Conformational model for ion permeation in membrane channels: A comparison with multi-ion models and applications to calcium channel permeability

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ABSTRACT The permeation properties of ion channels existing in several conductive states were analyzed. Each state was represented by the one-ion model. A special emphasis was placed on features, assumed to be indicative of a multi-ion mode of channel occupancy such as a deviation of concentration dependence of channel conductance from the Michaelis-Menten equation, an anomalous mole fraction effect, a strong voltage dependence of ion block and coupling of unidirectional fluxes (anomalous Ussing flux ratio). The conformational model was shown to have all these properties. The ion permeation through voltage-sensitive calcium channels fulfilled all the characteristics of the model proposed.

INTRODUCTION

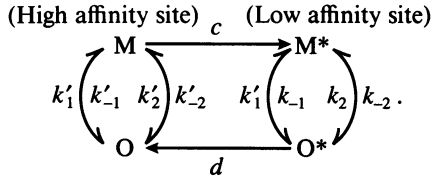
Proteins can exist in a variety of conformational states. For membrane ion channels, this structural flexibility is believed to underlie the channel gating properties: opening, closing, inactivation or desensitization, induced either by changes in membrane voltage or agonist binding to the receptor in question. For almost every known ion channel, several conductance levels have been observed (for a recent review, see Meves and Nagy, 1989) and each of them corresponds to a specific conformation of the channel protein. Theoretical aspects of ion permeation in open channels existing in two conductive states were first discussed by Lauger et al. (1980), who showed that their permeation properties comprise a variety of intermediate states between two extremes: ion carriers (like valinomycin) and channel pores (like gramicidin A).

The aim of the present work was to elaborate the original ideas put forward by Lauger and others (Lauger et al., 1980; Ciani, 1984; Lauger, 1985; Mironov, 1988; Lux et al., 1990). New mechanism of channel permeation suggested in the present paper was analyzed for experimental protocols previously assumed to be indicative of multiple occupancy and ion–ion interactions within the channel (Heckmann, 1972; Aityan et al., 1977; Hille and Schwartz, 1978; Urban et al., 1980; Eisenmann and Horn, 1983). The evidence for the channel to be a multi-ion pore was based on one of the following criteria: (*a*) the Ussing flux ratio exponent is larger than unity; (*b*) in the presence of two permeating ions with their constant total concentration, the channel current has an anomalous mole fraction dependence; the conductance-concentration relationship does not correspond to the Michaelis-Menten equation, revealing a maximum (*c*) or possessing another slope (*d*); (*e*) the ion block has an unusually strong voltage dependence. Anomalies (*b*) and (*c*) have been already demonstrated for a channel with two conducting states. The calculations made in the present work indicate that a model proposed possesses all the lines of evidence assumed earlier to be inherent to multi-ion pores.

THEORY

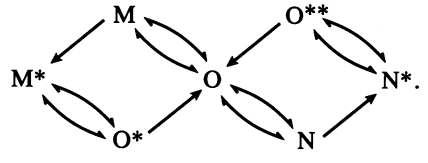
General assumptions

The basic assumption of the model is that the first ion passing through the open channel makes a specific pathway which exists for some period of time and is accessible only to similar ions. Put another way, when the ion binds to the empty channel the whole complex has two possibilities: either to release a bound ion to the bathing compartments with the channel conformation unchanged or to enter a new channel state with other permeability properties which can be transformed into the initial one via the second conformational transition. For only one type of permeant ion, the minimal kinetic model contains four distinct states:



$$k'_1\,k'_{-1}\,k'_2\,k'_{-2}\qquad k'_1\,k_{-1}\,k_2\,k_{-2}\,. \tag{1}$$

The binding sites are denoted as the "high-affinity" and "low-affinity," respectively, which corresponds to voltage-sensitive calcium channels considered here as a model application. Symbols O and O* denote the empty channel, and M and M* stand for the occupied channel. Small cycles (O) ⇄ (M) and (O*) ⇄ (M*) describe ion translocation. According to the suggested mechanism, for two permeant ions the kinetic scheme is to be obtained by connecting separate diagrams for each ion via an empty state (O).



(2)

For clarity, the rate constants are not indicated here.

The kinetic scheme was first used by Lux et al. (1990) to explain the voltage dependence of Ca block of sodium current through the calcium channel. From a formal

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perspective Scheme 1 resembles the model of Lauger et al., (1980). However, the latter assumes that the channel experiences random fluctuations between two conformational states whereas in the present work the channel behavior is deterministic, resulting in kinetic differences for the case of two permeant ions. In addition, the conformational transitions in the model proceed only in one direction, starting from the occupied channel in the first state and from the empty channel in the second one. Thus, the channel cycling is reminiscent of enzyme catalysis which can be considered as essentially irreversible and this similarity is further explored in the Discussion section.

One permeant ion

The steady ion flux is determined by the occupancies of states which are found from the system of linear algebraic equations for a state diagram 1,

$$\{M\} = \frac{a}{(b+c)}\{O\}$$

$$\{M^*\} = \frac{a\cdot c\cdot(\alpha+d)}{\beta\cdot d\cdot(b+c)}\{O\}$$

$$\{O^*\} = \frac{a\cdot c}{d\cdot(b+c)}\{O\}$$

$$\{O\} = 1\Big/\left\{1+\left[\frac{a}{(b+c)}\left(1+\frac{c}{d}+\frac{c\cdot(\alpha+d)}{d\cdot\beta}\right)\right]\right\}, \quad (3)$$

with a and b defined as $a = k_1' + k_{-2}'$, $b = k_2' + k_{-1}'$, $\alpha = k_1 + k_{-2}$, $\beta = k_2 + k_{-1}$ (Lauger, 1973). Steady flux J through the channel is a sum of fluxes in the two conformational states

$$J = k_1'\{O\} - k_{-1}'\{M\} + k_1\{O^*\} - k_{-1}\{M^*\}$$
$$= \frac{a}{b+c}\left[(k_1'(k_2'+c) - k_{-1}'k_{-2}')/a + (k_1k_2 - k_{-1}(k_{-2}+d))\frac{c}{d\beta}\right]\{O\}. \quad (4)$$

