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The influence of dynamics of ionic channel protein on its selectivity function

A.P. Demchenko [a], N.N. Kositsky [a] and V.I. Teslenko [b]

[a] *A.V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian S.S.R., Kiev 252030 and* [b] *Institute for Theoretical Physics, Academy of Sciences of the Ukrainian S.S.R., Kiev 252130, U.S.S.R.*

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Computer simulation was applied for modelling the sodium channel selective filter. The geometric parameters, electrostatic interactions and channel protein polar group dynamics were studied with respect to their effect on permeability and selectivity function. The most important parameters were shown to be the filter width, electrical charge on the binding site and the dielectric constant. Appropriate selection of all three parameter values permitted a qualitative description of the experimentally obtained data on Na^+ channel selectivity. The dynamics of the dipolar groups in the channel protein molecule were treated in terms of the Debye model of dipolar relaxations. The dynamics of the dipoles exerted the most significant effect on channel permeability and selectivity. It is shown that when the dynamics occur on a scale slower than that of the motion of ions, the channel will exhibit a low degree of permeability and its selectivity will be lost. The model predicts the appearance of an effect arising from the saturation of electric current with increasing concentration of the permeant ion species. The saturation current decreases at slower rates of dipolar relaxation. Therefore, the effective operation of ion channels requires the channel protein to be capable of undergoing rapid motion.

1. Introduction

One of the most intriguing properties of the cell membrane is the existence of ion selectivity, i.e., the ability to allow ions of one kind to permeate through the channel while blocking the transmission of others. This is the result of specific ion channel proteins forming narrow, water-filled pores of diameter within the range 5–10 Å [1]. In order to elucidate this phenomenon, the problem concerning the manner in which the channel's selectivity is affected by the dielectric properties of the pore medium, and the particular conditions under which this can take place, must be resolved. Eisenmann [2] was the first to suggest that channel selectivity in biomembranes is governed by the change in free energy that occurs at the stage of ion exchange between the aqueous solution and the binding site within the channel. In order to provide an explanation for the fact that the K^+ channel presents a barrier to the passage of Na^+ and, vice versa, that the Na^+ channel is impermeable to the transfer of K^+, Eisenmann and coworkers considered the situation for the cases of various binding sites, differing in their radius of interaction, and obtained values for the equilibrium free energy differences with respect to Na^+/K^+ specificities in vacuo amounting to almost the order of 5 kcal/mol [2,3].

However, in their calculations on the interaction of cations with the multipole of a single water molecule, the above authors used an approximation that did not take into account the interaction of the cation with other water molecules as well as with the multipoles of mobile protein groups and immobilized water molecules adjacent to the binding site within the channel. To devise a suitable means of evaluating the macroscopic effect of

Correspondence addres: A.P. Demchenko, A.V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian S.S.R., Kiev 252030

polar media on the cation-site interaction in the simplest possible form, the value of ϵ, the effective dielectric constant of the channel, must be included in the calculation procedure. Recently reported empirical estimations and numerical microscopic calculations have demonstrated that this value was of sufficient magnitude, viz., $\epsilon = 10$–20 [4]. Since the value of the free energy difference in the case of the method of Eisenmann is purely the difference between the electrostatic energies, then a channel medium of dielectric constant ϵ results in a 10-fold decrease in this value, thus leading to the almost complete loss of Na^+/K^+ specificity (e.g., 5 kcal $mol^{-1}/10 = 0.5$ kcal mol^{-1}).

It has been shown in a number of studies [4–6] that the motion of an ion within the channel should be regarded as being continuous. To obtain the explicit potential energy profile of the ion along the channel, the electrostatic and Van der Waals interaction energies of the ion with its binding sites and the channel walls at each point within the channel interior must be determined. Once this has been done, one should then take into account the spatial dimensions of the channel [5] and evaluate the influence of the dielectric screening of ion-site interactions within the channel as well as clarifying the behaviour concerning the dynamics of this screening with respect to intramolecular motion in the channel protein and the motion of ions along the channel.

Many workers have succeeded in describing channel selectivity by suggesting the existence of several energy barriers along the path of the ion through the channel and by selecting the heights of the barriers to fit the experimental findings. However, the question as to the elucidation of the physical nature and molecular mechanisms of regulation of these heights in real ion channels remains unresolved.

In the present paper, we consider a rather simple three-site model for the ion channel selective filter. In accordance with the model, proposed by Hille for sodium channels, we consider the narrowest filter cross-section to be rectangular with dimensions of about 3×5 Å. We obtain the explicit potential energy profiles for different ions with variation of the geometric, electrostatic and dielectric filter parameters. For these profiles we evaluate the three permeability ratios P_{Li^+}/P_{Na^+}, P_{K^+}/P_{Na^+} and $P_{NH_4^+}/P_{Na^+}$, our observation agreeing well with the data of Hille.

We then deal with the effect of the channel dielectric constant in greater detail, including the case of its possible dispersion. Finally, we discuss the consequences arising from the above in relation to the interpretation of a number of current-dependent phenomena in ion channels.

