

Brownian Dynamics Simulation of pH-Effects on Conductance Through Potassium Channels

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Abstract. A new theory termed “tunnel-acid-group-potential” (TAGPT), explaining the effect of pH_o and pH_i on ion conductance through different membrane channels, is presented. It is suggested that shifts in pH_o and pH_i lead to changes in values of negative charges generated by acid groups of side chains of some polar (Glu^- , Asp^-) amino acid residues lining the tunnel part of the channel. The resulting modification of electrostatic field influences the heights of rate-limiting energy barriers (for ion transport) in the transition zones between the tunnel and the vestibules, followed by changes in channel conductivity.

Key words: Ion channels — Pores — Titration — Channel conductance — Brownian dynamics

Introduction

Cytoplasmic pH_i , as well as extracellular pH (pH_o) are known to influence ionic currents of the plasma membranes in various cells, depressing these currents with the increasing acidification of bathing solutions (Drouin and The 1969, Mozhaeva and Naumov 1970, 1972, 1983, Hille 1973, 1984, Woodhull 1973, Shrago 1974, Schauf and Davis 1976, Carbone et al 1978, Meech 1979, Wanke et al 1979, Moody 1981, 1984, Roos and Boron 1981, Moody and Hagiwara 1982, Stampe and Vestegaard-Bogind 1985, Byerly and Moody 1986, Deutch and Lee 1989, Peers 1989, Kume et al 1990). Investigators have not come to an agreement on why acid solutions lower ionic conductances. The existing theories consider two principal effects. First, low pH might reduce single-channel conductance by titrating one or more negative acid groups within the narrow part of the channel, blocking it sterically – the “steric acid-group-theory” (SAGT) (Hille 1973, 1984, Woodhull 1973). Second, low pH

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might reduce ionic conductance by titrating some of surface charged groups which normally attract an ion atmosphere of transferring ions to the mouth of the pore—the surface-potential theory (SPT) (Adrian 1969; Mozhaeva and Naumov 1970; 1972; Shriager 1974; Schauf and Davis 1976; Bell and Miller 1984; Dani 1986; Cai and Jordan 1990; Naranjo et al 1994). As to the former theory it has been shown recently (Veresov 1992a,b; 1994) that the cross-section of the narrowest part of the channel (tunnel or pore or P-region elsewhere) is substantially larger than proposed earlier (see e.g. Hille 1984) and as a result the hydrogen ion with a small crystal radius cannot be a good channel steric blocker. The other theory (SPT) suggests that the decrease of the negative surface charge can be expected to lower the concentration of cations near the channel entrances and therefore reduce the conductance for these cations. At least one objection can be put forward in this connection. The traditional analysis by SPT takes into account the effect of surface potential on the ion concentration at the channel mouth, often ignoring the influence of the surface charge on the electromotive forces all along the channel. These two effects have opposite consequences for the conductance. Neglecting any of them can lead to an overestimation by SPT of the influence of the surface charge and even to misinterpretation. Thus, for example, reconstitution of K channels of the sarcoplasmic reticulum into lipid bilayers formed from neutral or negatively charged phospholipids has shown that complete removal of the negative surface charge leads to only a partial loss of conductance by a single channel (Bell and Miller 1984). Studies on frog skeletal muscle (Hutter and Warner 1972) which showed that the anion conductance of the muscle is reversibly abolished by low values of pH contrary to the predictions of the surface-potential theory can also be considered as corroborating the above thesis.

At the same time it was shown theoretically (Veresov 1992a,b; 1994) as well as experimentally using site directed mutagenesis (Imoto et al 1988) that the presence of one or more rings of negative charges is necessary for electrostatic reduction of the energy barrier at the tunnel entrance to the level when the channel fluxes can be detected. On the contrary, in the absence of negative charges or when they are small, these barriers will be too large to give any significant fluxes.

Since acidification of the bathing solutions is accompanied by an increase in the protonation of the titratable groups lining the tunnel and by a proportional decrease of the negative charge of these groups with the respective flux reduction caused by the correspondent change of the electrostatic field, one can speak about the alternative possibility of conductance depression by low pH that we shall term tunnel-acid-group-potential in contrast to steric acid group.