When permeant ions are present only from one side of the membrane, e.g., when $C_{in} = 0$ and $C_{out} = C$ where C is the permeant ion concentration in the external compartment, a simple expression for ion flux can be derived. Assuming that ions enter the channel in each conformational state with the same on-rate constant k_{on} ($a = \alpha = k_1 = k_{on}C$) and conformational transitions proceed faster than ion exit from the high-affinity site in the first conformational state ($c, d \gg b$) the above equation is reduced to

$$J = \frac{ak_2(1+a/d)}{\beta[1+a(1/c+1/d+1/\beta)+a^2/\beta d)]}. \quad (5)$$

Terms containing a^2 in numerator and denominator give a quadratic concentration dependence. Therefore, ion flux (and channel conductance) cannot be described by empirical equation of a Michaelis-Menten type. The latter, however, is obtained for $a/d \gg 1$

$$J = \frac{ak_2}{\beta d(1/c+1/d+1/\beta)+a}, \quad (5a)$$

for which an apparent dissociation constant can be defined as

$$K_{app} = \beta d(1/c+1/d+1/\beta)/k_{on}, \quad (5b)$$

which is different from a true dissociation constant for the low-affinity site $K_{app} = k_{-1}/k_{on}$. The two equations given above can be compared with those for one-ion channel with only one conformational state and the low-affinity site which according to usual conventions is denoted below as '2B1S' model (two barriers and one binding site),

$$J' = \alpha k_2/(\alpha+\beta) \quad (6a)$$

$$K_{app}' = \beta/k_{on}. \quad (6b)$$

Two permeant ions

The occupancy numbers for all channel states are given by Eq. 3, except of the central one $\{O\}$ in a state diagram 2 which contains in the denominator a similar term for another ion (N)

$$\{O\} = 1\Big/\left\{1+\left[\frac{a_M}{(b_M+c_M)}\left(1+\frac{c_M}{d_M}+\frac{c_M\cdot(\alpha_M+\beta_M)}{d_M\cdot\beta_M}\right)\right] + \left[\frac{a_N}{(b_N+c_N)}\left(1+\frac{c_N}{d_N}+\frac{c_N\cdot(\alpha_N+\beta_N)}{d_N\cdot\beta_N}\right)\right]\right\}. \quad (7)$$

The total flux is a sum of two ion fluxes

$$J = k_{1M}'\{O\} - k_{-1M}'\{M\} + k_{1M}\{O^*\} - k_{-1M}\{M^*\} + k_{1N}'\{O\} - k_{-1N}'\{N\} + k_{1N}\{O^{**}\} - k_{-1N}\{N^*\}. \quad (8)$$

All resulting equations are simple but cumbersome and therefore the explicit expressions are omitted here.

Ratio of unidirectional fluxes

Isotopes are physically distinct species distinguished by their mass number, and even in the presence of one type of permeating ion, the channel behavior will be similar to the case of two permeant ions considered above. When isotopes are present from opposite sides of the membrane, each unidirectional flux J^+ and J^- is to be derived from Eqs. 3, 7, and 8, by setting the rate constants $k_{-2}^+ = k_1^- = 0$. (Here superscripts + and − substitute for indices M and N in diagram 2 and correspond to isotopes present in the external and the internal compartment, respectively). Assuming again that $c, d \gg b$ the flux ratio can be written as

$$\frac{J_+}{J_-} = \frac{k_1^{+\prime}}{k_2^{-\prime}} \frac{k_2^{+\prime} + c^+ + c^+ k_1^+ k_2^+ / d^+ \beta^+}{k_{-1}^{-\prime} + k_{-1}^-(k_{-2}^- + d^-)c^-/d^-\beta^-}. \quad (9)$$

For the channel with only one conformational state ($c^+ = c^- = 0$) it is reduced to the familiar Ussing formula

$$J_+/J_- = k_1^{+\prime} k_2^{+\prime} / k_{-1}^{-\prime} k_{-2}^{-\prime} = \text{const} \cdot \exp(n'V), \quad (9a)$$

with $n' = \sigma_1^+ + \sigma_2^+ + \sigma_{-2}^- + \sigma_{-2}^-$ being determined by the exponential voltage dependence of rate constants via the relationship $k_i = k_i^0 \exp(-\sigma_i V)$ where k_i^0 is the rate constant at zero membrane potential, σ_i is "the electrical distance," and V is the dimensionless voltage in units ze_0F/RT where all symbols have their usual meaning. The Ussing flux ratio exponent n' equals to 1 if, and only if $\Sigma\sigma_i = 1$, a condition, which holds for a channel with fixed potential barriers and energy wells independent of how complicated the potential profile is (Lauger, 1973). However, if electrical distances are not the same for unidirectional fluxes, the flux ratio exponent may not satisfy this identity. The conformational model provides a logical background for this hypothesis. Consider a channel which in the first conformational state has the energy profile independent of the side of an ion entry, but in the second conformational states it is different, depending on the side of ion entry. For example, a single binding site in the channel may have the same position, but energy peaks can be differently located, giving rise to the difference in the voltage dependence of corresponding rate constants. Neglecting the contribution from the first conformational state, Eq. 9 gives for the flux ratio

$$\frac{J_+}{J_-} = \frac{k_1^{+\prime} k_1^+ k_2^+ / \beta^+}{k_{-2}^{-\prime} k_{-1}^- k_{-2}^- / \beta^-}. \quad (9b)$$

It can be shown, that n' determined from this equation as a slope at $V = 0$ mV, satisfies the inequality $0 \le n' \le 2$, with lower and upper bounds corresponding to the two limiting cases as described in Fig. 5 *B*.

Model parameters

The model parameters were chosen as appropriate to calcium channels. Dissociation constants for Ca and Ba binding to the high-affinity and to the low-affinity sites were taken from the experimental data (Kostyuk et al., 1983): $K'_{Ca} = 1$ μM and $K'_{Ba} = 10$ μM, $K_{Ca} = 1$ mM and $K_{Ba} = 10$ mM, respectively. The rate constant for ion entry into the channel is to be represented as a product of bimolecular on-rate constant k_{on} times ion concentration in the corresponding bathing compartment, i.e., $k_1^0 = k_{on}C_{out}$ and $k_{-2}^0 = k_{on}C_{in}$. According to usual conventions, the compartments were denoted as "external" and "internal." All the on-rate constants were set to a diffusion limit of $k_{on} = 3 \cdot 10^8$ M^{-1} s^{-1}. Off-rate constants k_{-1}^0 and k_2^0 were obtained through the relationship $K = k_{-1}^0/k_{on} = k_2^0/k_{on}$. For simplicity, the channel was assumed to have a symmetric energy profile with all electrical distances equal to ¼. In calculations of the Ussing flux ratio they were varied as indicated in Fig. 5. Rate constants for all conformational transitions were assumed to be voltage independent and equal to 260 ms^{-1}. These values formed a basic set of parameters. Some of them were varied to analyze the behavior of the model in more detail. In most calculations only one parameter was changed as specified in legends to figures and in the text.

RESULTS

One permeant ion

Fig. 1 shows how the channel current in the conformational model depends on the concentration of permeant ions. For a basic set of parameters the calculated dose-response curve was shifted leftward in comparison with that for the one-ion 2B1S model shown by the dotted curve in Fig. 1. This could be expected from the difference in apparent dissociation constants given by Eqs. 5*b* and 6*b*, respectively, due to additional pathway to exit from the first occupied state {M} in diagram 1. The concentration dependence of current was insensitive to variations in the dissociation constant for the first conformational state, satisfying an inequality $K' < K/10$ which means that this state has really the high-affinity site. For $K' \ge K$ the concentration dependence in the midactivity range markedly changed. Now both conformational states of the channel were conductive and the first one produced the equal or even larger current. (A shallowed energy well results in a larger channel current, see Eq. 6*a*). A sum of two overlapping curves of a Michaelis-Menten type for each conformational state gives a bell-shaped dose-response curve.

