027$ (mV^{-1}), $V_R=-17$ (mV), $V_A=-40$ (mV), $\ln L=6.64$ and $M=0$. (b) $N=24$, $q=0.035$ (mV^{-1}), $V_R=-24$ (mV), $V_A=-30$, $\ln L=5.22$ and $M=0$.

channel opening as a result of the completion of the charge movement and predicts that the relation

$$f_A = f_Q^3$$

should be satisfied at any clamp voltage. However, the above equation does not hold as can be readily seen in Fig. 2. We shall give an explanation for the observed relationship on the basis of the present model.

Before numerical analysis, we briefly discuss the steady-state relationship between the normalized charge transfer and the fractional population in the active conformation. For this purpose it is sufficient to consider only the resting and active quaternary conformations. The parameters V_R and V_A in equation (1) have the phenomenological meaning that a half of the charges is transferred at the potential V_R or V_A if the quaternary conformation is frozen in either the resting or active conformation. As can be seen from equation (4), the f_Q vs. V curve jumps from the curve given by $G_R/1 + G_R$ to that by $G_A/1 + G_A$ when the quaternary conformational transition from the resting to active conformation occurs. The condition (5) is necessary for the transition. Thus, the quaternary conformational transition occurs possibly in two ways; (i) N is small but $V_R - V_A$ is large or (ii) N is large but the difference $V_R - V_A$ is small. In the case (i), f_Q and f_A are almost the same at any voltage. This is because the population is distributed approximately in only two states (the R_Q and A_N states in Fig. 1) at any voltage and the orientation change of dipoles and the quaternary conformation change take place in parallel. In contrast, when the difference in constraints is very weak ($V_R \approx V_A$), f_Q is approximated by the form $e^{aV+b}/1 + e^{aV+b}$ and the f_Q vs. V curve looks like as if there were no quaternary conformational transition. However, it should be noted that the transition can take place sharply when N is so large that the relation (5) is satisfied. Thus the case (ii) seems appropriate for explaining the observed relationship between the sodium conductance and charge transfer.

The fit is satisfactory for $N = 10$ and the result is shown in Fig. 2(a). Larger values of N , e.g. $N = 24$, also account for both f_Q and f_A data with suitable values of parameters, as shown in Fig. 2(b). The estimated values of parameters shown in the figure legend, therefore, should be taken as illustrative ones. For a unique determination, more detailed measurements are needed, in particular, at large negative and positive membrane potentials. The characteristic relationship between f_A and f_Q is also recognized on squid giant axon. In this case a small N (e.g. $N = 4$) also cannot fit both of the data but a relatively larger N (e.g. $N = 20$) accounts for the data (see Fig. 3).

The present model explains the observed relationship between f_A and f_Q in the following way. In the resting conformation a part of the charges is transferred by the orientation change of some dipole moments in response to a small depolarizing step voltage. At a middle range of voltage the channels are opened by the quaternary conformation change and the conductance change saturates. At a high membrane potential, the other remaining dipoles change their directions in the active conformation. Thus, the charge transfer does not saturate at the stage when almost all of the channels are opened (i.e. $V = -10$ mV on frog or $V = 20$ mV on squid). This explanation is further supported by the kinetic measurements (Fig. 3 in Neumcke

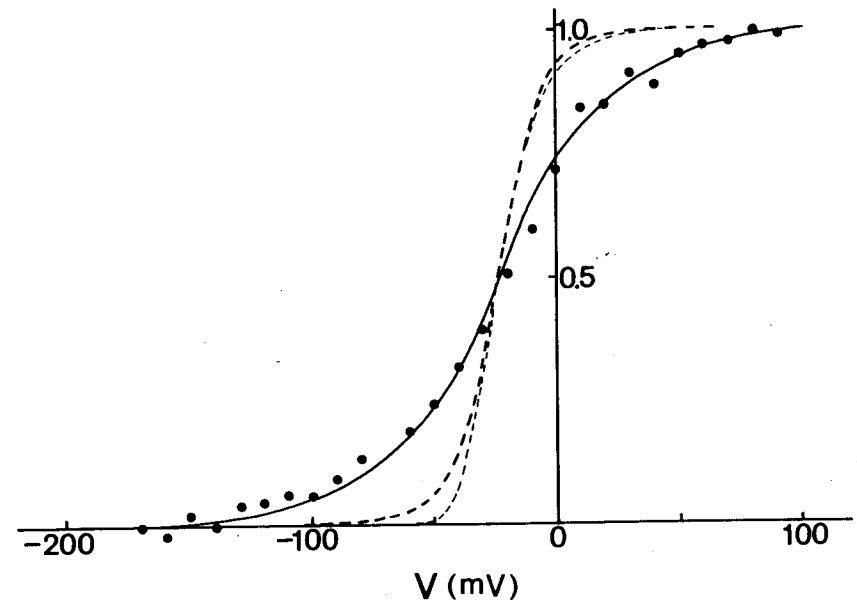


Fig. 3 Normalized charge transfer and sodium conductance on squid giant axon. The measured data (●) of the charge transfer are taken from Armstrong and Gilly (1979). The calculated f_Q curve is shown by the solid line. The thin broken line shows the normalized steady-state sodium conductance m_{∞}^3 in the Hodgkin-Huxley model (1952), choosing the resting potential at -62 mV. The f_A curve computed from equation (2) is shown by the thick broken line. $N = 20$, $q = 0.015$ (mV^{-1}), $V_R = -10$ (mV), $V_A = -35$ (mV), $\ln L = 7.39$ and $M = 0$.

et al., 1976 and Figs. 2 and 12 in Armstrong & Gilly, 1979) in which an appreciable gating current is recognizable when the inward sodium current reaches the maximum. This property is neglected in the reaction scheme proposed by Armstrong and Gilly (1979). The present model predicts two relaxation times in the kinetics of the gating current. This is also consistent with the recent observation that two time constants are recognizable in the ON charge movement at higher membrane potentials (Dubois & Schneider, 1982), as will be discussed in the following subsection.