2. Theoretical backgrounds and methods

2.1. Three-site model of sodium channel selective filter

To consider quantitatively the physically simplest case that reproduces the most important aspects of the selectivity of the channel, we begin by making the following principal assumptions.

2.1.1. Filter geometry

Initially, we introduce the geometric parameters of the selective filter (see Fig. 1). Recent studies on the permeability and blockage of sodium channels have shown that the most appropriate model of a selective filter is a sequential three-site structure with the distance separating neighbouring sites being of the order of 10 Å [6]. We suggest the filter structure to be symmetrical with respect to the central site and the sites as being identical with the charges $Q = -0.3$ (in units of proton charge). The central part of the filter has the narrowest cross-section. Such a geometry was selected for the sake of convenience. The divergence in position of the central binding site and the site of the steric barrier has no substantial influence on our results. The cross-section is smallest at the filter center with dimensions of about 3×5 Å. Among the water molecules surrounding the cation within the filter, we distinguish the three nearest which are associated with the cation in a rigid three-tetrahedron–one-sphere cluster. This cluster passes through the filter without any distortion of its initial structure.

2.1.2. Filter walls

We consider the filter walls to be absolutely rigid. We assign a fixed value to the filter dimen-

sion along the $0Z$ axis of 3 Å which is close to the size of a water molecule, $\Delta \approx 2.9$ Å. Thus, the ionic cluster can move only in two directions, viz., along the $0X$ and $0Y$ axes (see fig. 1). The possible variations in coordinate y for a given coordinate x would be limited by the following inequalities:

$$\rho_i \leqslant y_i(x) \leqslant H - (\Delta + \rho_i), \tag{1}$$

where ρ_i is the radius of the monovalent cation species i. Here, $y_i(x)$ represents the dependence on i.

2.1.3. Energy reference level

In order to calculate the potential energy profiles for different cations passing through the filter from its left peripheral site to that on the right, the energy reference level must be established. Before entering the filter, the ion experiences progressive transitions from the bulk solution through the channel entrance to close contact with the peripheral sites [7–10]. The energy involved in this process depends on many factors: diffuse-double-layer potentials, local and net charge potentials, image potential, hydration and dehydration of the ion and its binding site and electrostatic interaction of peripheral sites with the cation moving from the solvent side. The first three potentials are believed not to be specific for most monovalent cations. The latter three interactions are even less specific due to the comparatively large value of the dielectric constant for the channel entrance medium [4]. Thus, during the movement of a cation from the bulk solution to the peripheral binding site, the very large change in ion-site interaction energy is almost totally compensated by the corresponding change in hydration energy. For these reasons, we consider the energy reference levels for the various monovalent cations being investigated to be approximately equal at their equilibrium positions near the peripheral binding sites. The reference level also includes contributions from the energy of interaction of the three binding sites with all water multipoles associated with the ion within a cluster as well as those occupying the filter interior. We believe that this interaction energy does not vary noticeably during the passage of a cation-water cluster through the filter in single-file coupling with other water molecules.

2.2. Cation-site interaction

The energy of interaction $W_i(r_i)$ of the monovalent cation species i at a position $r_i = (x, y_i(x))$ with three binding sites $j = 1$–3 generally includes the contributions arising from repulsion of the electron shells, and attraction due to dispersive forces and electrostatic charge. The first two terms correspond to the Lennard-Jones potential, the final one denoting the Coulombic potential. It includes the dielectric constant ϵ which describes the screening of the interaction by the environment:

$$W_i(r_i) = \sum_j \left[A_i/R_{ij}^{12} - B_i/R_{ij}^{6} - 332\ Q_j/\epsilon\ R_{ij} \right], \tag{2}$$

where $W_i(r_i)$ is determined in units of kcal mol^{-1} Q_j denotes the charge residing on site j (in units of proton charge), A_i and B_i Lennard-Jones parameters and R_{ij} the distance between cation i and binding site j (in Å).

In the suggested two-dimensional approximation for each $y_i(x)$, we are interested in determining the minimum value of the interaction energy, i.e., $W_i^0(x) = W_i(x, y_i^0(x)) = \min W_i(r_i)$. In the evaluation thereof, coordinate $y_i(x)$, depending on the ion species within the limits of the inequalities expressed by eq. 1, serves as the variable parameter, thus providing the equilibrium value of $y_i^0(x)$. The possibility of performing such a calculation of y_i^0 for every given x is justified by referring to the well-known fact that equilibrium in the plane of the cross-section is always attained more rapidly than in the case of translational movement [11]. On knowing $y_i^0(x)$ introduced in the Cartesian system (fig. 1A), we can easily obtain the expression for R_{ij}:

$$R_{ij}(x) = \left\{ \left[H + |\, j - 2\,| \cdot h - y_i(x) + \rho_0 \right]^2 + \left[(j-1)L - x \right]^2 \right\}^{1/2}, \tag{3}$$

which for given H, h, L and ρ_0 (see fig. 1A) becomes a one-to-one function of the coordinate x, thus describing the quasi-one-dimensional movement of a cation within the filter.

