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A New Approach to the Selectivity of Ion Channels: Nonlocal Electrostatic Consideration¹

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As mentioned in the classical B. Hille's book [1], to calculate the change of solvation free energy for ion entering into the cell membrane, the classical Born treatment is usually used [1]. In the same book, one can find the table of empirical experimental values of standard hydration energies of ions. However, only recently Noskov and Roux [2] used these empirical data to evaluate the free energy of ion transfer into the ion channel selectivity filter. There is also a brief review of theoretical approaches to modify the Born formula in [1]; however, important, in our opinion, theoretical treatment of ion salvation by Dogonadze-Kornyshev [3] is not mentioned. The peculiarity of Dogonadze-Kornyshev approach [3] is a consideration of the spatial dispersion of water dielectric permittivity. Consequentially, the relation between the electric field induction and tension around the ion becomes nonlocal, and the Poisson equation describing the potential distribution turns into the integro-differential equation. It also shown in [3] that "the free energy of solvation is less than given by the Born formula, due to the correlation of polarization fluctuations in space." Electrostatic treatment, which accounts for spatial dispersion ε , is called nonlocal electrostatics (NE) [4]. Using the NE approach, the modified Born formula has been derived [5]. According to [5], when calculating the ion hydration energy, one should substitute into the Born formula the effective dielectric permittivity ε_{eff} , which depends on the ion radius. The $\epsilon_{\rm eff}$ value is considerably smaller than the static dielectric permittivity of water ε_{w} . The importance of using the NE approach in the study of ionic channels of biomembranes was pointed out in [6, 7]. However, the static dielectric permittivity of water $\varepsilon_{\rm w} = 80$ is often used in the Born equation in analysis of ionic channel functioning [7-10]. Moreover, neither exact values of hydration energies nor the Born formula modifications are taken into consideration. For numerical calculations related to entering of ions into the channel, the Poisson equation is used in its classical form, i.e., the ε_{w} value is taken to be 80 even in the water cavity of the K⁺-channel [9, 11].Currently, a definite interest has been drawn to using the NE for solving the problem of electrostatic interaction in proteins [12, 13]. Our work is the first attempt to apply NE in analysis of ion stabilization in the selectivity filter of channels and stabilization of cations in the water cavity of the K⁺-channel. The last problem was studied in [1, 9, 11, 14].

According to [15], the free energy of ion transfer from the volume of solvent into the pore of polymer membrane $\Delta W_{\rm SP}$ has two terms: $\Delta W_{\rm SP} = \Delta W_{\rm Bo} + W_{\rm im}$. The proof of this division of ΔW_{SP} is based on the procedure called the Güntelberg charging cycle. ΔW_{Bo} is the Born's term that is conditioned by the difference between the average ε_p in the pore and ε_{eff} for the ion in water. The energy W_{im} is the work performed against the image forces connected with the influence of the pore walls. For the analysis of selectivity, the dependence of the energy on the ion radius r is important. This dependence is contained in the $\Delta W_{\rm Bo}$ term but not in $W_{\rm im}$ [15]. Later we will consider only $\Delta W_{\rm Bo}$, as we are primarily interested in the selectivity connected with different radii of ions. For the complete analysis of selectivity, it is necessary to establish the dependence of the ion interaction energy in the selectivity filter on coordinate x along the channel [2, 11]. However, there is one more problem associated with evaluation of the change in the free energy in ion transfer from water into the selectivity filter. In solving the classical equation of Poisson–Boltzmann for the water cavity in the channel instead of its nonlocal analogue, the authors [9, 11] introduce a principal error into the evaluation of $\Delta W_{\rm SP}$. To evaluate $\Delta W_{\rm SP}$, it is necessary to make use of the average effective ε_{p} in the channel selectivity filter. The

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Fig. 1. (a) Dependences of changes in solvation energies on the dielectric permittivity in the channel ε_p , calculated for the transition of cations Na⁺ (dashed lines) and K⁺ (solid lines) from the bulk of water into the selectivity filter of the ionic channel and (b) differences between the changes in solvation energies, calculated by the classical and modified Born formulas depending on the ion radius.

evaluation of ε_p by the Frölich–Kirkwood theory of dielectrics made in [8, 11] results in $\varepsilon_p \sim 2-6$ for the selectivity filter in the channel. The average dipole moment of the channel polar group determines ε_p and, as we will show later, the channel selectivity. However, the dependence of energy on *x* determines the dynamics of the permeating ion [2]. The calculation of the change solvation energy ΔW_{Bo} for the case of transfer of an ion with radius *r* from the bulk of water with ε_w into an ionic channel with ε_p is usually made according to the classical Born formula (1) with $\varepsilon_w = 80$ as in [7, 10]:

$$\Delta W_{\rm Bo}(r) = \frac{z^2 e^2}{8\pi\varepsilon_0 r} \left(\frac{1}{\varepsilon_{\rm p}} - \frac{1}{\varepsilon_{\rm w}}\right),\tag{1}$$

where *e* is the electron charge, *z* is the ion valence, and ε_0 is the dielectric permittivity of vacuum. Making use of the NE method, Kornyshev [5] has derived the modified Born formula, which in our case (ion transfer from water into the selectivity filter of an ionic channel) can be written as:

$$\Delta W_{\rm NE}(r) = \frac{z^2 e^2}{8\pi\varepsilon_0 r} \left(\frac{1}{\varepsilon_{\rm p}} - \frac{1}{\varepsilon_{\rm eff}(r)}\right). \tag{2}$$