The aim of this work is a theoretical analysis of this hypothesis of channel flux regulation by pH on the basis of the previously developed structural models of potassium channels (Veresov 1992a,b; 1994) as well as with the use of stochastic molecular dynamics simulation of direct ion passage through these channels. Fur-

thermore the new theory of pH-dependence of conductance we are going to present shall be termed the "tunnel-acid-group-potential theory" (TAGPT)

Materials and Methods

It is generally accepted that potassium channels have wide entrances – vestibules and narrow regions linking them – tunnels (or pore region, P-region) (Bezawilla and Armstrong 1972, Latorre and Miller 1983, Yellen 1987) It has been assumed that this structure is formed by cyclic association of four homologic subunits with rotational symmetry around a central pore, either of which includes 7 transmembrane segments (Fig. 1A) 6 α -helices H1, H2, H3, S4-L, H4, H6, and one β -loop β H5 (or S5-S6 linker or S5-S6 loop or SS1-SS2 segment elsewhere) (Tempel et al 1987, Guy and Raghunathan 1989, Guy 1989, Veresov 1992a, 1994) Also it has been suggested (Tempel et al 1987, Guy and Raghunathan 1989, Guy 1989, Hait-

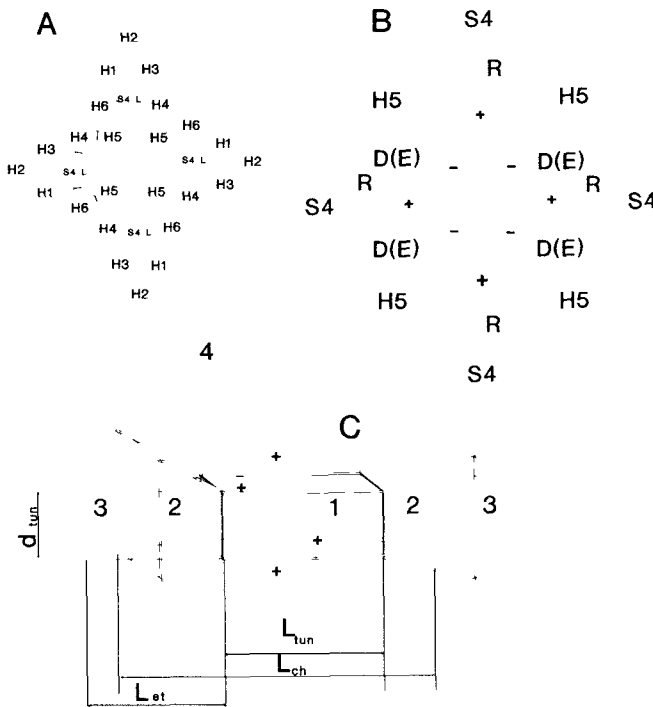


Figure 1. Diagram of generation of the model potassium channel (A) Rotationally symmetric association of transmembrane segments (B) Ion pair formation inside the tunnel (C) A calculated model of K^+ -channel with tunnel (1) vestibules (2) entrance tubes (3) and a layer of ion pairs (4) L_{tun} , L_{ch} , L_{et} and d_{tun} are tunnel length, channel length, entrance tube length and tunnel diameter, respectively

mann et al 1991, Yool and Schwarz 1991) that hydrophilic segments SS2s of the four β -hairpins H5 participate in the formation of the tunnel lining and that positively charged groups of segments S4-L form ion pairs with negative charges of segments H5 stabilizing the channel structure (Fig 1B), the hydrophobic helices H1, H2 H3, H4, H6 constitute a hydrophobic periphery of the corresponding channel former. The model tunnel used in the calculations (Fig 1C), agrees with the above mentioned commonly used structural concepts and corresponds to channel MB004 suggested by Veresov (1995) and based on the channel former amino acid sequence (Baumann et al 1987, Tempel et al 1987) the principles of membrane protein folding (Guy and Seetharamulu 1986, Guy 1989), and electrophysiological data (e.g. Yellen 1987). The tunnel was viewed as a parallelepiped with repulsive walls, carrying four (or less because of protonation) ion pairs of Arg^+ -Glu $^-$ (or Arg^+ -Asp $^-$ type), which were located at the square vertices within the plane perpendicular to the channel axis that reflected four-fold rotational symmetry of channel forming. The ion pairs were modeled by the dipoles with 3Å interpole distances, which corresponds to ion pair geometry at the protein-solute interfaces (Barlow and Thornton 1983, Singh et al 1987). As to the vestibules, then modeling was conjugated with the simulation of entry processes, and will be mentioned below in the section describing the simulation technique.