For modelling the situation of a cation passing though the filter, we used cyclic computer simula-

tion. By varying the value of $y_i(x)$ in eq. 3 for fixed x under the conditions of eq. 1, we find $y_i^0(x_0)$ which corresponds to the minimum value of the energy $W_i(x_0)$ expressed by eq. 2. By processing x in eq. 3 from 0 to $2L$ for all known $y_i^0(x)$, we calculate the complete 'quasi-static' potential energy profile for the cation along the filter $W_i(x)$.

The profile $W_i(x)$ resulting for given values of A_i, B_i and ϵ includes a major portion of the information about the specificity for a given cation and the channel permeability within the filter. The constants A_i and B_i are characteristic of the intimate Van der Waals interaction of different cations with the suggested anionic sites on the filter. Their values are unambiguously coupled with the position $\rho_m^{(i)}$ of the energy minimum on the curve representing the non-covalent interaction:

$$\rho_m^{(i)} = (2A_i/B_i)^{1/2};\ E_m^{(i)} = -B_i^2/4A_i$$

By supposing the binding site to contain a carbonyl oxygen atom with a crystallographic radius of $\rho_0 = 1.4$ Å, we use

$$\rho_m^{(i)} = 0.55(\rho_0 + \rho_i)$$

thus describing the interaction of 'soft spheres' for the case where the separation between anion and cation undergoing Van der Waals interaction is about 10% greater than the sum of the crystallographic radii of the cation ρ_i and anion site ρ_0 [12].

A considerable effect is exerted by the translational movement of a cation within the filter on the magnitude of the energy as the result of a dynamic factor associated with a possible correlation between the passage of the ion and the relaxational movement of charged atomic groups within the channel. These processes constitute the so-called 'dynamic' part of the potential energy profile included in the form of a frequency dependence of the dielectric constant $\epsilon = \epsilon(\nu)$ as discussed below.

2.3. Dielectric properties of channel protein

Since the three-dimensional structure of the sodium channel protein at the atomic level remains unresolved, the exact values of the electrostatic potential arising due to protein cannot be calculated at present. Therefore, we shall deal with the electrostatic screening of charge in ion channels at the macroscopic level by employing the dielectric constant of the medium ϵ, and the dynamics of this screening via the use of the effective relaxation frequency ν_R. The Debye equation [13] has been extensively applied in investigations on the physics of dielectrics with respect to the interaction between a dielectric and an alternating electric field. The dielectric constant dispersion $\epsilon(\omega)$ may be expressed by:

$$\epsilon = \epsilon(\omega) = \epsilon_0 - \frac{\epsilon_0 - n^2}{1 + (\nu_R/\omega)^2}, \qquad (4)$$

where ω is the frequency of the alternating electric field, ϵ_0 the static dielectric constant, n^2 the dielectric constant at the frequency of light, and ν_R the characteristic frequency of dipolar relaxation.

In the Debye approximation, the relaxation frequency of a dielectric medium is considered not to depend on the nature of the perturbation. Application of the Debye theory is effective not only in the description of experiments on the interaction of a dielectric medium with an alternating electric field, but also in spectroscopy, where an electric field inside the dielectric is created on electronic excitation of the chromophore [14]. We consider that eq. 4 may describe the behaviour of the relaxation for sufficiently rapid introduction of ionic charge into the selected region. At rates of ion transfer through the channel ν_i, that are comparable in magnitude relative to the relaxation rate in the channel protein ν_R, the dielectric dispersion may be expressed by eq. 4, assuming $\omega = 2\pi\nu_i$. Applying eq. 4 for evaluation of the energy profile $W(r)$ via eq. 2, we obtain the values of the permeabilities as functions of ion transfer rates ν_i. Estimates made previously on the basis of electrophysiological experiments [5] suggest a lower limit of $\nu_i = 10^7\ s^{-1}$ for the possible range of transfer rates of the various permeable cations through the open channel.

For employing eq. 4 in our studies, the frequencies characteristic of the intramolecular dynamics of dipolar groups within the channel protein ν_R,

should be estimated. Since these values are unavailable, we resorted to the use of published data on the dynamics of globular proteins in solutions. Fluorescence molecular relaxation spectroscopy [15,16] allows the determination of the characteristic times of dipolar motions in proteins. The available data suggest the most appropriate time range for such motions to be between tens and hundreds of nanoseconds. Specifically, for tetrameric melittin [17], the dipolar-orientational relaxation time τ_R in the environment of a tryptophan residue is 3 ns at 40 °C and 40 ns at 25 °C. Therefore, the first crude estimate for the relaxation frequency can be made: $\nu_R = 1/\tau_R \approx 10^9\ s^{-1}$.