Apparent dissociation constants determined as midpoints for calculated curves are dependent on the rate constants for conformational transitions. With a decrease of model parameters c or d a progressive rightward shift of dose-response curves was observed as shown in Figs. 1, *B* and *C*, respectively. In the latter case the slope was also altered, indicating another type of deviation from the Michaelis-Menten equation. This new result can be used to explain on the alternative (possibly simpler) basis the relevant experimental data like the dependence of single Ca channel conductance on Ba activity measured by Yue and Marban (1990).

Changes in membrane voltage or in the dissociation constant of the low-affinity binding site produced a parallel shift of dose-response curves along the concentration axis (data not shown), resembling the behaviour of the 2B1S model.

Two permeant ions

Anomalous mole fraction effect

Fig. 2 presents the results of calculations of ion current when two different permeant ions were present in the external compartment with their total concentration

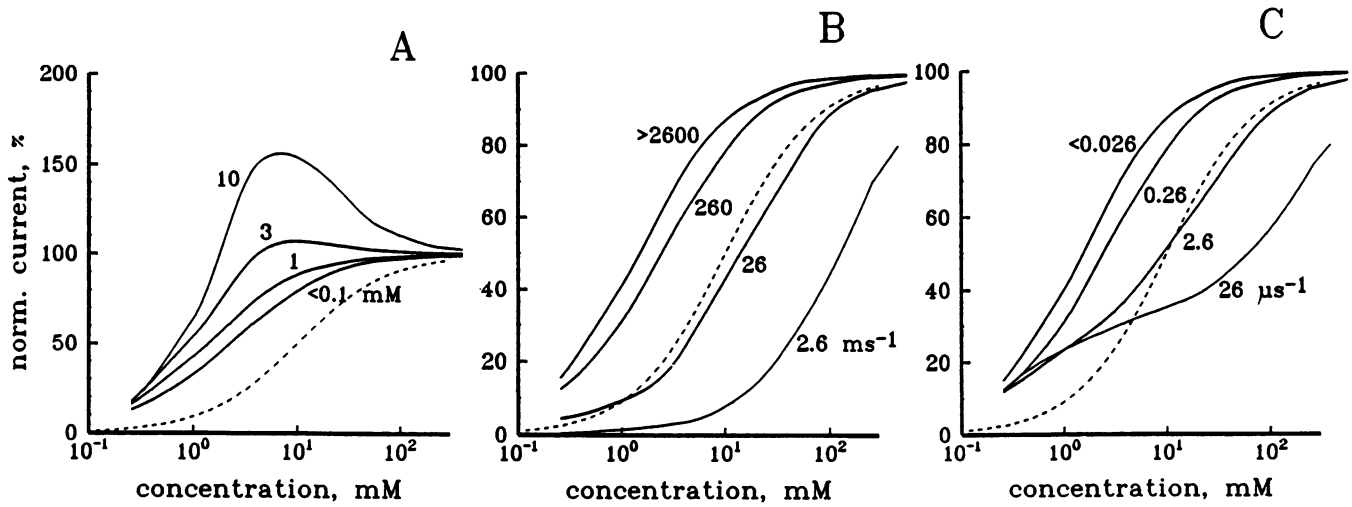

FIGURE 1 Concentration dependence of the channel current for the conformational model. All the calculations were made with the basic set of parameters for Ca listed in the Theory section with variations indicated near each curve. Calculated current at −25 mV was normalized to its limiting value at high concentrations. Permeant ions were present only in the external compartment ($[Ca]_{in} = 0$) and their concentration was plotted on the abscissa. For each frame only a single parameter was varied: dissociation constant for the high-affinity binding site (*A*), rate constants for the first conformational transition (*B*), and for the second conformational transition (*C*), with the numerical values indicated near each curve. Dotted line in each frame shows the concentration dependence for the one-ion model (2B1S) with the dissociation constant of 10 mM.

kept constant. In most cases, the total current goes through a minimum as a function of the ratio of ion concentration. This phenomenon has been called (Hagiwara et al., 1977; Hille and Schwarz, 1978) an anomalous mole fraction effect (AMFE). Both the position and the depth of a minimum were sensitive to variations of model parameters. Figs. 2, *A* and *B*, show that AMFE virtually disappeared with the increase in total concentration of permeant ions and at negative membrane potentials, the only parameters that can be varied in the experiment. This behavior is in accord with data obtained for calcium channels (Chesnoy-Marchais, 1985; Friel and Tsien, 1989).

Other variables of the model represent an intrinsic property of ion channel, but may depend on the channel type or be experimentally modified. AMFE became more pronounced with an increase in the rate constants of conformational transitions (Fig. 2, *C–E*). Similarly to the above described concentration dependence of current, variations in the dissociation constant for the high-affinity binding site did not affect AMFE (data not shown). In contrast, altering the dissociation constant for the low-affinity site produced marked changes (Fig. 2 *F*) which are similar to those observed with variations in the total concentration of permeant cations (Fig. 2 *A*). Both effects reflect the changes in the occupancy of low-affinity conformational states. Overall, the results of calculations indicate that, for the conformational model, AMFE exists only for some combination of model parameters and it can be expected to manifest itself only in some experimental conditions.

For a basic set of model parameters with $K' \ll K$ only states with the low-affinity binding sites contribute to the total current. In the conformational model with two permeable ions, the channel flickers between different states, resulting in a decrease of the total current in a midactivity range. Thus, AMFE in the conformational model arises from correlations among conductive states selectively permeable for a given ion, so in the kinetic sense its explanation sounds similar to that provided by the multi-ion model (Hille and Schwarz, 1978). However, in the former case AMFE does not require high n' values for the Ussing flux ratio, a feature that can be used to distinguish between these two models.

Permeant ion and blocking ion

In a simple 2B1S model the blocking ion gets stuck for a long time in the channel, thus, shifting the equilibrium to the blocking (nonconductive) state of the channel. For the conformational model this corresponds to the case when a large cycle in the kinetic subdiagram for the blocking ion *X* is interrupted by setting the rate constant $c_X = 0$. This assumption means that ion binding to the channel state is not sufficient to trigger its conformational transition. It is reminiscent of competitive inhibition of enzymes where drugs bound to the active site cannot leave it via the pathway normally used for substrate transformation.