(b) Kinetic relationship between sodium conductance and gating current

Fig. 4 illustrates the kinetics of the normalized charge transfer $f_Q(t)$ and conductance $F_A(t)$ which were measured by Neumcke *et al.* (1976) on frog node of Ranvier. They obtained their normalized conductance data also by compensating the inactivation which follows a single exponential relaxation. The time course of $f_Q(t)$ is well described by a single exponential at the lower clamp voltage, but at the higher voltage two relaxation times are slightly observed. It is especially noticeable that the normalized conductance saturates before the normalized charge transfer does at the higher potential. This tendency is not derived from the simple molecular inter-

pretation of the Hodgkin-Huxley model, as was emphasized by Neumcke *et al.* (1976). According to the Hodgkin-Huxley model, the channel is opened after the rearrangement of gating structure is completed, and therefore the normalized conductance should saturate after the charge transfer. The observed relation, however, clearly demonstrates that the gating current also flows in the open state. This tendency is well reproduced in our model. Most of the channel proteins take the R_O state at the holding potential and part of electric charges are transferred by the dipole reorientation in the resting quaternary conformation. The quaternary conformational transition is expected to occur at some intermediate step of $R_j \rightarrow A_j$. Then, the remaining dipoles are reoriented during the process from A_j to A_N in the active quaternary conformation. The calculated curves of $f_A(t)$ and $f_Q(t)$ are also shown in Fig. 4 by solid lines. The quaternary conformational transition occurs mainly at the step of $R_{12} \rightarrow A_{12}$ for the estimated values of parameters. In these simulations and others presented hereafter the initial condition for the integration of the master equation is set such that the population distribution is in thermal equilibrium at the holding potential. Our adjustable parameters are l_R , l_A , l_I , l_{RA} and l_{AI} , since the remaining parameters have been estimated in the analysis of the steady-state data.

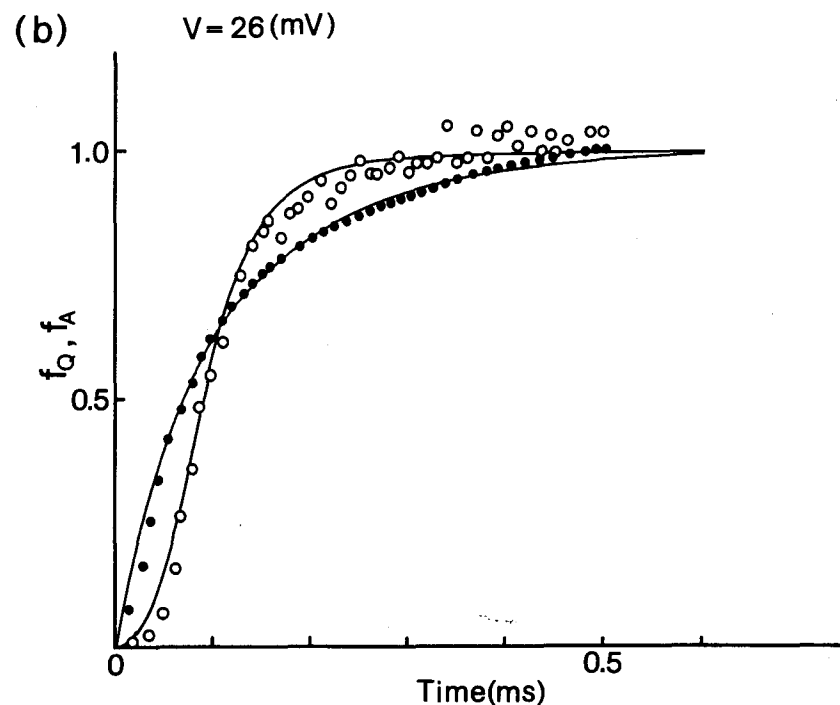
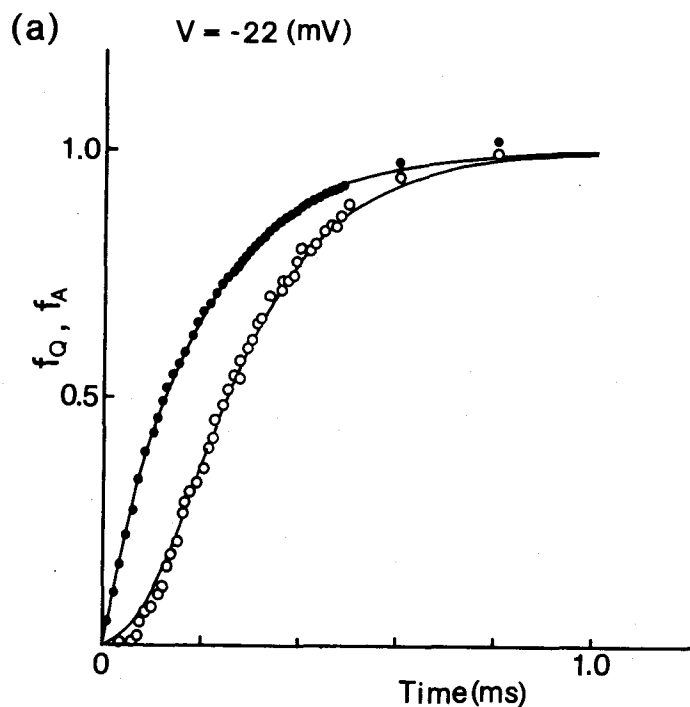


Fig. 4 Time course of the normalized charge transfer and conductance. The normalized data for the charge movement (\bullet) and for the macroscopic conductance (\circ) are taken from Neumcke *et al.* (1976). The calculated curves are shown by solid lines. Used values in the calculation are (msec^{-1}): (a) $l_R = 3.2$, $l_A = 3.5$, $l_{RA} = 30.0$ for $V = -22$ (mV) and (b) $l_R = 1.8$, $l_A = 1.0$, $l_{RA} = 30.0$ for $V = 26$ (mV). In this experiment the holding potential is -98 (mV).