2.4. Channel permeability

Another contribution to the translational energy of an ion within the selective filter is provided by the reorientation of water molecules in the cation-water cluster and recombination of molecules between the cluster and filter interior. These processes constitute the socalled 'diffusive' portion of the translational energy of the ion. As a consequence of both steric hindrance within the filter and single file conditions, the possibility of the reorientation process occurring may be excluded from our consideration. The recombination characterized by low activation energies (2.5 kcal/mol^{-1} as estimated by Warshel and Russel [4]) induces rapid diffusive jumps of the cation-water cluster within times covering the range 10^{-11}–10^{-10} s at the corresponding distances of 1–2 Å. This process can readily be taken into consideration in the usual manner [11] by phenomenological introduction of the local diffusion coefficient $D_i(x)$ inside the channel commitantly with that in the bulk solution d_i for each ion species i. In wide channels, d_i should be comparable to D_i, whereas in narrow channels D_i is smaller. We assume that $D_i(x) = \gamma(x) d_i$, where the distribution coefficient $\gamma(x)$ is independent of the ion species i. The use of this assumption does not appear to cause significant error in the following continuous solution [7], since the values introduced for the radii of permeable cations are of similar magnitude ($\rho_{Na^+} \approx 0.9$ Å and $\rho_{K^+} \approx 1.3$ Å, see fig. 1).

In the basic continuous theory, the flux of the monovalent ion species i is described by the following equation [18]:

$$J_i = -D_i S\left[\frac{dc_i}{dx} + \frac{e}{kT} c_i \frac{d(\varphi + \phi_i)}{dx}\right] \tag{5}$$

where $D_i(x)$ represents the diffusion coefficient of the ion, $S(x)$ the area available to the ion within the channel, $C_i(x)$ the concentration of the ion, $\varphi(x)$ the applied external voltage and $\phi_i(x)$ the intrinsic electrochemical potential of the channel. In the steady-state approximation eq. 5 can be integrated easily [18]:

$$J_i = \left(C_i^{(1)} \exp(eV/kT) - C_i^{(2)}\right) / \int_{-\infty}^{\infty} \left[\exp\{(\varphi + \phi_i)/kT\}/D_i S\right] dx \tag{6}$$

where $C_i^{(1)}$ and $C_i^{(2)}$ denote the bulk concentrations on sides 1 and 2 ($\varphi^{(1)} = V$, $\varphi^{(2)} = 0$), and $\phi_i^{(1,2)} = 0$ the zero energy reference level for each ionic species i. The integral in eq. 6 contains all of the terms expressing the selective properties of the channel. In the case of a quasi-homogeneous channel, $D_i = \gamma d_i$ = constant, $S_i(x) = S$ = constant $\phi_i(x) = (1/e) W_i(x)$ if the channel is sufficiently large, $2L \gg S^{1/2}$ and in the low-field limit ($eV \ll kT$) we can estimate the single-channel permeability P_i as:

$$P_i = \gamma_i d_i S / \int_0^{2L} \exp(W_i(x)/kT)\, dx. \tag{7}$$

Thus, in our approximation the permeability ratios $P_i/P_{i'}$ include information on the energy profiles $W_i(x)$ and bulk diffusion coefficients d_i. In the limiting case where the transmembrane electric potential and the concentration of the ion species at one side of the membrane are equal to zero ($V = 0$, $C_i^{(2)} = 0$, $C_i^{(1)} = C_i$), the expression for the diffusive unidirectional ionic current J_i takes its simplest form:

$$J_i = P_i C_i \tag{8}$$

The channel current is proportional to the ion transfer rate ν_i,

$$J_i = e\nu_i. \tag{9}$$

Once the value of ν_i has been selected, the channel current is then determined from eq. 9

with the channel permeability $P_i(J_i)$ being calculated via eq. 7. This allows one to determine the permeant ion concentration from eq. 8.

3. Results

3.1. Energy profiles and permeability ratios

Fig. 1B demonstrates the 'static' energy profiles in the channel for various ions. During their passage through the channel, different ions have differing energy profiles. In the case of highly permeable ions such as Li^+ and Na^+, the profile possesses two energy barriers, the heights of which are completely determined by variations in the energy due to electrostatic attraction of the cations to anion sites. This energy is inversely dependent on the radius of the cation; therefore, for Li^+, both barriers are greater than those for Na^+. In the case of ions of low permeability (NH_4^+, K^+), an additional barrier appears, whose size is largely determined by the value of the first ('repulsion') term in eq. 2. This energy barrier is directly proportional to the cation radius. The abnormal position for NH_4^+ is explained by its greater ability to form hydrogen bonds with water molecules, leading to a decrease in the effective radius of the ion-water clusters by 0.1 Å.