Figs. 3 and 4 show that the voltage and the concentration dependence of ion block for this mechanism is different from the predictions of the 2B1S model. First, the latter always demonstrates a monotonic relief of block at positive potentials as shown by the dotted line in Fig. 3 *A* and Fig. 4, *A* and *C*. For the conformational model such behavior was seen only for the block of the outward

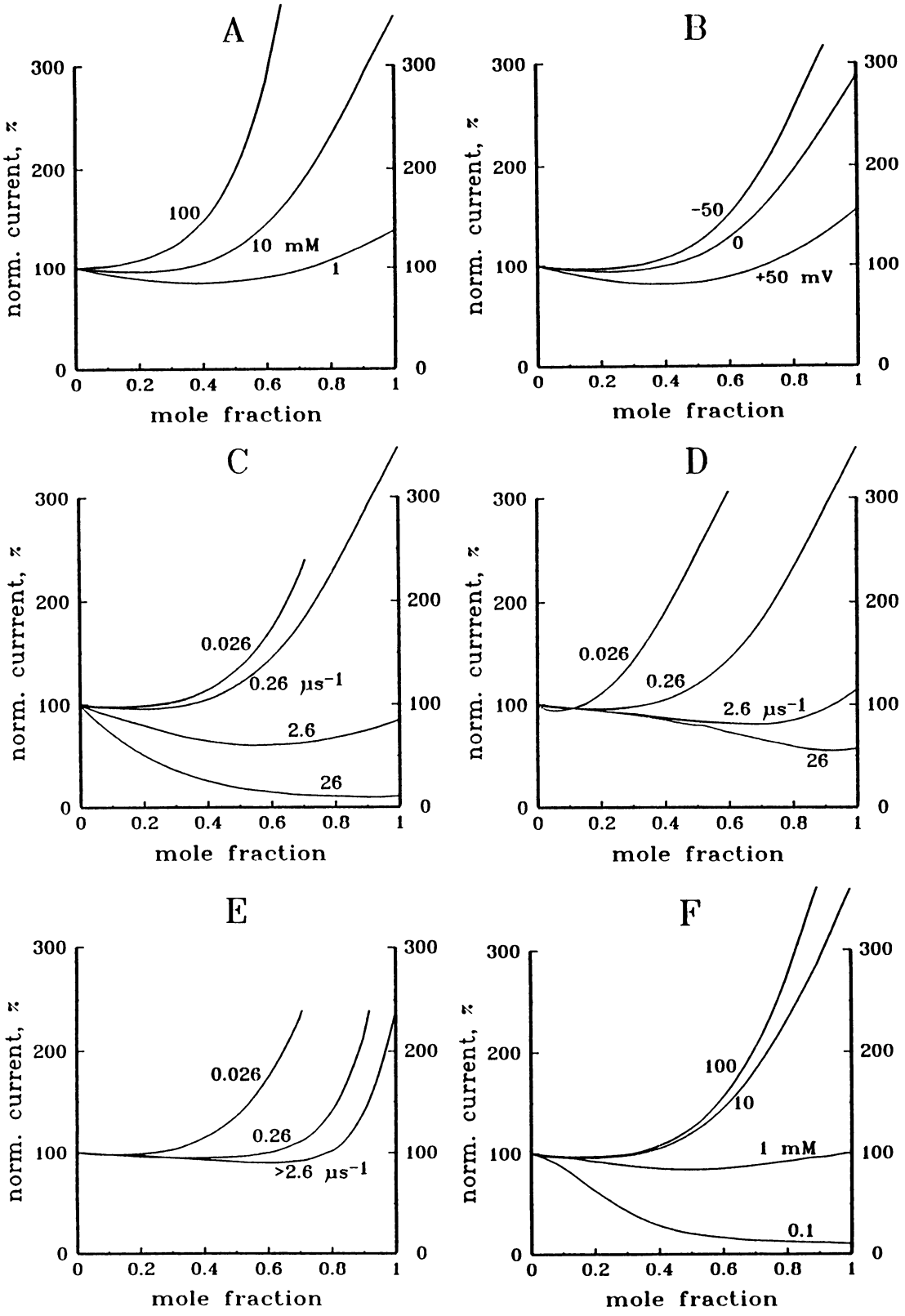

FIGURE 2 Anomalous mole fraction effect for the conformational model. Two permeant ions (Ca and Ba) were present only in the external compartment ($[Ca]_{in} = [Ba]_{in} = 0$) and except in (*A*) their total concentration was 10 mM. The current calculated at −25 mV was normalized to the amplitude of pure calcium current and was plotted vs the mole fraction of Ba. All the calculations were made with the basic set of parameters for Ca and Ba listed in the Theory section with variations of total concentration of divalent cations (*A*), membrane voltage (*B*), rate constant for the first conformational transition *c* (*C*), rate constant for the second conformational transition *d* (*D*), dissociation constant for the low-affinity site for Ba (*F*). In (*E*) both rate constants for conformational transitions were varied with $c = d$. Corresponding numerical values of model parameters are indicated near each curve.