Fig. 5 illustrates the full time courses of the macroscopic sodium conductance and the intramembrane charge movement calculated with the same parameter values used in Fig. 4. Here, the inactivation of sodium channel is not compensated and transitions to the inactive quaternary conformation are taken into consideration. An appreciable charge displacement flow is recognized at the time when the conductance reaches its maximum value at the higher potentials in accordance with the experiment by Armstrong and Gilly (1979).

(c) ON and OFF kinetics at the same potential

According to the Hodgkin-Huxley (1952) description, the rearrangement of gating structure occurs with a unique time constant at a fixed voltage. However, Dubois and Schneider (1982) found on frog node of Ranvier that ON charge movement after a voltage pulse was considerably slower than OFF kinetics measured after a repolarization to the same voltage. Our model can reproduce the difference between

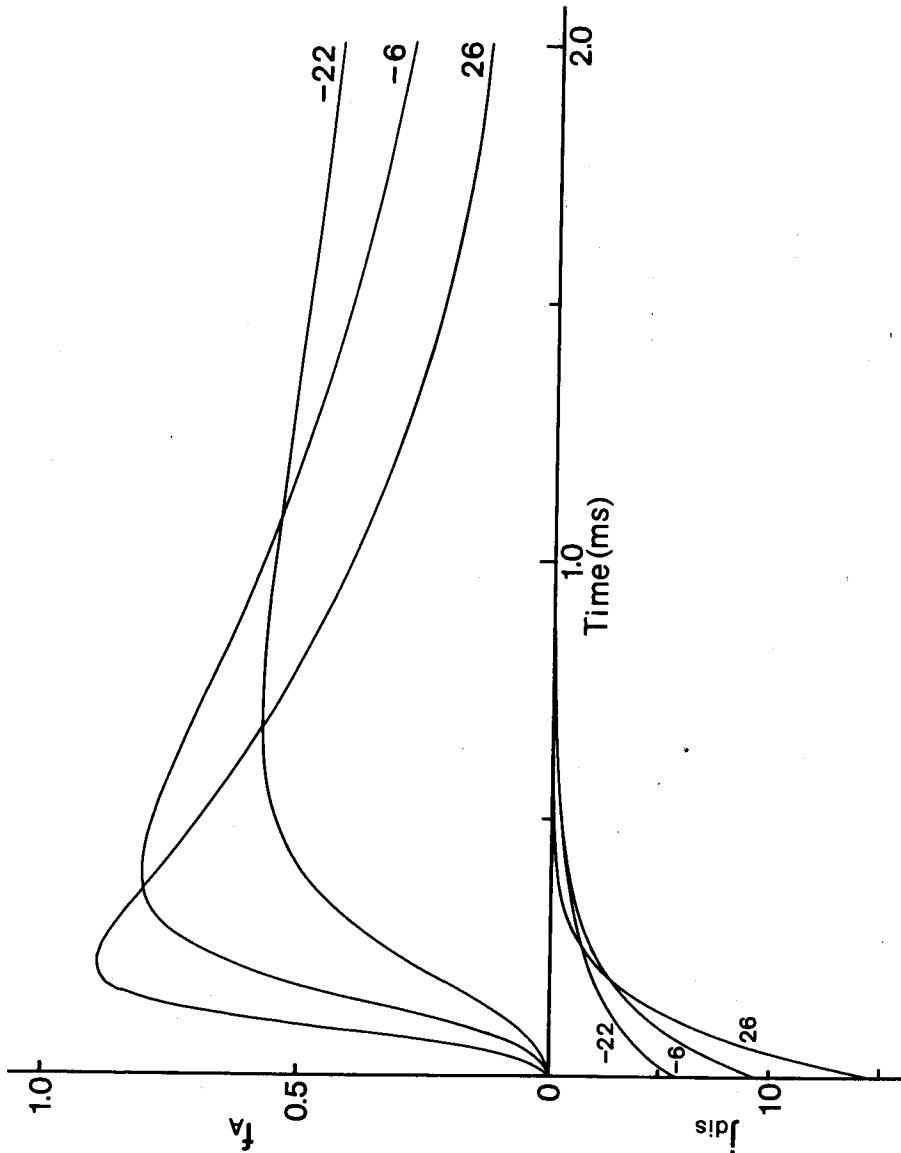


Fig. 5 Comparison between time courses of the conductance and charge displacement flow. Upper curves show the macroscopic sodium conductance changes and lower ones show the intramembrane charge flows on the application of the indicated step voltage (mV) from the holding potential -98 (mV). Used values are (msec^{-1}): $l_R=3.2$, $l_A=3.5$, $l_I=0.028$, $l_{RA}=30.0$, $l_{AI}=0.162$ for $V=-22$ (mV), $l_R=3.0$, $l_A=2.3$, $l_I=0.028$, $l_{RA}=20.0$, $l_{AI}=0.162$ for $V=-6$ (mV) and $l_R=1.55$, $l_A=1.43$, $l_I=0.028$, $l_{RA}=30.0$, $l_{AI}=0.162$ for $V=26$ (mV).