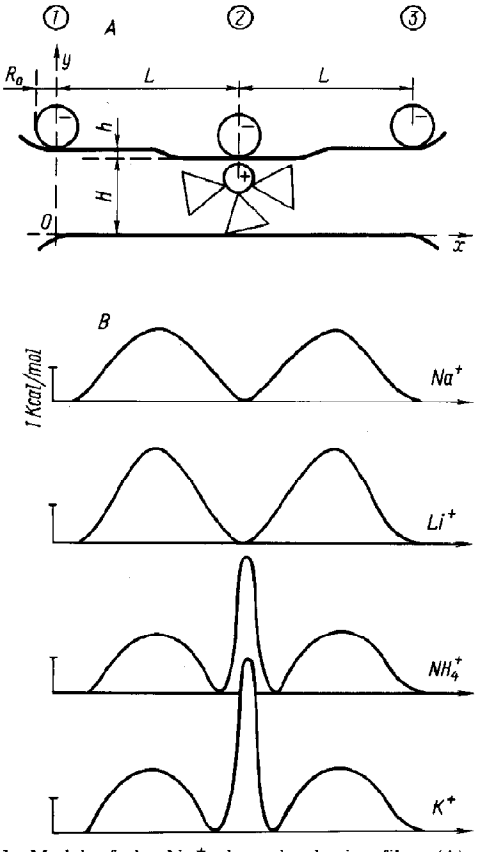

Fig. 1. Model of the Na^+ channel selective filter (A) and energy profiles for different permeant ions (B). (A) Cross-section of selective filter: (large circles) Anionic binding sites, (small circles) permeant cation, (triangles) water molecules. (B) Energy-distance functions calculated using eqs. (1–3) for i = Li^+, Na^+, NH_4^+, K^+ with the following parameters: $\rho_i = 0.6$, 0.95, 1.34, 1.33 Å [5]; $A_i = 3214$, 20480, 129300, 107600; $B_i = 57$, 137, 344, 293; $\Delta = 2.87$ Å; $\rho_0 = 1.40$ Å [5]; $H = 5.0$ Å; $h = 0.5$ Å; $L = 15$ Å; $Q_j = -0.3$; -0.3; $-0.3e$. $\epsilon = 10$.

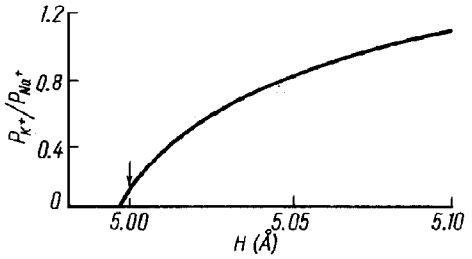

Fig. 2. Dependence of permeability ratio P_{K^+}/P_{Na^+} on width of filter cross-section H.

Table 1 provides a comparison of experimental and theoretical data on permeability ratios for the Na^+ channel obtained via eq. 7 employing static potential profiles simulated by using eqs 1–3. As shown by fig. 1B, the energy profiles calculated are in good agreement with the selectivity observed.

Figs. 2 and 3 show the effects of variations in the filter geometry on the value of the K^+/Na^+ permeability ratio. From fig. 2, it follows that an increase in filter width H (see fig. 1A) by 0.1 Å leads to the complete loss of the channel selectivity only. This effect is due to the removal of the central (steric) barrier on the energy profile of K^+. A similar effect arises if the distance L between filter anion sites is increased by 15–20 Å (see fig. 3). However, in this case, the loss of channel selectivity results from the predominance of the

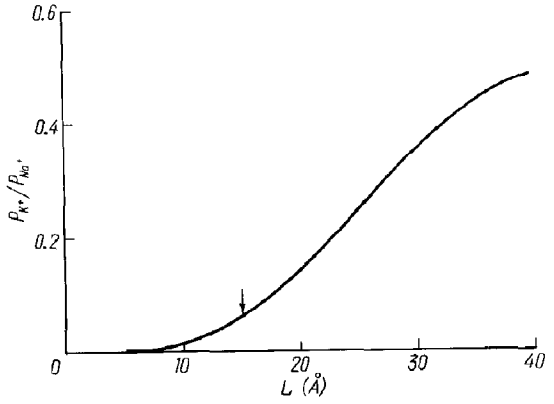

Fig. 3. Dependence of permeability ratio P_{K^+}/P_{Na^+} on distance between binding sites L.

integral contribution of electrostatic barriers over the steric term in eq. 7 for distances $L > 30$ Å. Variation of h does not produce any significant effect.

A dramatic influence of relative heights on the electrostatic barriers is caused by an increase in the site charge Q. This effect is equivalent to the corresponding decrease in the channel dielectric constant ϵ. As follows from fig. 4, at values of $Q \approx 0.8$, the permeability ratio P_{K^+}/P_{Na^+} approaches the value of 1.2, i.e., the channel conductivity for K^+ in the Na^+ channel becomes comparable to that for K^+ in the K^+ channel.

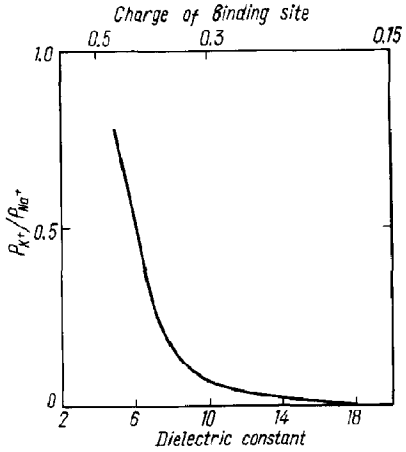

Fig. 4. Dependence of permeability ratio P_{K^+}/P_{Na^+} on charge Q (in units of proton charge) of the binding site (at ϵ = constant = 10) and dielectric constant ϵ of the medium (at Q = constant = 0.3).