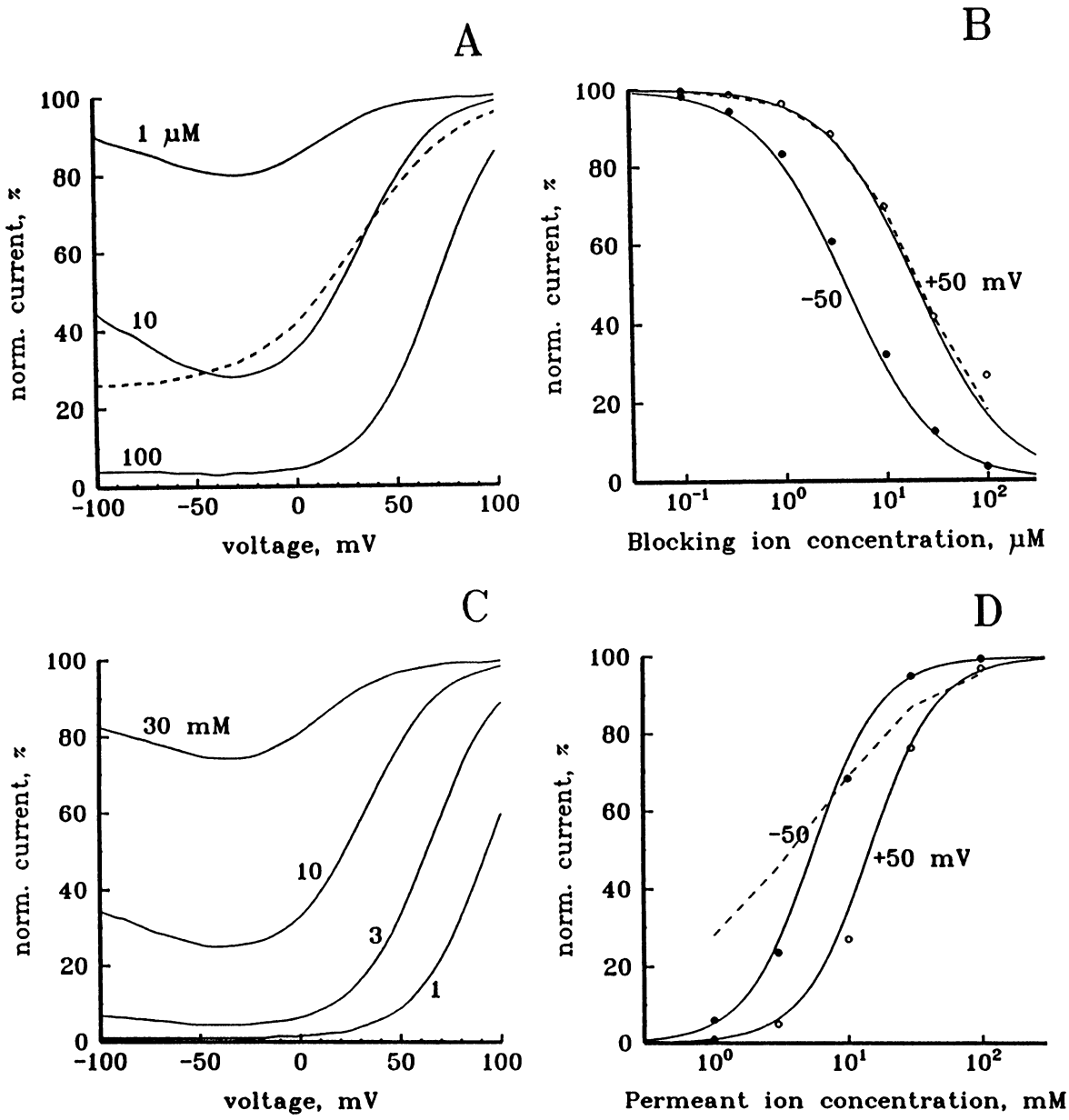

FIGURE 3 The external ion block for the conformational model in symmetrical conditions. All the calculations were made with the basic set of parameters listed in the Theory section for Ca as a permeable ion. For blocking ion X all parameters were the same except the dissociation constants for the high-affinity binding site $K'_X = 0.01$ μM and the rate constant for the first conformational transition $c_X = 0$. $[X]_{in} = 0$. (*A*) Voltage dependence of block calculated for different concentrations of the blocking ion indicated near each curve. $C_{in} = C_{out} = 10$ mM. Superimposed is a dotted curve showing the block for the 2B1S model calculated for $[X]_{out} = 10$ μM with $K_{Ca} = 1$ mM and $K_X = 0.3$ μM. (*B*) Circles show the concentration dependence of block for two voltages (as indicated near each curve) plotted from the results of calculations exemplified in (*A*). Smooth curves were drawn according to a Michaelis-Menten type equation $I_{norm} = K_X[X]/(K_X + [X])$ with apparent dissociation constant K_X of 4 μM at −50 mV and 20 μM at +50 mV. A dotted line calculated at +50 mV using the 2B1S model with parameters listed in *A*. (*C*) Voltage dependence of block calculated for different concentration of the permeant ions ($C_{in} = C_{out}$) indicated near each curve. $[X]_{out} = 10$ μM. (*D*) Circles show the concentration dependence of block for two voltages (as indicated near each curve) plotted from the results of calculations exemplified in *C*. Smooth curves were drawn according to the Hill equation $I_{norm.} = ([Ca]/(K_{Ca} + [Ca]))^n$ with $n = 1.7$ and the apparent dissociation constant $K_{Ca} = 3$ μM at −50 mV and 15 μM at +50 mV. Dotted curve calculated at +50 mV using the 2B1S model with parameters used in *A*.

current, and for current in symmetric ionic conditions the block relief occurred both for positive and negative voltages. The latter effect can account for the voltage dependence of ion block observed for calcium channels (Fukushima and Hagiwara, 1985; Lansman et al., 1986; Lux et al., 1990).

In comparison with the 2B1S model for positive membrane potentials ion block within the conformational model had a steeper voltage dependence which was treated as an indirect evidence in favor of multi-ion model (Hille and Schwarz, 1978). To quantify the effect, the fraction of blocked channels to not blocked was ap-