The effects of pH on conductance were considered on the basis of the model presented above. The ionic equilibrium for the system including N_c channels was calculated using diagram shown in Fig 2, where negative sites correspond to the internal ionogenic carboxylic groups of the Glu $^-$ or Asp $^-$ side chains. In accordance with Washell's results (Washell and Russel 1984, Washell et al 1984) it was supposed that the existence of states with uncompensated charge within the protein (e.g. pairs $\text{Arg}(\text{neutl})$ -Asp $^-$, Arg^+ -Asp(neutl), $\text{Arg}(\text{neutl})$ -Glu $^-$, Arg^+ -Glu(neutl)) is unlikely. Such states were not considered, and are not included in the diagram in Fig 2. The ionic equilibrium was calculated using the following equations

$$\begin{aligned}
 & [\text{A}] + [\text{AH}] + [\text{AH}_{2c}] + [\text{AH}_{2d}] + [\text{AH}_3] + [\text{AH}_4] = N_c \\
 & [\text{AH}_3] = [\text{AH}_4] \frac{k_6}{[\text{H}^{+*}]} \\
 & [\text{AH}_{2d}] = [\text{AH}_3] \frac{k_5}{[\text{H}^{+*}]} = [\text{AH}_4] \frac{k_5 k_6}{[\text{H}^{+*}]^2} \\
 & [\text{AH}_{2c}] = [\text{AH}_3] \frac{k_4}{[\text{H}^{+*}]} = [\text{AH}_4] \frac{k_4 k_6}{[\text{H}^{+*}]^2} \\
 & [\text{AH}_1] = [\text{AH}_{2d}] \frac{k_3}{[\text{H}^{+*}]} = [\text{AH}_4] \frac{k_3 k_5 k_6}{[\text{H}^{+*}]^3} \\
 & [\text{A}] = [\text{AH}_1] \frac{k_1}{[\text{H}^{+*}]} = [\text{AH}_4] \frac{k_1 k_3 k_5 k_6}{[\text{H}^{+*}]^4} \quad (1)
 \end{aligned}$$

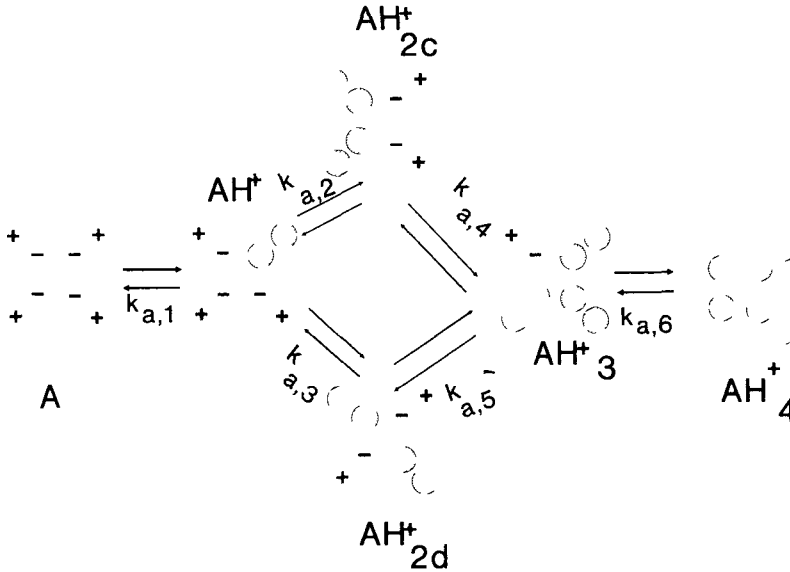


Figure 2. Diagram of ionization equilibrium between possible states. Shown are completely deprotonated state (A), one-fold protonated state (AH⁺), two-fold protonated states (AH⁺_{2c} and AH⁺_{2d}), three-fold protonated state (AH⁺₃) and completely protonated state (AH⁺₄). k_a (n = 1, 2, ..., 6) are equilibrium constants between these states.

where [A], [AH₁], [AH_{2d}], [AH_{2c}], [AH₃], [AH₄] stand for the numbers of channels in specific protonation state in accordance with Fig. 2. N is the number of channels considered. k₁ / k₁, k₂, k₆ are equilibrium dissociation constants in correspondence with Fig. 2. [H⁺] is proton concentration at the center of the ring of charges on the tunnel axis.