The conclusion that the interaction of an anion with cations in K^+ channels takes place with a stronger electrostatic field as compared to sites in Na^+ channels is exactly the reverse of the situation as derived on the basis of Eisenmann's selectivity sequence [3]. This disagreement may be due to the fact that Eisenmann calculated only the equilibrium free energy of the cation within potential wells whereas we have determined the entire energy profile taking into account the heights of the potential barriers. It appears that the heights of the potential barriers are more sensitive than the depths of the potential wells to the field strength of the binding sites.

Thus, according to calculations on the basis of our model for a selective filter, a change in the geometric parameters (filter length L and width H) influences channel selectivity. The selectivity is substantially reduced when these parameters are increased. The values $L = 15$ Å and $H = 5$ Å are optimal for the description of experimental data (see table 1). They are set at fixed values for further study of the effects of other parameters.

3.2. Dielectric permittivity effects

The interaction of a permeant ion with the binding site in a selective filter is governed by the dielectric properties of the medium, and the magnitude and rate of orientation of dipole moments. These properties will differ according to the relation between the ion transfer rate and the rate of orientation of medium dipoles (fig. 5).

Table 1

Experimental and calculated values of permeability ratios P_i/P_{Na^+} for different cations

Ion species (i)	P_i/P_{Na^+} [a] (experiment)	P_i/P_{Na^+} [b] (calculation)
Li^+	0.93	0.94
Na^+	1.0	1.0
NH_4^+	0.16	0.15
K^+	0.08	0.1

[a] Data from ref. 27.

[b] Calculated from eq. 7 and eqs. 1–3 with the parameters given in the legend to fig. 1.

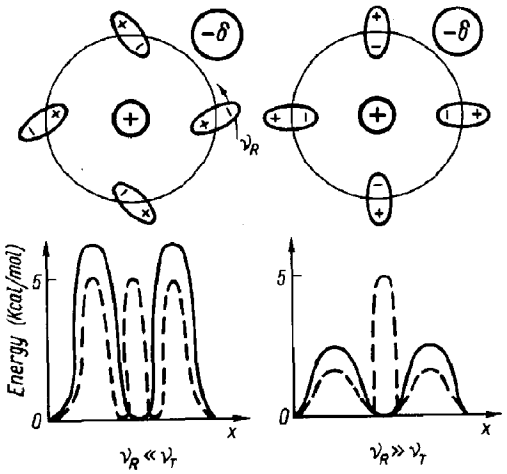

Fig. 5. Scheme illustrating the dynamic interaction of a permeating cation with selective filter charge $(-\delta)$ and channel dipoles and energy profiles for highly permeable Na^+ (———) and poorly permeant K^+ (- - - - -) in the cases of fast ($\nu_R \gg \nu_i$) and slow relaxations ($\nu_R \ll \nu_i$).

If motion of the ion is slow with respect to the channel protein's dipolar relaxation rate ($\nu_i = \nu_T \ll \nu_R$), the orientation of dipoles reduces the extent of electrostatic interaction within the selective filter. This facilitates thermoactivated ion diffusion through the barrier. The ion passes through the filter in a quasi-equilibrium fashion. The exact picture may be as follows: the permeant ion interacts with the binding site, then dipolar relaxation occurs, decreasing the ion-binding site interaction energy. This reduces the barrier heights and allows ion diffusion to occur more readily.

The situation is different when the motion of the ion is fast relative to dipolar relaxation. The rotations of dipoles do not keep pace with the ion's motion, the ion becomes entrapped by the selective filter within a deep energy well and remains there until dipolar relaxation occurs, reducing the barrier height. Only then is it free to move. This results in autoregulation of the ion transfer through the channel.

Assuming that in eq. 2 the dynamics of protein dipoles is determined by the frequency dependence of the dielectric constant (eq. 4), we obtain the channel permeability as a function of the rate (ν_i) of ion transfer through the channel. For Na^+,

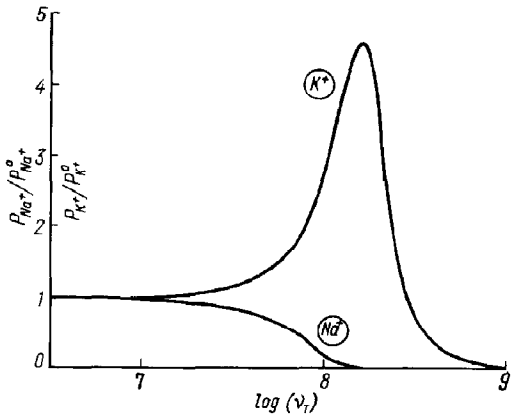

Fig. 6. Dependence of channel permeability normalized at $\epsilon = \epsilon_0$ ($\nu_i \ll \nu_R$) for Na^+ $P_{Na^+}/P^0_{Na^+}$ and K^+ $P_{K^+}/P^0_{K^+}$ on ion transfer rate ν_i.

which is a highly permeable species in the Na^+ channel, P_{Na^+} is high for low rates of ion transfer ν_{Na^+} and decreases abruptly with increasing ν_{Na^+} values (fig. 6).