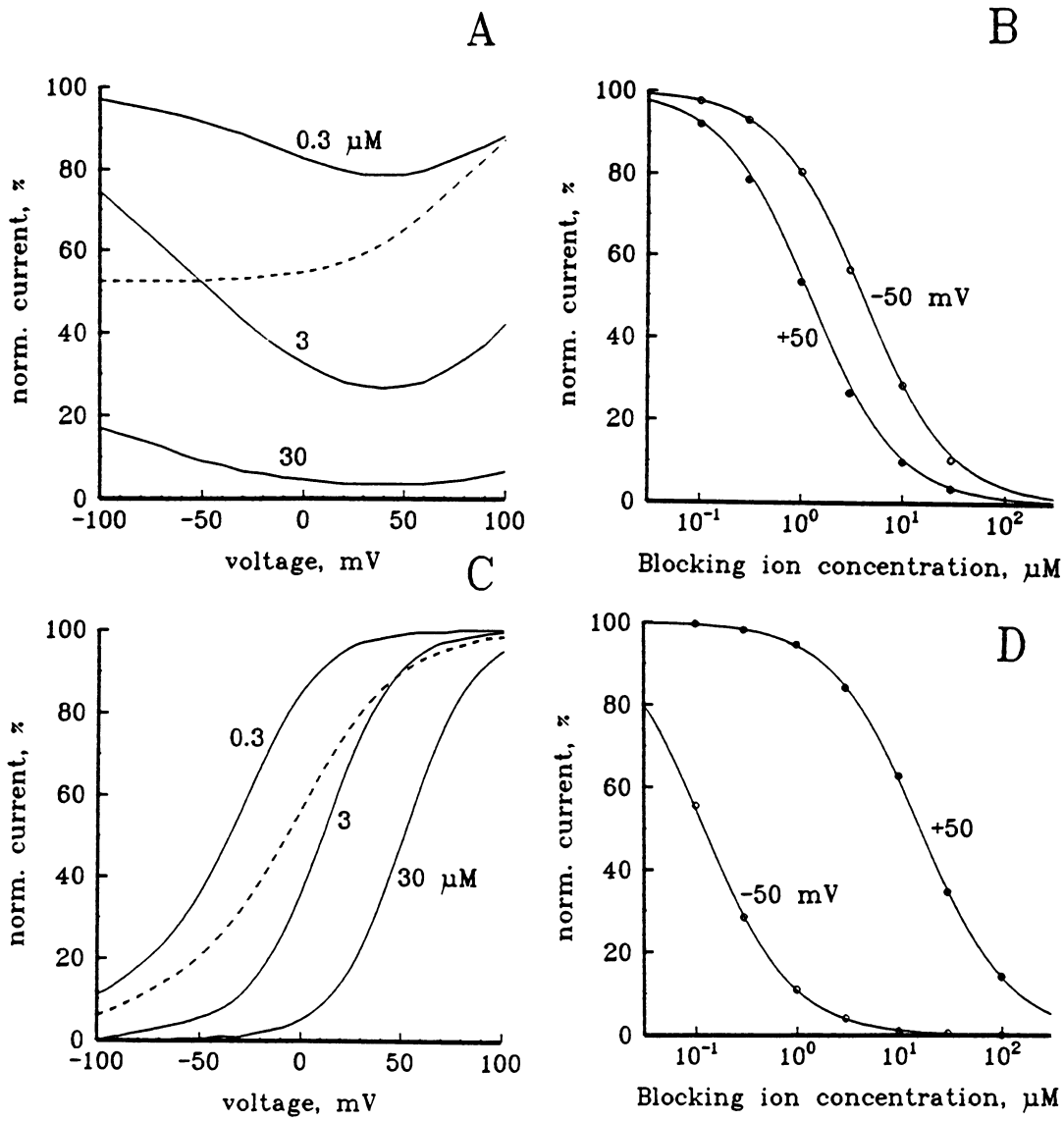

FIGURE 4 External ion block of unidirectional currents for the conformational model. All the calculations were made using the conformational model with parameters given in legend to Fig. 3. $[X]_{in} = 0$. $[C]_{in} = 0$ for *A* and *B*, and $[C]_{out} = 0$ for *C* and *D*. (*A*) Voltage dependence of external ion block of the inward current calculated for different concentrations of the blocking ion (X) indicated near each curve. $X_{out} = 10$ μM. $C_{out} = 10$ mM. $C_{in} = 0$. Dotted curve shows the block for the 2B1S model calculated for $[X]_{out} = 3$ μM with $K_{Ca} = 1$ mM and $K_X = 0.3$ μM. (*B*) Circles show the concentration dependence of block for two voltages (as indicated near each curve) plotted from the results of calculations exemplified in *A*. Smooth curves were drawn according to a Michaelis-Menten type equation $I_{norm.} = K_X[X]/(K_X + [X])$ with the apparent dissociation constant $K_X = 4$ μM at +50 mV and 1.2 μM at −50 mV. (*C*) Voltage dependence of external ion block of the outward current calculated for different concentrations of the blocking ion indicated near each curve. $X_{out} = 10$ μM. $C_{in} = 10$ mM. $C_{out} = 0$. Dotted curve shows the block for the 2B1S model calculated with parameters listed in *A*. (*D*) Circles show the concentration dependence of block for two voltages (as indicated near each curve) plotted from the results of calculations exemplified in *C*. Smooth curves were drawn according to a Michaelis-Menten type equation $I_{norm.} = K_X[X]/(K_X + [X])$ with the apparent dissociation constant $K_X = 0.12$ μM at −50 mV and 16 μM at +50 mV.

proximated by the exponential dependence $\exp(z'V)$, where z' can be considered as the effective valence of the blocking reaction. For curves shown in Fig. 4 *C* the values of z' for the 2B1S model and that of the conformational model differ by a factor of 2.

The block increased with the concentration of the blocking ion and with the decrease in the permeant ion concentration. The concentration dependence of block could be well fitted by a Michaelis-Menten type equation as exemplified by solid curves in Fig. 3 *B* and Fig. 4, *B* and *D*. Apparent dissociation constants employed in such fits show large variations depending on membrane voltage and the ion composition of bathing solutions. They were larger than the actual value of K_X and sometimes this difference reached two orders of magnitude.

The block relief by increasing the concentration of permeant ions had a steeper dependence in the conformational model than in the 2B1S model. Fig. 3 *D* shows

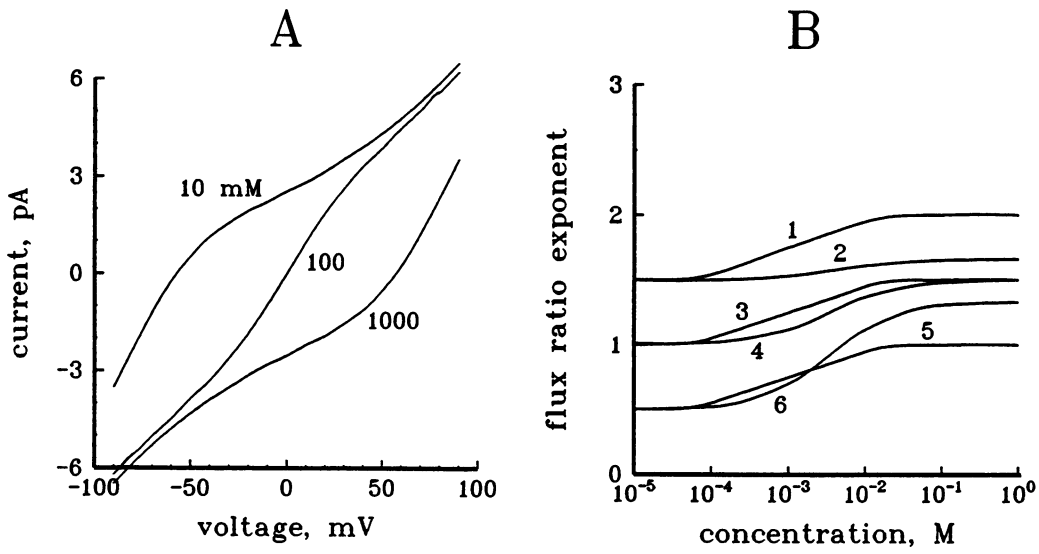

FIGURE 5 Ussing flux ratio for the conformational model. All the calculations were made with the basic set of parameters for Ca listed in the Theory section but the charge of permeant cation was set to +1. (*A*) Voltage dependence of the total channel current calculated with the basic set of model parameters. Electrical distances were chosen as follows: in the first conformational state all $\sigma_i^+ = 0.25$, in the second conformational state $\sigma_1^+ = \sigma_2^+ = 0.5$, $\sigma_{-1}^+ = \sigma_{-2}^+ = 0$ for an ion entering the channel from the external compartment, $\sigma_1^- = \sigma_2^- = 0$, $\sigma_{-1}^- = \sigma_{-2}^- = 0.5$ for an ion entering the channel from the internal compartment. C_{out} = 100 mM and C_{in} is indicated near each curve. (*B*) Concentration dependence of the exponent in the Ussing flux ratio. Permeant ions were present in the external and the internal compartments in equal concentration plotted on abscissa. Calculations were made for the basic set of model parameters with the following modifications: dissociation constant in the first conformational state was 1 μM (curves 1, 3 and 5) and 1 mM (curve 2, 4 and 6); in the first conformational state all $\sigma_i^+ = 0.25$, and in the second conformational state $\sigma_1^+ = \sigma_2^+ = 0.5$, $\sigma_{-1}^+ = \sigma_{-2}^+ = 0$, $\sigma_1^- = \sigma_2^- = 0$, $\sigma_{-1}^- = \sigma_{-2}^- = 0.5$ (curves 1, 2); all $\sigma_i^+ = \sigma_i^- = 0.25$ (curves 3, 4); $\sigma_1^+ = \sigma_2^+ = 0$, $\sigma_{-1}^+ = \sigma_{-2}^+ = 0.5$, $\sigma_1^- = \sigma_2^- = 0.5$, $\sigma_{-1}^- = \sigma_{-2}^- = 0$ (curves 5, 6).

that the dose-response curves could be well fitted by the Hill equation with the index n = 1.7. Interestingly, a similar relationship was found for Ca block of sodium current through the calcium channel with variations in the internal Na concentration (Yamashita et al., 1990).