The solution of equations (1) yields

$$\begin{aligned}
 [AH_1] &= \frac{N_c}{F_d} \\
 [AH_3] &= [AH_1] \cdot 10^{-(pK_6 - pH)} \\
 [AH_{2d}] &= [AH_3] \cdot 10^{-(pK_5 - pH)} \\
 [AH_{2c}] &= [AH_3] \cdot 10^{-(pK_4 - pH)} \\
 [AH_1] &= [AH_{2d}] \cdot 10^{-(pK_2 - pH)} \\
 [A] &= [AH_1] \cdot 10^{-(pK_1 - pH)}
 \end{aligned} \tag{2}$$

pH is hydrogen ion index that corresponds to H⁺. F_d was calculated as

$$F_d = 1 + \frac{k_6}{[H^+]} + \frac{k_5 k_6}{[H^+]^2} + \frac{k_4 k_6}{[H^+]^2} + \frac{k_3 k_5 k_6}{[H^+]^3} + \frac{k_1 k_3 k_5 k_6}{[H^+]^4}$$

The pK_a values in equation (2) differed from pK_a values of the corresponding amino acids. They were calculated as (Warshell and Russel 1984; Warshell et al. 1984)

$$pK_{a,i} = (pK_{a,mt})_i + \sum_j \frac{332q_i q_j}{2 \cdot 303RT R_{ij} \epsilon_{\text{eff}}} \quad (3)$$

where $(pK_{a,mt})_i$ is the pK_a value of the i -th titratable group when other charges are absent. R_{ij} is the distance between sites i and j . ϵ_{eff} is the effective value of the dielectric constant. The value $pK_{a,mt1} = 3.9$ was used, corresponding to the pK_a value of carboxylic acid of the aspartate side chain. The use of aspartate as a negative charge generator within the P-region is based on amino acid sequence of the H5 segment (or S5-S6 loop elsewhere) of the potassium channel former (Tempel et al. 1987).

The value $\epsilon_{\text{eff}} = 10$ (Mouoi 1991) was taken for the interaction inside the ion pairs. In other cases, the value $\epsilon_{\text{eff}} = 80$ was employed, in agreement with interactions via water solution. The proton concentration within the channel near the ionized groups presented in equations (1) and (2) can differ from values measured in bathing solutes. The calculation of the intrachannel proton concentration was carried out assuming constancy of the axial component of proton fluxes J_{H^+} (this results from the continuity equation), e.g. assuming

$$\frac{dJ_{H^+}}{dz} = 0 \quad \Rightarrow \quad \frac{d\mu_{H^+}}{dz} = \text{const} \quad (4)$$

where μ_{H^+} is the proton electrochemical potential at the point z on the channel axis. From (4) one can write the following expression for μ_{H^+}

$$\mu_{H^+} = \mu_{1,H^+} + \frac{\mu_{0,H^+} - \mu_{1,H^+}}{\delta_1 + \delta_2} \delta_1 = \frac{\mu_{1,H^+} \delta_2 + \mu_{0,H^+} \delta_1}{\delta_1 + \delta_2} \quad (5)$$

In (5) μ_{H^+} is the proton electrochemical potential at point z on the channel axis. μ_{0,H^+} and μ_{1,H^+} are proton electrochemical potentials on extracellular and intracellular phases respectively. δ_1 and δ_2 are the distances between the charge ring localization plane and the tunnel entrances (see Fig. 1). As follows from equation (5)

$$H^+ \cdot e^{F\varphi/RT} = \frac{H_1 \delta_2 + H_0 \delta_1}{\delta_1 + \delta_2} \quad (6)$$

Equation (6) links the values of pH_0 and pH_1 with those of proton concentration inside the channel.

There are some problems with the selection of the values of δ_1 and δ_2 . Since the influence of pH_1 on conductance is much stronger than that of pH_0 (Mozhaeva and Naumov 1970, 1983; Hille 1973, 1984; Carbone et al. 1978; Wanke et al. 1979

Moody 1984) it was supposed that $\delta_1 < \delta_2$. From earlier results (Yellen 1987, Veresov 1992a, 1994) one can also suggest that $8A < \delta_1 + \delta_2 < 15A$. The selection of δ_1 and δ_2 was done by computer experiment using two restrictions described above: a) $\delta_1 < \delta_2$, b) $8A < \delta_1 + \delta_2 < 15A$.