An interesting dependence of permeability on ν_i is observed for ions displaying low permeability. K^+ permeability P_{K^+} (see fig. 6), gradually increases at higher transfer rates ν_i until the latter reaches a value of $\nu_i \simeq 1.5 \times 10^8$ s^{-1}. This increase is due to a reduction in the height of the central (steric) selective barrier (fig. 1B). This is due to a decrease in dielectric screening at higher rates, greater electrostatic interaction and lowering of the electrostatic energy well. Maximum permeability occurs at ν_i values where the heights of the

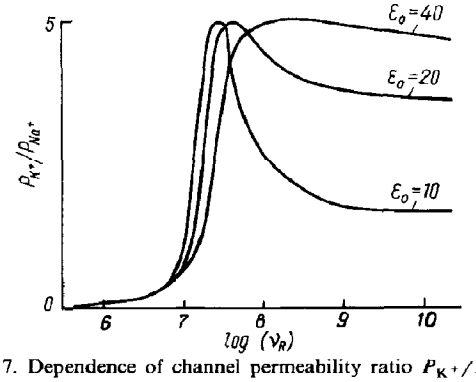

Fig. 7. Dependence of channel permeability ratio P_{K^+}/P_{Na^+} on relaxation rate constant ν_R for different values of the static dielectric constant. Transfer rate $\nu_i = 10^7$ s^{-1}.

steric and energy barriers are equal. For increase in the rate ν_i the K^+ permeability, after reaching its maximum value, decreases sharply, similarly to the case for Na^+.

The curves in fig. 6 have been plotted with the supposition that the rate of dipolar relaxation is fixed: $\nu_R = 10^9\ s^{-1}$. The selection of other values for ν_R does not lead to qualitatively distinct phenomena, the curves being simply shifted along the frequency axis.

The rate of relaxation ν_R of dipoles in the channel-forming protein depends on a number of physical parameters describing the conditions under which the channel operates, e.g., temperature, membrane or channel microviscosity, etc. Fig. 7 demonstrates Na^+ channel selectivity as a function of relaxation rate. A rise in ν_R lowers the channel selectivity thus increasing the P_{K^+}/P_{Na^+} ratio. This reaches a maximum at a characteristic value $\nu_R \approx 7 \times 10^7\ s^{-1}$ which depends on ϵ_0. With further increase in ν_R, the P_{K^+}/P_{Na^+} ratio decreases, tending toward the level of saturation which also depends on ϵ_0.

Thus, strong selectivity requires a high polarity for the environment of a mobile ion within a selective filter.

3.3. Concentration effects in ionic current

The existence of non-linearity in the dependence of ionic current on permeant ion concentration is one of the most important consequences of our model. Ionic current may be calculated from the permeability vs. tranfer rate functions (see fig. 6) by using eqs. 4 and 9. Its concentration dependence in the cases of Na^+ and Li^+ is illustrated in fig. 8. The curves are characterized as showing saturation and can be approximated by the Langmuir relation:

$$J(C) = J_{max}(1 + K_d/C)^{-1}, \qquad (9)$$

where J_{max} is the saturation current and K_d the apparent dissociation constant ($J(K_d) = 1/2\ J_{max}$). The level of the saturation current for the less permeant Li^+ ($P_{Li^+}/P_{Na^+} = 0.93$) is 1.2 pA lower than that for Na^+.

The magnitude of the saturation current J_{max} in our model is an essential function of the static

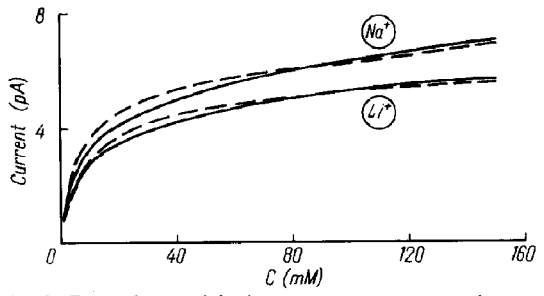

Fig. 8. Dependence of ionic current on permeant ion concentration. (———) Calculations, (- - - - - -) approximation according to the Langmuir equation.

dielectric constant ϵ_0. Plots of the ionic current vs. permeant ion concentration at different ϵ_0 values are presented in fig. 9. With a rise in ϵ_0, the saturation current J_{max} increases and the apparent dissociation constant K_d decreases. A decrease in K_d is attributed to the smaller extent of ion interaction with the binding site in the selective filter.

It is essential that, if dipolar relaxation within the channel is rapid, one may observe a linear dependence of current on ion concentration without any indication of saturation. This case is illustrated by fig. 10, where these functions are displayed for different relaxation rates. At $\nu_R = 10^{10}\ s^{-1}$, a very steep increase in current is observed with $J_{max} \approx 36$ pA. For lower ν_R values the saturation current is much smaller.