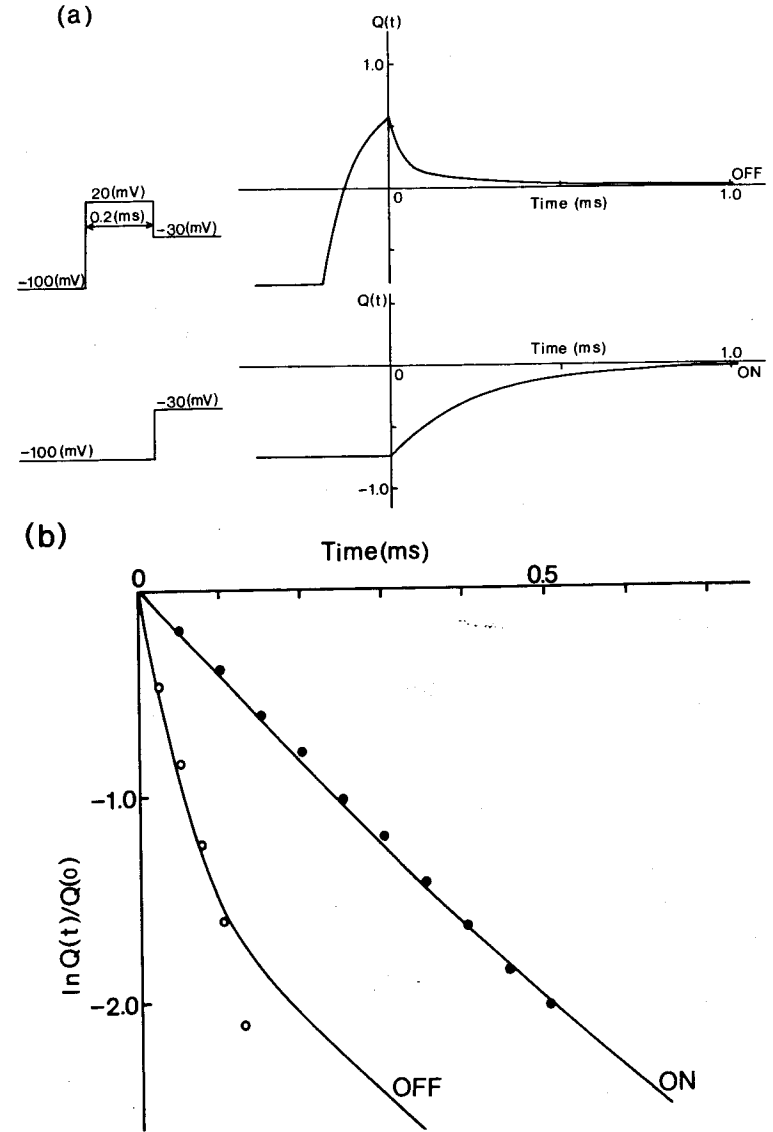


Fig. 6 (a) ON and OFF charge movements calculated at the same potential. Upper curve shows the OFF intramembrane charge displacement flow calculated by equation (6) at -30 (mV). Lower curve shows the ON charge displacement flow at the same potential. Pulse programs are shown on the left. Used values are (msec^{-1}): $l_R=2.30$, $l_A=14.0$, $l_I=0.028$, $l_{RA}=2.0$, $l_{AI}=0.162$ at -30 (mV) and $l_R=1.85$, $l_A=1.23$, $l_I=0.028$, $l_{RA}=25.0$, $l_{AI}=0.162$ at 20 (mV). (b) Semi-logarithmic plot of the ON and OFF charge movements. Normalized data for the ON (\bullet) and for the OFF (\circ) charge movement are taken from Dubois & Schneider (1982). The solid lines show the semi-logarithmic plot of the ON and OFF time courses drawn in (a).

the ON and OFF time constants, as shown in Fig. 6(a) and (b). Our simulation is compared with their measurement of ON and OFF charge movements at -30mV in Fig. 6(b). In our model, the dipole reorientation proceeds with different time constants depending on the quaternary conformation. The ON time constant is analyzed to correspond to $\tau(R)$ which is the relaxation time for the dipole reorientation occurring in the resting quaternary conformation, since most of the channel molecule takes the R_O state at the holding potential -100mV . On the other hand, OFF charge movement proceeds much more rapidly with the time constant $\tau(A)$, since most of the dipole reorientation occurs in the active quaternary conformation after the repolarization to -30mV .

Although only a single exponential relaxation is seen in the experimental OFF time course at -30mV , our kinetic scheme rather predicts two exponentials in OFF charge movement when the clamp voltage satisfies $\tau(R) > \tau(A)$, as illustrated in Fig. 6(b). It is easily understandable that ON charge movement is described by two exponentials at a voltage satisfying $\tau(R) < \tau(A)$. In consistent with this, two time constants are certainly recognized in the measurement of ON charge movement at higher membrane potentials than 20mV (Dubois & Schneider, 1982).

(d) Charge immobilization

It is well-known that the inactivation of sodium channels affects the gating current by immobilizing the gating charge and that the immobilization proceeds in parallel with the inactivation (Armstrong & Bezanilla, 1977; Nonner, 1980; Dubois & Schneider, 1982). The immobilization is followed by measuring ON charge movement during a voltage pulse and comparing it to OFF charge movement after the pulse. Fig. 7 shows the ON and OFF charge movements calculated from equation (12). We can observe that the fast component in the OFF charge movement is decreased after the longer-duration pulse. In this calculation the value for I_I is chosen to be much smaller than that for I_R or I_A and the dipole reorientation after the pulse proceeds much more slowly in the inactive conformation; the fast component in the OFF kinetics is attributed to the dipole reorientation in the active and resting conformations and the slow component results from that in the inactive conformation. The inactivation is caused by the transition from A_n to I_N state in our simulation. The population in the I_N state is increased during longer pulses. Thus, the fast component in the OFF charge movement is decreased after the longer-duration pulse. By this explanation it is easily understandable that the immobilization of the fast component proceeds in parallel with the inactivation.