The average potassium conductances $G_{K,av}$ were calculated in the following manner:

$$G_{K,av} = g_{K,AH_4}p(AH_4) + g_{K,AH_3}p(AH_3) + g_{K,AH_{2d}}p(AH_{2d}) + g_{K,AH_{2c}}p(AH_{2c}) + g_{K,AH_1}p(AH_1) + g_{K,AP}(A) \tag{7}$$

where g_{K,AH_i} is the channel conductivity for the channel state AH_i , $p(AH_i)$ are the probabilities of the state AH_i ($i = 0, 1, 2c, 2d, 3, 4, AH_0 \equiv A$), $p(AH_i)$ were calculated as:

$$p(AH_x) = \frac{[AH_x]}{\sum_y [AH_y]} \tag{8}$$

The potassium conductances g_{K,AH_i} for each possible state of protonation of the potassium channel were calculated by the Brownian Dynamics method in the Langevin approach. The finite-difference version of the stochastic Langevin equations in the diffusion limit (i.e. assuming that force F_j is constant during time step Δt and $\beta\Delta t \gg 1$ (β is friction coefficient)) was used. The Langevin equations for this case can be written in the following form (Eimak and Buckholz 1980, Cooper et al. 1985):

$$r_j(t_0 + \Delta t) = r_j(t_0) + D/kT \cdot F_{0j} \cdot \Delta t + B_R(\Delta t) \tag{9}$$

where $D = \mu\beta/kT$, $B_R(t) = 1/m\beta \int R(t_0 + t)dt$, r is the ion position, R is the random force with gaussian distribution, D is the diffusion coefficient in the absence of counterion effects, F is external force due to counterions' interaction with the channel former and water, Δt is the time step, subscript zeros indicate values at the beginning of the time step.

The potential energy of interaction of the ion, occupying the position (x, y, z) with the channel former was calculated as the sum of the energy of the ion-coulomb interactions with the channel former charged groups and the potential energy of ion repulsion from the channel walls. The following expression for potential energy (E_{ic}) of this interaction was used:

$$E_{ic} = \sum_i z_i \varepsilon_j / r_{ij} + V_0 [(2x/a_0)^{12} + (2y/a_0)^{12}] \tag{10}$$

where

$$a_0 = (d_{tun} - 2\sigma_{io})(V_0 / \varepsilon_{io})^{1/12}$$

$$\sigma_{io} = (\sigma_i + \sigma_o) / 2$$

$$z_{io} = (z_{oo} - z_{ii})^{1/2}$$

The non-ionic part of relationship (12) is a parallelepiped analog of potential function for a repulsive interaction between the ion and the channel wall as described by Fisher and Brickmann (1983). The electrostatic parameters were taken from Mackay et al. (1984): σ_{oo} , σ_{ii} , τ_{oo} , τ_{ii} from Fisher and Brickmann (1982). The scaling factor $V_0 = 5 \cdot 10^{-21}$ J was taken from Fisher and Brickmann (1983). d_{tun} is the tunnel diameter; the subscripts denote o – oxygen, i – ion.

Forces acting on the ion upon dehydration were evaluated using the energetic profiles of potassium ions in a model channel obtained earlier (Veresov 1992a). The single channel potassium conductance was calculated when equal potassium ion concentrations in internal and external phases $c_o = c_i = 0.4$ mol/l were assigned. The use of high concentrations of transported ions permitted to reduce the simulation time and at the same time to make the obvious extrapolation to lower concentrations. The processes of ion entries into the tunnels were considered using the entrance tubes (Ermaik and Buckholz 1980; Cooper et al. 1985) which are the extensions of the tunnels into external and internal bathing solutions. They included the axial parts of the vestibules and had the parallelepiped form with cross-sections identical to those of model tunnels. The length of the tube (L_{it}) was calculated as

$$L_{it} = 1 / (x_{itfj} \cdot y_{itfj} \cdot c \cdot N_A) \quad (11)$$

where L_{it} is the entrance tube length, x_{itfj} and y_{itfj} are effective tunnel diameters in x and y directions, c is the molar concentration of ions in bathing solutions, N_A is Avogadro's number. This length ensures that the volume of the tube is such that it contains one ion on the average at any moment.