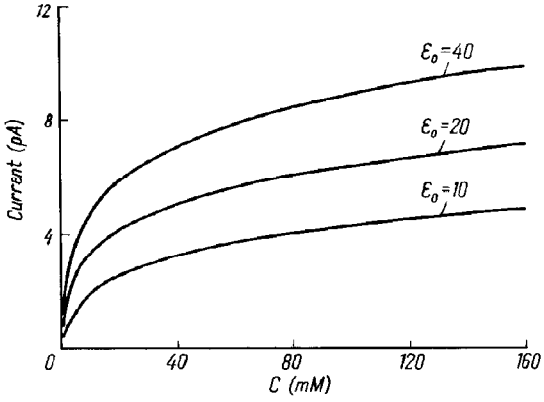

Fig. 9. Dependence of ionic current on Na^+ concentration at different ϵ_0 values.

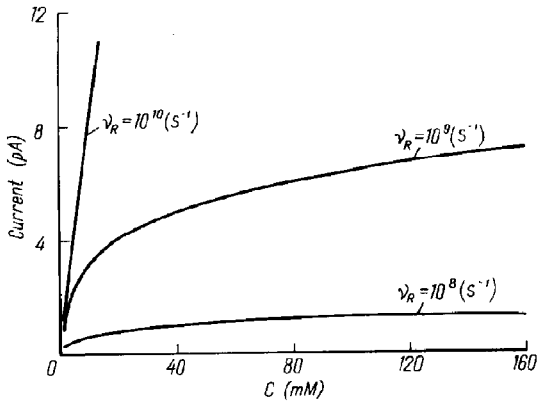

Fig. 10. Dependence of ionic current on Na^+ concentration for different relaxation rate constants ν_R.

The relationships obtained are in qualitative agreement with experimental data [19,20]. For different ions in Na^+ channels, non-linearity (with saturation) of the ionic current as a function of concentration is observed.

4. Discussion

Similarly to other models [21,22], in the present model, channel permeability and selectivity are considered to be determined by the energy profile for permeant ions. The profile is characterized by having several potential barriers. As in other models, we study unidirectional ion motion within a single-ion approximation and do not consider the direct interaction between ions. However, in other models, the origin of the energy barriers and the regulation of their heights with respect to the permeant ion species have not been regarded. In contrast, we have obtained the energy profile of the channel on the basis of predetermined geometric and electrostatic parameters of the selective filter (number of binding sites and their charges, distance between them, channel width, dielectric permittivity of the medium).

Different models suggest the Eyring mechanism of transfer over the energy barrier to be valid (for a review, see ref. 18). This reduces the limits for spatial range of ion transfer by several Ångströms (1–2 Å). Ion jumps over greater distances are difficult to consider within the framework of Eyring's formalism. We use the concept of an electrodiffusion mechanism for ion transport [22]. This allows one to study a wide range of geometrical parameters for a selective filter and to demonstrate the manner in which the model molecular parameters influence the energy profile of ion within a channel. The selective filter width, charge of the binding site and dielectric permittivity of the medium are the most important of these.

As is well know [4,23], the interior of a protein is a strongly polar dielectric medium with high concentrations of different dipolar groups having various motional times in the nano- and microsecond ranges [24]. Thus, the dipole moment of a peptide group is 3.5–3.6 Debye, tyrosine 1.55 Debye, tryptophan 3.4 Debye and protein-bound water 1.85 Debye. If the electrostatic equilibrium in a protein is disturbed by the absorption of a quantum of light by tryptophan residues, the rotations of dipolar groups in time can be detected by the fluorescence method [16,17]. Meanwhile the existing theories of ion transport do not take into consideration the possibility that the ion's motion can disturb the dielectric medium of the channel. We suggest a possible mechanism for the way in which the motions of dipolar groups in an ion channel can influence its conductivity and selectivity. The macroscopic treatment in terms of the dielectric permittivity is an approximation which does not require knowledge of a channel protein's structure or details at the microscopic level of protein dipoles and charges, as well as the detailed characteristics of the channel protein dynamics.

The concentration dependence of channel currents is often characterized by saturation. The effects of saturation of current are usually explained by ion binding in the channel and blocking ion transport. A quantitative description in terms of traditional approaches is difficult because of the necessity to consider two or more interacting ions [19,20]. In our model channel permeability is limited by the rate of relaxation in the channel protein, this being the mechanism determining the magnitude of the saturation current. The rate of ion transfer is governed by the equilibrium polarization necessary for transfer of each ion. This will be in equilibrium for the permeating

ion following in sequence. The ion is retarded and free to move after a new step of relaxation. This is the mechanism for individual recognition of each ion by the filter and of definite intervals between such ions during their motion. Using this approach, there is no need to consider occupied-vacant ion binding sites. The ionic current should depend on single-ion transfer rates.

At present, the influence of rapid protein dynamics on the functional properties of proteins is a matter of extensive discussion [24–26], with considerable attention being focused on specific ligand binding and enzyme catalysis. This paper extends the discussion to ion channel functioning. We suggest that, for the most effective operation of a channel (high transfer rate associated with high selectivity), the rate of protein dipole motions should be faster than that of transfer. If not, then dielectric blocking of the channel and loss of selectivity should take place.

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