4. Effects of Scorpion Toxin on Sodium Channel

(a) Voltage-dependent binding

The scorpion toxin shows a voltage-dependent binding to the sodium channel as shown in Fig. 8 (Catterall, 1977a, 1979). It is emphasized by Catterall (1979) that the

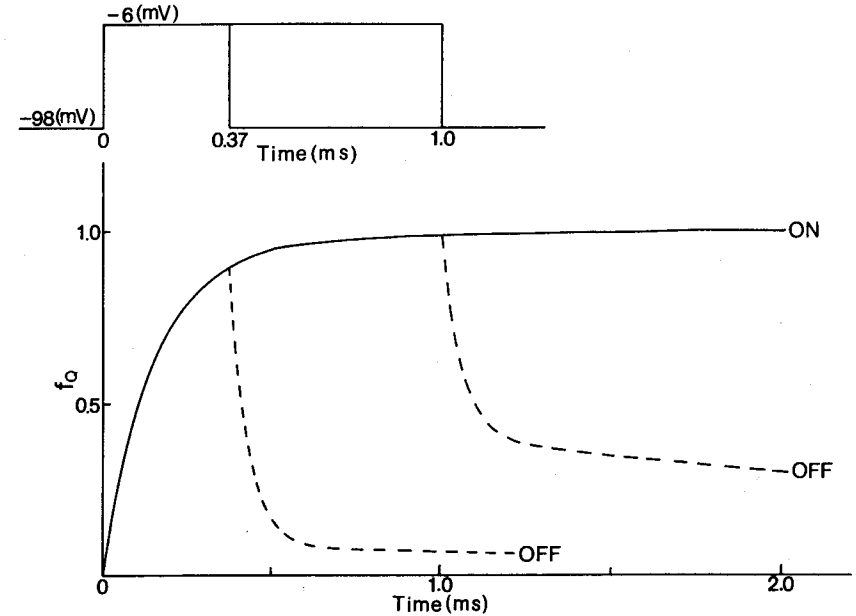


Fig. 7 Charge immobilization for the OFF gating current. The solid line shows the ON charge displacement flow at -6mV , which is calculated by equations (10) and (11). Two broken lines show the OFF charge displacement flows after the shorter-duration pulse (0.37 msec) and the longer-duration (1 msec), respectively. Pulse programs are also shown on the upper. Used values at -6mV are the same as those given in Fig. 5.

dependence is correlated with the activation parameter m_∞ rather than the inactivation parameter h_∞ in the Hodgkin-Huxley equation. However, the kinetic effect of the toxin appears only in the inactivation process (e.g. Okamoto *et al.*, 1977). Thus, it seems difficult to understand both the voltage-dependence and kinetic effect in the molecular picture of the Hodgkin-Huxley equation. In contrast, the present allosteric model provides a straightforward explanation in the following way. We regard the toxin as an allosteric effector, which is usually defined as a molecule showing a preferential binding to one of quaternary conformations. We consider that a binding site for the toxin is provided in each quaternary conformation, since it is shown that the toxin binds to a sodium channel one to one (Okamoto *et al.*, 1977; Catterall, 1979). The partition function including toxin binding then becomes

$$(15) \quad Z(S) = L(1 + S/K_d)(1 + G_R)^N + (1 + S/K_d)(1 + G_A)^N + M(1 + G_I)^N,$$

where S represents the concentration of free toxin. The fractional binding of the toxin is calculated from

$$(16) \quad y = S \frac{\partial}{\partial S} (\ln Z(S))$$

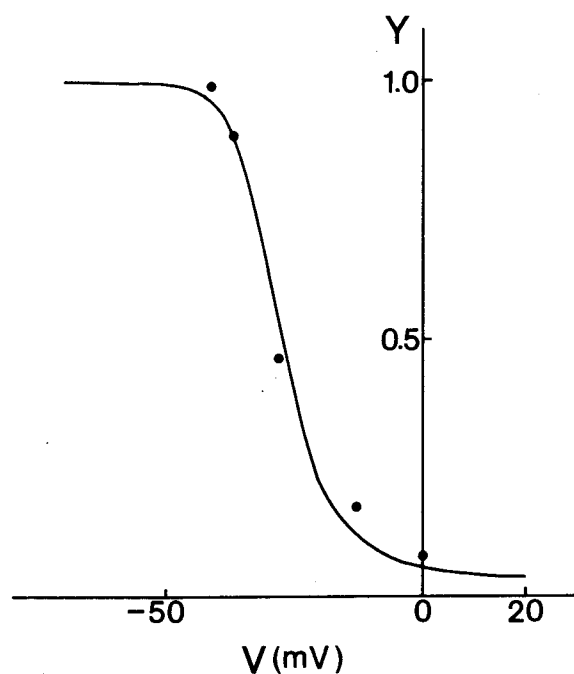


Fig. 8 Voltage-dependent binding of the scorpion toxin to neuroblastoma cell. The measured fractions (●) of the specific binding are taken from Catterall (1977a, 1979). The fraction y calculated from equation (17) is shown as a function of the membrane potential by the solid line. The values for K_d and S are also taken from Catterall (1977a), i.e., $K_d = 0.7$ (nM) and $S = 0.56$ (nM). Fitted values are $V_I = -33.6$ (mV) and $\ln M = -2.0$. Other values of parameters are the same as those given in the legend of Fig. 2(b).