Ratio of unidirectional fluxes

Fig. 5 presents the results of calculations of unidirectional fluxes within the conformational model. In all calculations, made with different variations of model parameters, the reversal potential always satisfied the Nernst law as exemplified by Fig. 5 *A*. The ratio of unidirectional fluxes exhibited an exponential voltage dependence (data not shown). As expected from the qualitative analysis made in the Theory section, the Ussing flux ratio exponent n' appeared to be highly sensitive to changes in the voltage dependence of rate constants k_i describing an ion permeation. Fig. 5 *B* shows the concentration dependence of n' calculated for the basic set of parameters with equal electrical distances in all conformational states (curves 3 and 4), and for two extreme cases where the rate constants in the second conformational state had a voltage dependence, depending on the direction of ion passage (see also Theory). For the potential profiles described above the calculated values of n' increased from lower limiting values equal to 1, 1.5 and 0.5, respectively, to different upper limits, ranging from 1 to 2.5. The calculated concentration dependence of n' is in line with measurement of isotope fluxes for the potassium channels (Hodgkin and Keynes, 1955; Begenisich and de Weer, 1980) and gramicidin channels (for review, see Finkelstein and Andersen, 1981). The value of n' increased with a decrease of the dissociation constant for the high-affinity site in the first conformational state (Fig. 5 *B*). This result may apply to gramicidin channels where Cs ions, binding to the channel stronger than Na ions do, yield a higher value of n'.

As to the variation of other model parameters, it appeared that n' showed only weak dependence on the rate constants for the conformational transitions. It slightly decreased with the increase of the rate constant for the first conformational transition and with the decrease the rate constant for the second conformational transition (data not shown).

DISCUSSION

Channels as enzymes

In contrast to prevailing views in biochemistry, conformational models are not widely accepted in membrane biology. Ion channels are usually treated as rigid structures, providing an aqueous pore in the membrane, the opening of which is determined only by a gating machinery assumed to function independently. However, the channel gating is often observed to depend on the type of permeating ion, as demonstrated by Nelson (1984) and

Chesnoy-Marchais (1985) for the calcium channels. In the conformational model, it is assumed that the channel permeability is directly influenced by the permeating ions. Proteins form highly ordered structures and their conformations are readily affected by ion binding, e.g., as shown for metalloenzymes. Strong electrostatic or donor-acceptor interactions between the binding ion and amino acid residues in the channel pore can induce the polarization of the whole membrane protein. Resulting conformational change can give another energy profile of the ion pathway or, in other words, will change the channel permeability and perhaps selectivity.

The basic assumption of the present model is that the open channel is permeable at every instant only to a given ion species and the first ion passing through the channel makes a specific path, accepting (selecting) only similar ions. The concept presented is based on features known for enzymes catalysis. Enzymes, suggested recently to include also ion channels (Eisenberg, 1990), are believed to undergo a transient strain in the whole protein upon substrate binding which can be maximal in the active site. The net result in enzyme catalysis is a lowering of the activation energy for a substrate transformation. For ion channels this might correspond to a creation of a selective pore for ion translocation. The suggestion that a specific pathway in the channel protein exists for each type of permeating ion recalls a reptation theory in polymers (De Gennes, 1979) which was recently applied to describe the channel gating (cf. Millhauser, 1989, and references therein).

Both enzymes and membrane channels have a high selectivity. For enzymes this means that a substrate fits exactly the active site which is equivalent that enzymes have a high substrate affinity. However, for membrane channels a strong binding conflicts with large ion fluxes measured. From Eq. 7*a* maximal flux in one-ion channel can be estimated as $J = k_{on}K_d$ which for typical values of $J \approx 10^6$–10^7 ions/s and $k_{on} = 10^8$–10^9 M^{-1} s^{-1} gives a lower limit of $K_d = 10$ mM. The apparent dissociation constants measured for ion channels are about this value or even larger. One-ion models with a weak binding can explain large channel fluxes and the selectivity appears by assuming different potential barriers for permeant ions (Hille, 1984). However, macrocyclic ligands (Lehn, 1985) which are often used to draw deductive parallels about the ion channels, have dissociation constants in submillimolar range. One way to resolve this discrepancy is to assume a strong electrostatic repulsion between ions in the channel. For instance, energy wells for ion(s) in the channel can be deep, corresponding to a strong binding and high selectivity, but ion–ion repulsion makes them shallower, leading to large fluxes. An alternative explanation is delineated in the present paper.

Weak points of multi-ion models

Multi-ion models were developed and successfully applied to describe a variety of ion transport phenomena, but they pose conceptual difficulties on the molecular level. Firstly, ion transport in membrane channels is thought to occur in a single-file mode. This conclusion is based upon the observed geometrical arrangement of model polypeptide channels and the results of studies, using the permeant ions of different size and shape as probes for channel dimensions (Hille, 1984). In such narrow channels a multiple ion occupancy should be strongly prohibited as discussed by Jakobsson and Chiu (1987). In gramicidin channels ion hydration by intrachannel water results in the alignment of single-file water molecules, corresponding to a configuration which is unfavorable to accept another ion in the channel (cf. Brickmann and Fischer, 1983; Jakobsson and Chiu, 1987). For putative membrane channels which are believed to have large vestibules and a short narrow part, acting as a selective filter, several closely spaced ions, imposing a strong electrostatic field, must affect the orientation of water molecules, needed both to separate ions in the channel and to hydrate them at the same time. Such highly ordered structures would impede the transport of ions and water. From the experimental point of view, these effects, together with ion–ion repulsion, should substantially retard the rate of ion entry into the occupied channel, an effect, that has never been clearly demonstrated for ion channels assumed to have a multiple occupancy.

Similarly, ion–ion repulsion in proteins was not confirmed. For example, Ca-binding proteins serving as a prototype for Ca channels, have four high-affinity binding sites separated by 0.8–1.2 nm (Strynadka and James, 1989). Even if protein screens electrostatic interactions as efficient as water does (Mironov, 1983), the successive dissociation constants for Ca should increase upon the sequential filling of the binding sites. However, the corresponding values measured by various biophysical methods are of similar magnitude and reveal no charge dependence in experiments with trivalent lanthanides that can also occupy Ca-binding sites (Forsen et al., 1986).