The following calculation strategy was used. First, 20 ions were randomly distributed within 20 entrance tubes, ten from each side of the model tunnel. Then the simulation of ion movements was done using the expression (9) and its unidimensional analogs for the case of movement inside the entrance tubes. $B_R(\Delta t)$ had zero mean and its standard deviations were $6D \cdot \Delta t$ for three-dimensional and $2D \cdot \Delta t$ for one-dimensional movements. In the simulation the routine GAUSS from Program Library of the Mathematical Institute of Academy of Sciences Belarus was called as a subroutine and scaled properly to yield the necessary random numbers to simulate $B_R(\Delta t)$ in (9). The ions in the channels passed randomly until leaving the channel exit. Subsequently the reinitialization procedure was performed: a new ion was distributed randomly in the channel and the simulation was continued. One million steps were realized for each channel and for each state of protonation. This corresponded to the real time of 500 μ S when the time step 5pS was used. To improve accuracy the procedure of step-breaking was applied for the regions where the force gradients were large. This took place in the dehydrational near-tunnel zones of vestibules. The forces in these zones were calculated based on linear interpolation of forces in the breaking points. The number of acts of breaking were

found by computer experiments, and they were within the 10–30 interval. Diffusion coefficients for expression (9) and its one-dimensional analog were selected in agreement with the procedure described earlier (Veresov 1992a,b). These coefficients were $1.844 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for the movement within the entrance tubes and $0.505 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for diffusion inside the tunnel.

Results and Discussion

As indicated, the average potassium conductances for the potassium channel were calculated using the value of $\text{p}K_{a6}$ ($\text{p}K_{\text{mti}} = 3.9$). This corresponds to the value of $\text{p}K$ of carboxylic groups of aspartate side chains. As already noted, there was some uncertainty as to the choice of δ_1 and δ_2 in the framework of experimentally based limits.

a) $\delta_1 < \delta_2$ b) $\delta_1 + \delta_2 < 15 \text{ \AA}$. The variation of the values δ_1 and δ_2 within these limits showed that agreement between experimental (for axons or frog nodes of Ranvier (Hille 1973, Woodhull 1973, Shriager 1974, Schauf and Davis 1976, Carbone et al. 1978, Wanke et al. 1979)) and theoretical data for dependences of the average conductances from pH_o and pH_i (titration plots in Figs. 3 and 4) can only be obtained when $\delta_1 \sim 2 \text{ \AA}$ and $\delta_2 \sim 10 \text{ \AA}$. A good fit of calculated and experimental results allows to conclude that the effect of pH on the cation transport

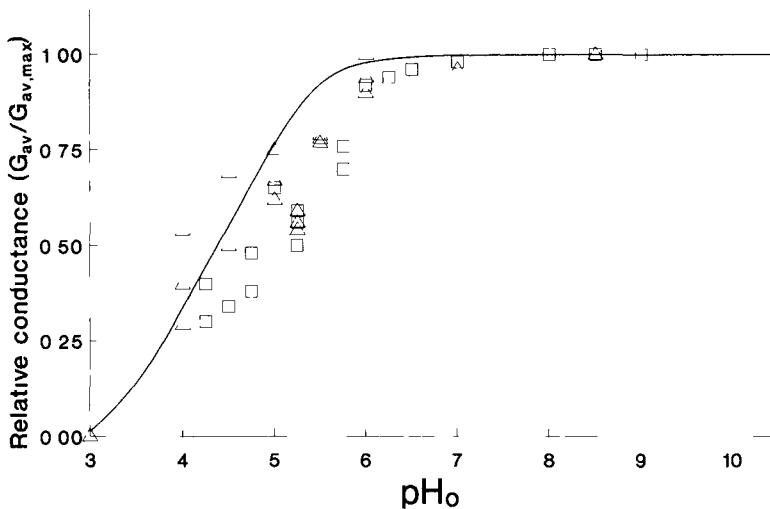


Figure 3. Relative conductance ($G_{av}/G_{av,max}$) as a function of internal solution pH (pH_i). Data points (triangles) represent titration values of the macroscopic peak G_K of the squid axon membrane from (Wanke et al. 1979). The continuous curve was obtained using BD-simulation of direct ion passage for each state in Fig. 2 and equations (2–8), $\text{pH}_o = 7$, $\text{p}K_{a\text{mti}} = 3.9$.