In equation (15), a common dissociation constant K_d for the toxin is taken for the resting and active conformations, since the activation is not significantly modified by the binding of the toxin. Since the binding slows the inactivation and is inhibited completely at large positive voltages, the affinity for the inactive conformation is taken to be zero.

In the present fitting the adjustable parameters are M and V_I , since the remaining parameters have been already estimated in the preceding section. The value for K_d is taken from that at large negative potential given by Catterall *et al.* (1976). As shown in Fig. 8, the voltage dependence of the binding is well described by treating the toxin as an allosteric effector. The inactive conformation is realized at large positive voltages, and the toxin bound to the resting or active conformation is released upon the quaternary conformation change into the inactive conformation. Catterall (1977b) has already treated the scorpion toxin as the allosteric effector in describing the interaction between this toxin and alkaloid neurotoxins. In his model, however, the

gating current is left out of consideration and it appears difficult to explain the sigmoidal change of sodium conductance.

(b) Slowing of inactivation

With the above choice of the dissociation constants for the respective quaternary conformations, the effect of the toxin on the kinetics of the sodium conductance change can also be reproduced, as shown in Fig. 9. In this calculation, we have used the transition probabilities given by equations (8) and (9) in which the free energies of each state are shifted by binding energies of the toxin and other values are not modified in the presence of the toxin. The toxin binding decreases the transition probability from A_N to I_N state by the larger affinity for A_N state and results in a slowing of the inactivation process. The decreased transition probability enhances the peak of the macroscopic conductance. This is consistent with experiment (e.g. Okamoto *et al.*, 1977).

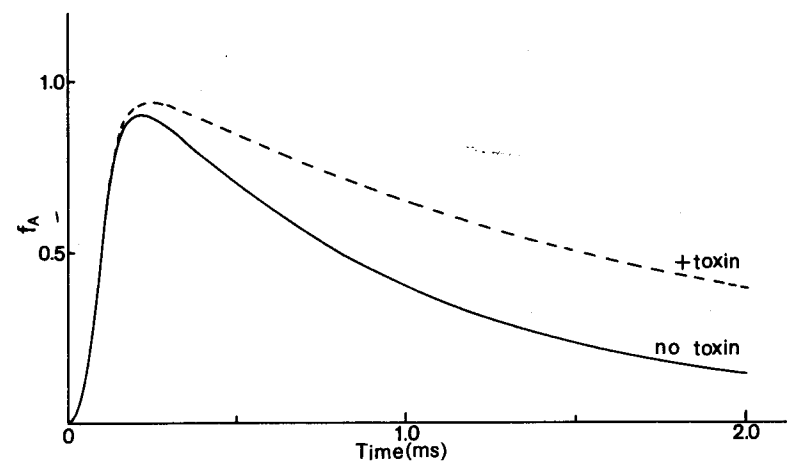


Fig. 9 Drug effect of the scorpion toxin. The solid line shows the time course of the sodium conductance at 26 (mV) in the absence of the toxin. The broken line indicates the time course in the presence of the toxin. The dissociation constant of the toxin for R or A quaternary conformation is chosen to be 0.71 (nM) and the toxin concentration is 3.0 (nM). Other values for the transition probabilities are taken to be the same as those in Fig. 5. The holding potential is taken at -98 (mV).

5. Discussion

We have presented the model in terms of the dipole reorientation. By this assumption, it is easy to understand the experimental finding that the charge transfer is described by a transition between two states with a linearly-voltage dependent energy difference. Here, we will attempt to estimate the magnitude of the dipole

moment assumed in the present model. The dipole moment is effectively described by two charges, $+ne$ and $-ne$, located in a separation distance l , where e is the electron charge. We obtain $n=0.1\sim 0.2$ for squid axon, using $1300\text{ e}/\mu\text{m}^2$ of the maximum charges transferred by the gating current (Armstrong & Gilly, 1979), $300\text{ }\mu\text{m}^{-2}$ of the channel density (Conti *et al.*, 1975) and $N=10\sim 20$. The separation distance can also be estimated from the value of q obtained in the present numerical analysis. Although it is very difficult to calculate the local electric field experienced by the dipole, we use the Lorentz field as an example by assuming that the dipole is exposed to and surrounded by water. Since q is expressed as $nle(\kappa+2)/3kT\delta$, where κ is the dielectric constant of water and δ is the thickness of the membrane, we obtain $l=7\sim 14\text{ }\text{\AA}$ at room temperature, using $\kappa=80$, $\delta=100\text{ }\text{\AA}$ and $q=0.015\text{ mV}^{-1}$. The separation distance thus estimated suggests that our dipole originates from a local region of the subunit, probably, the side chains of the polypeptide. This local nature of the dipole is not incompatible with the assumption that our dipoles change their directions independently in a quaternary conformation.

As an alternative origin of the gating current, it is also possible to consider that the charge movement arises from adsorption or desorption of ions by the channel protein, as long as we are concerned with its voltage dependence. Suppose that an adsorption site is provided at the inner surface of each subunit. The adsorption produces an outward current and desorption results in an inward current. Even in this case, we need no alteration in the present formulation by modifying the meanings of parameters; (i) the affinity for ion is modulated by the quaternary conformation (the difference between V_R and V_A in equation (1)) and (ii) the ion concentration at the inner surface would be altered by the insertion of electrode for voltage clamp and the chemical potential thus modified is proportional to the membrane potential (the appearance V in equation (1)). In this interpretation our model becomes equivalent to the original model by Monod *et al.* (1965), in which the ligand binding induces the quaternary conformational transition. The possibility of ion adsorption would be more suitable, if we concentrate our attention on the experiment showing that the internal K^+ rich solution triggers the excitation (Tasaki, 1968). However, we cannot distinguish between the ion adsorption and the dipole reorientation by the present energetic consideration alone. Therefore, it may be safe to state that the gating current originates from some tertiary conformation change occurring in a local region of the subunit. It has been reported that the gating particles are heterogeneous (Khodorov, 1979; Bekkers *et al.*, 1984). For the sake of simplicity of the model, however, we have neglected this heterogeneity.