Predictions of the conformational model

Previous conformational models have been shown to possess some features that earlier seemed possible to explain only in terms of multi-ion models. Lauger et al. (1980) indicated the way to obtain a maximum in the concentration-conductance relationship observed for gramicidin and potassium channels. Anomalous mole fraction effects are intrinsic to the conformational model as shown by Kostyuk and Mironov (1986) and Mironov (1988) for calcium channels. Using the conformational model, Lux et al. (1990) described a bell-shaped voltage dependence for Ca block of the sodium current through the calcium channel.

Common to all these models is the assumption of several conducting states of the channel which transform

into each other via conformational transitions. It should be emphasized that both the kinetic schemes employed and the model parameters used are different in all the above mentioned models. The present model deals with a specific mechanism of ion permeation which takes into account the channel 'memory.' The analysis has been extended to demonstrate a constellation of all transport properties that earlier seemed explicable only using multi-ion models. All the calculations were made using parameters appropriate to voltage-sensitive calcium channels because the mechanisms of ion permeation and selectivity are still the matter of debate (see Kostyuk et al., 1983; Kostyuk and Mironov, 1986; Mironov et al., 1986; Mironov, 1988; Lux et al., 1990; vs Almers and McCleskey, 1984; Hess and Tsien, 1984; Lansman et al., 1986; Friel and Tsien, 1989; Yue and Marban, 1990; Yamashita et al., 1990).

Anomalous extrema on curves, describing the dependence of current on ion concentration and the mole fraction are also explained by the model. In addition, it was shown that the concentration-conductance relationship may deviate from a Michaelis-Menten type equation. The relief of block by increasing the permeant ion concentration has a slope, corresponding to the Hill equation, rather than belonging to the Michaelis-Menten type. Ion block demonstrated a steeper voltage dependence in comparison with the one-ion model, a feature that invoked a multi-ion model (Hille and Schwarz, 1978). All these experimental protocols employing different blocking and permeant ions can be used to distinguish between the conformational and the multi-ion models. However, it might well be that resulting differences in the predicting ability between the both models will be only quantitative, not qualitative. For calcium channels such analysis was performed for Ca and Mg block of the sodium current (Lux et al., 1990) and the choice has been made in favor of the conformational model. A comparison between the multi-ion model and the kinetic model with lazy state for the potassium channel allowed rejection of the former model for description of AMFE observed in K^+/Tl^+ mixtures (Draber et al., 1991).

The conformational model produced an anomalous Ussing flux ratio exponent obtained with a specific voltage dependence of rate constants for ion translocation in different conformational states. Such coupling of unidirectional fluxes was earlier considered as the major indicator of the simultaneous presence of several ions in the channel, and the exponent in the Ussing flux ratio was assumed to give directly the channel occupancy. The development of multi-ion models was inspired by measurements of isotope fluxes through potassium channels in the squid axon membrane (Hodgkin and Keynes, 1955). In these experiments the exponent in the Ussing flux ratio was $n' \approx 2.5$ (see also Begenisich and deWeer, 1980), which is close to the limiting value of the present model.

Weak points of the conformational model

The mechanism suggested in the present work satisfies the energy conservation law: energy gained due to ion binding to the high-affinity site is used to produce an activated protein with a low-affinity site and then the energy released after protein transformation to the initial state. Large cycles in the kinetic schemes 1 and 2 function only in a clockwise direction, thus, the principle of microscopic reversibility (Onsager, 1931) is in doubt. For biopolymers this condition may not necessarily hold. Examples of experimental evidence of violation of Onsager's principle in biological systems include a deterministic gating of Cl channels (Richard and Miller, 1990; and references therein) and the association of oligomeric proteins (Erijman and Weber, 1991). Biophysical studies of hemoproteins led to the introduction of the concept of two types of motion in proteins, equilibrium fluctuations and 'functionally important motions' which are responsible for transitions between different conformational states. These nonequilibrium processes called 'proteinquakes' (Frauenfelder et al., 1988) were shown to be similar to glass transitions (Stein, 1985).

All conformational models predict the extra noise arising from flickering between different conformational states (Lauger et al., 1980; Lauger, 1985). For several channel types the increased noise for the open channel has been observed (cf. Sigworth, 1985). However, a detailed analysis is required before claiming that these effects are related to fluctuations in the channel conductance in a way suggested by conformational models. The present model predicts that the flickering should be the most prominent in biionic conditions at the potential of current reversal or at the minimum for the anomalous mole fraction effect when fluxes in each conductive conformational state are of similar magnitude.

Future research needed

At present, it seems that only the multi-ion or conformational models can explain various phenomena observed while studying the permeation and selectivity of membrane channels. Therefore, future studies are welcome that would allow to distinguish between these two models. Some differences in model predictions are mentioned above. In addition, derivation of working hypotheses may be guided by molecular modeling. Much work has been done to investigate the mechanisms of ion permeation using the methods of molecular dynamics or Brownian motion. Studies of model gramicidin channels have already shown that the channel flexibility can be an important factor (cf. Brickmann et al., 1983). These entropy effects may play a crucial role in determining the channel selectivity as discussed by Eisenmann and Horn (1983). Such ab initio studies should also address the issue of ion–ion interactions within the channel and the problem of existence of long-lived conformational states for a flexible channel with intrachannel water.

Application of various spectroscopy methods have provided many intimate details of the structure and affinity of specific binding sites in enzymes. NMR-spectroscopy has been already applied to study the ion binding in gramicidin channels (Urry et al., 1989). Spin labels, recently employed for alamethecin channels (Archer and Cafiso, 1991) have shown that channel rearrangements are important determinants of the voltage dependence of channel conductance. Fluorescence measurements were extensively used to gain structural insights into conformational transitions in Ca-binding proteins (Forsen et al., 1986). Keeping in mind the presence of high-affinity Ca binding sites in voltage-sensitive Ca channels, the application of fluorescence methods may not only provide information complementary to that obtained in electrophysiological experiments, but also give new data hitherto unknown.

Structural manipulation of channel proteins with chemical and genetic tools are now widely used to establish the importance of given functional groups and elements (cf. Miller, 1990). The perturbations producing changes in the channel selectivity can be treated either as a modification of a selective filter or a shift in the equilibrium of allowed protein states with different permeability properties. Some experimental data, like specific action of omega-conotoxin on different conducting states of the calcium channels (Carbone and Lux, 1988), may be easier to explain using the latter assumption.

The major value of current models is to suggest ideas for new, more informative, experimental studies. If the results do not support the model, alternatives will be developed and tested. This interactive process should allow one to understand some of the basic mechanisms of ion channel permeation and selectivity.

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