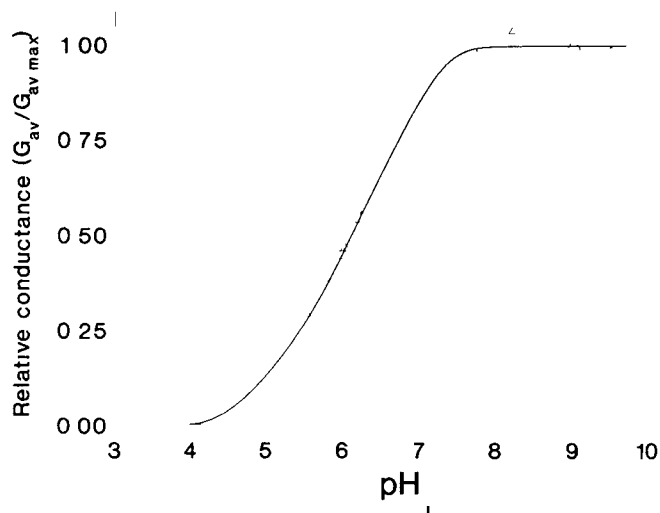


Figure 4. Relative conductance ($G_{av}/G_{av\ max}$) as a function of external pH (pH_o). Data points represent titration values of the macroscopic peak G_K of the frog nodes of Ranvier (Hille 1973) (triangles) and of *Myxicola* giant axons (Schauf and Davis 1976) (squares). The continuous curve was obtained using BD-simulation of direct ion passage for each state in Fig. 2 and equations (2.8) $pH_i=7$, $pK_{a\ intr}=3.9$

can be described by electrostatic blockage-deblockage of ionic channels (“tunnel acid group-potential theory”). According to this theory, the following sequence of events occurs when pH_o and (or) pH_i change. First, the magnitudes of negative charges generated by side chains of polar (Glu⁻ or Asp⁻) amino acid residues lining the narrowest part of the channel (tunnel), change as a result of protonation (or deprotonation). This leads to changes in the actions of the electrostatic field generated by these negative charges on the heights of rate-limiting energy barriers in the transition zones between the tunnel and the vestibules with resulting changes in channel conductivity.

The calculations of the energetics of ion-protein interactions involve several assumptions which should be kept in mind in estimating the limitations of the presented version of TAGPT. These assumptions show what future research should focus on to improve such calculations. Undoubtedly, the three-dimensional model used is a typical channelog (channel-like analogue, Eisenman and Alvarez 1991) representing a rough approximation of the real structure, and its refinement is desirable, in the view of the complexity of the system considered which includes protein forming the channel, lipids and the solvent, this is currently a substantial problem. Another aspect to be mentioned is the preservation of the “frozen” structure during the computations. It is obvious that the use of the contrary concept of

mobile atoms within the model channel former leads to a very computer-intensive level of simulation requiring more powerful computational means. Moreover, such a level of simulation is hardly justifiable for a channellog which approximates the real structure rather inexactly. It is clear that the application of more elaborated models is necessary to estimate the errors introduced by these above two assumptions.

One more point must be stressed in relation to the quantitative interpretation by-TAGPT of the titration data caused by pH_o changes. Since it can be assumed that pH_i changes with the pH_o , caution is required in using this theory, these changes have to be accounted for upon applying equation (5). Thus, experimental studies performed under controlled both pH_o and pH_i would be more suitable for their analysis on the basis of TAGPT. At the same time, there is some experimental evidence that cytoplasmic pH is relatively unaffected by changes in pH of the extracellular medium, unless such changes are brought about by CO_2 enrichment (Caldwell 1958, Spyropoulos 1960, Bisher and Ohki 1972). In the experimental studies discussed here with data shown in Figs. 3 and 4, the effects of CO_2 were not studied; consequently the use of $\text{pH}_{i,7}$ in eq. (5) can be considered an admissible approximation.

Within the scope of these insights one can propose a new (based on the formation of ion pairs) view of the mechanism of channel gating. Thus, it can be supposed that in the closed state of a channel the positively charged arginine side chains of voltage sensors S4s are moved apart from negative charges of aspartate (or glutamate) side chains of segments H5s due to the action of electric field. In this case there is unpaired positive charge within the tunnel that results in greater barriers at the tunnel entrances and nonconductivity of the channel. When the depolarizing pulse is applied, the unlike charges on segments S4s and H5s attract each other forming the ion pairs and, as indicated above, reducing the rate limiting energy barriers, thus making the channel ionconductive.

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