In the observed gating current there is an initial rising phase for the ON charge movement (e.g. Armstrong & Gilly, 1979). Many authors believe that this phase is not an artifact either of the clamping system or of the procedure subtracting out the symmetric displacement current (Bezanilla & Taylor, 1978; Armstrong & Gilly, 1979). Armstrong & Gilly (1979) have interpreted the rising phase as representing an intrinsic property of the gating system. On the other hand, Hoyt (1982) has considered that it results from a dielectric relaxation process within the membrane and represents the response of the gating system to a separate membrane phenomenon. If the rising phase arises from the gating system itself, we should modify the transition

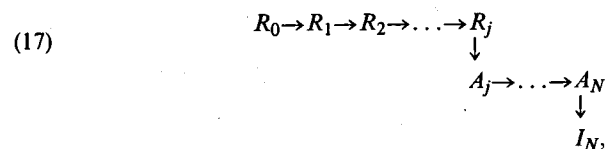
probability between the tertiary conformations such that

$$w(R_0 \rightarrow R_1) < w(R_1 \rightarrow R_2),$$

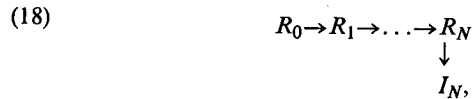
assuming that l_R in equation (8) depends, for example, on the configuration of all the dipoles. Then, we can obtain the initial rising phase in the time course of the ON charge movement.

Our sodium channel is opened in the following sequence on the application of a depolarizing voltage. A local conformation change such as the dipole reorientation or the ion adsorption occurs in response to a change of membrane potential, inducing the tertiary conformation change of the subunit. The accumulation of the tertiary conformation changes alters the quaternary conformation into the other one forming an ion-conducting pore in the channel. The difference of the quaternary conformational constraints, V_R and V_A , plays the essential role in inducing this quaternary conformational transition. In the cooperative oxygenation of hemoglobin, which is the best-known example of allosteric proteins, it has been suggested that the strain of oxygen binding in the deoxy quaternary conformation is transmitted to the salt bridges between the subunits to break them and that the oxygen affinity for the deoxy conformation decreases by the formation energy of the salt bridge compared to the oxy quaternary conformation, in which the salt bridges cannot be formed for the stereochemical reason. Thus the strain modulates the oxygen affinity for the deoxy and oxy quaternary conformations. In the sodium channel it may also be possible to consider that the similar quaternary conformational constraint is exerted on the local dipole or the ion adsorption site. The scorpion toxin for the sodium channel is analogous to the DPG molecule for hemoglobin. This DPG module can bind to the deoxy quaternary conformation of hemoglobin but is released upon the transition to the oxy quaternary conformation (e.g. Herzfeld & Stanly, 1974). Furthermore, the formation of ion-conducting pore is also analogous to the broadening of the central cavity in the oxy quaternary conformation of hemoglobin (Perutz, 1970).

The activation and inactivation of sodium channels had been interpreted by assuming the presence of the independent activation and inactivation units, following the Hodgkin-Huxley equation. However, recent views disagree about the mechanism of inactivation; one is that the inactivation process starts after the channel is activated (Bezanilla & Armstrong, 1977; Armstrong & Bezanilla, 1977) and the other is that the channel does not necessarily require opening before it inactivates (Horn *et al.*, 1981). In the present allosteric model the opening and closing of a channel is regulated by the quaternary conformation of the entire channel protein and, therefore, the above two aspects can be unified in the kinetic scheme based on the allosteric theory. That is, following the sequence of transitions



the channel inactivates after it is opened. If we introduce a direct transition pathway from the resting to inactive quaternary conformations, e.g.,



then the channel does not require opening before it inactivates. It is inferred that the pathway (17) occurs more frequently than that shown by (18), since Bezanilla and Armstrong's result (1977) is obtained from the macroscopic voltage clamp experiment while the result by Horn *et al.* (1981) is obtained in a probability sense from the patch clamp experiment. For this reason, we have neglected the transition probabilities between the resting and inactive quaternary conformations as a first approximation for the average kinetic behavior. Aldrich *et al.* (1983) have proposed from single channel recordings that mammalian sodium channel activation process is slow and rate-limiting whereas inactivation is rapid. In order to examine whether their proposal is applicable to other sodium channels and further to characterize the molecular nature of the inactivated state in distinction to the resting state, we are preparing a detailed analysis of patch clamp data obtained in various kinds of ion channels.

A large number of subunits ($N=10\sim 20$) is required in the present numerical analysis assuming identical subunits for the channel protein. This assumption is made for reducing the number of parameters and for little structural information about the channel protein. However, it is to be noted that the present model scheme is able to account for a variety of experimental data and that the assumption of identical subunits is not essential to these explanations. It has been reported from electrophoresis studies that the sodium channel protein purified from the electric organ of the eel *Electrophorus electricus* is a single polypeptide of molecular weight (M_r) $\geq 260,000$ (Miller *et al.*, 1983), the protein from rat brain synaptosome consists of three peptides of M_r 's = 270,000, 39,000 and 37,000 (Catterall, 1982), and the mammalian sarcolemma protein is formed by four peptides of M_r 's = 130,000–230,000, 45,000, 39,000 and 38,000 (Barchi *et al.*, 1980). From cDNA sequence, Noda *et al.* (1984) have recently succeeded in determining the primary structure of the sodium channel protein of *El. electricus* electroplax. They have shown that the protein has four internal repeats and suggested that 16–24 helical segments form a sodium channel. The subunits in our theoretical model do not necessarily mean the "subunits" in the usual biochemical sense. They only mean tightly coupled units interacting weakly with each other (Kunisawa & Otsuka, 1978). Thus, it may be plausible to consider that the helical segments proposed by Noda *et al.* (1984) correspond to the subunits in our model system, if the single peptide is proved to be responsible for the function of sodium channel. At any rate, it may require future work to determine the functional unit, since it is not definitely excluded that a cluster of proteins form a functioning sodium channel. Most of the models so far proposed appear to be based on the consideration that there are molecules specific to each of the biological functions such as activation, inactivation and voltage sensing on the basis

of the one function-one protein hypothesis. In contrast to this view, the authors wish to emphasize that these functions of sodium channel are understandable from the allosteric properties of the multisubunit system; some functions originate from the conformation of the subunit but others from the conformation of the entire multisubunit system.

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APPENDIX A

Assumed Interactions and Free Energy

The states of the channel protein are specified by the variables θ and σ_i ($i = 1$ to N), where θ takes the value of -1 for the resting, 0 for the active and $+1$ for the inactive quaternary conformation, respectively, and σ_i takes the value of either -1 or $+1$ depending on whether the direction of the i th dipole is inward or outward. Under the clamp voltage V , the free energy $F(\theta; \{\sigma_i\})$ of the channel protein is assumed to be expressed in units of $-1/kT$ by

$$(A1) \quad -F(\theta; \{\sigma_i\})/kT = q(V - V_0) \sum_{i=1}^N \sigma_i + (H\theta + H'\theta^2) + (J\theta + J'\theta^2) \sum_{i=1}^N \sigma_i$$

where k stands for the Boltzmann constant and T is the absolute temperature. The first term is the electrostatic rotation energy of the dipole moment p in the electric field $(V - V_0)/\delta$, where δ is the membrane thickness. Thus, q is defined by $p/kT\delta$. The ion concentrations, for example, would affect the orientation of the dipole. Such effects are taken into consideration by introducing an effective potential V_0 . We have neglected the dipole-dipole interaction for its short range nature. Two parameters are generally necessary to express the relative intrinsic energies of the three quaternary conformations and these energies are defined as $-H + H'$, 0 and $H + H'$ for the resting, active and inactive quaternary conformations, respectively. The last term in equation (A1) represents an interaction between the quaternary conformation and each dipole moment. For this quaternary conformational constraint, the energy required for a dipole to change its direction is different depending on the quaternary conformation; these energies are defined as $2(-J + J')$, 0 and $2(J + J')$ for the resting, active and inactive quaternary conformations, respectively. The partition function can be readily calculated as

$$Z = \sum_{\theta} \sum_{\{\sigma_i\}} \exp\{-F(\theta; \{\sigma_i\})/kT\} \\ = L(1 + G_R)^N + (1 + G_A)^N + M(1 + G_I)^N.$$

Here,

$$(A2) \quad G_R = \exp\{2(J' - J) + 2q(V - V_0)\} \\ G_A = \exp\{2q(V - V_0)\} \\ G_I = \exp\{2(J' + J) + 2q(V - V_0)\} \\ L = \exp\{-N(J' - J) + (H' - H)\} \\ \text{and } M = \exp\{-N(J' + J) + (H' + H)\}.$$

G_R , G_A and G_I represent the statistical weights for the dipole reorientation from the inward to outward direction in the respective quaternary conformations, and can be rewritten in the form given in equation (1). L and M show the fractional populations in R_0 and I_I states relative to that in A_0 state, respectively. It is noted here that these relative populations are independent of voltage.

APPENDIX B

Normalized Fraction of Electric Charges Transferred by Gating Current

Each of the dipole moments arranged perpendicularly to the membrane surface contributes to a surface charge density. The charge density at the outer surface is calculated in the following way. The density is proportional to

$$Q(V) = \rho p \sum_{i=0}^N (2i - N) {}_N C_i (L G_R^i + G_A^i + M G_I^i) / Z,$$

at a given potential V , where ρ is the surface density of the channel protein and p is the magnitude of the dipole moment. We define a generating function as

$$\Phi(s) = L(s + G_R/s)^N + (s + G_A/s)^N + M(s + G_I/s)^N.$$

Then we obtain

$$Q(V) = - \left(\frac{\partial}{\partial s} \ln \Phi(s) \right)_{s=1}$$

$$= \rho N p \left\{ L(1 + G_R)^{N-1} (G_R - 1) + (1 + G_A)^{N-1} (G_A - 1) + M(1 + G_I)^{N-1} (G_I - 1) \right\} / Z.$$

The charge density at the inner surface is given by $-Q(V)$. The charges transferred by dipole reorientation are proportional to

$$\Delta Q = Q(V) - Q(V_H),$$

when a step depolarization pulse is applied from a holding potential V_H . The holding potential is usually taken to be very low, and $Q(V_H)$ is approximated by $-\rho N p$, since all the dipoles are then in the inward direction. This approximation is justified in the end of the present numerical analysis. The maximum charge transfer is given by

$$\Delta Q_{max} = 2\rho N p.$$

The normalized charge transfer f_Q is defined as

$$f_Q = \Delta Q / \Delta Q_{max},$$

and therefore we obtain equation (4).