Here, ΔW_{NE} —the change solvation energy for ion transfer into ion channel, corresponding to NE, ε_{eff} —effective dielectric permittivity, that depends on the ion radius *r* calculated by Eq. (3) derived in [5]:

DOKLADY BIOCHEMISTRY AND BIOPHYSICS Vol. 417 2007

$$\varepsilon_{\rm eff}(r) = \frac{\varepsilon_{\rm w}}{\left\{1 + \left(\frac{\varepsilon_{\rm w}}{\varepsilon_{*}} - 1\right)\left(1 - \exp\left(-\frac{2r}{\Lambda}\right)\right)\frac{\Lambda}{2r}\right\}}.$$
 (3)

Parameter ε_* in Eq. (3) is the short-wavelength dielectric permittivity. At room temperature, ε_* for water is \approx 5, and Λ is a correlation radius for orientational Debye degrees of freedom. The essence of parameter Λ is that the orientation of the dipole moments of water molecules up to Λ distance from the ion is not independent, and, according to [4], "the value of Λ is the order of the typical length of correlation in the chain of hydrogen connections, that is ~0.3-0.7 nm." Ion radii in calculations by Eqs. (1)–(3) are taken by us as well as in [3-5]according to the scale of Gourary and Adrian [1, 3]. Values $\varepsilon_{\text{eff}}(r)$, calculated by Eq. (3) at $\Lambda = 0.7$ nm, are $\varepsilon_{\rm eff}(r_{\rm Na}) = 5.82$ and $\varepsilon_{\rm eff}(r_{\rm K}) = 6.05$; at $\Lambda = 0.3$ nm, $\varepsilon_{\rm eff}(r_{\rm Na}) = 7$ and $\varepsilon_{\rm eff}(r_{\rm K}) = 7.6$. In the small environment of an ion, its potential reaches the maximum value, but it is there that the effects of nonlocal screening take place, and as a result, ε is considerably decreased. That is why the Güntelberg charging cycle in the case of NE gives the result for $\Delta W_{\rm NE}$ that considerably differs from the classical one [3-5].

Figure 1a shows the graphs of the change of free solvation energy in the transfer of Na⁺ and K⁺ cations from the bulk of water into the ionic channel with the dielectric permittivity ε_{p} . In these graphs ΔW_{Bo} and ΔW_{NE} calculated by Eqs. (1), (2) are given in kT units, k—the Boltzmann constant, T—absolute temperature, T =



Fig. 2. (a) Difference between the changes in solvation energies for Na⁺ and K⁺ as a function of the dielectric permittivity ε_p in the channel and (b) the ratio of distribution coefficients of ions between the bulk of water and the ionic channel.

300 K. It follows from Fig. 1a that the change in the solvation energy $\Delta W_{\rm NE}$ is considerably less than $\Delta W_{\rm Bo}$ calculated with $\varepsilon_{w} \approx 79$. The difference between these energies does not depend on the dielectric permittivity of the channel and changes with ion radius. This can be seen in Fig. 1b, where the difference $[\Delta W_{Bo} - \Delta W_{NE}]$ is represented in kT units. This difference is equal 38kTfor Na⁺ and 28kT for K⁺ (for $\Lambda = 0.7$ nm). The curves $\Delta W_{\rm NE}$ are calculated with the effective dielectric permittivity ε_{eff} ; they cross the zero line within the range of dielectric permittivity values which can be accepted for the ion channel. At the same time, the Born energy $\Delta W_{\rm Bo}$ is greater than 30kT in the range $\varepsilon_{\rm p} < 6$, as it can be seen in Fig. 1a. This fact is important for understanding the process of ion penetration across the energy barrier while entering into the channel from the bulk of water. This barrier, calculated by NE formulas (Eqs. (2), (3)) is substantially smaller than it follows from the classical Born formula (Eq. (1)). The next important conclusion from Fig. la consists in the fact that the curves $\Delta W_{\rm NE}$ for the cations Na⁺ and K⁺ cross one another within the range $3 < \varepsilon_p < 6$; however, the curve $\Delta W_{\rm Bo}({\rm Na^+})$ is really uppermost than $\Delta W_{\rm Bo}({\rm K^+})$ within the entire range. This fact is essential for the mechanism of cation selectivity of the channel. Let us introduce into consideration the values $\Delta\Delta W_{\rm Bo}$ and $\Delta\Delta W_{\rm NE}$, which represent the differences between the changes in the solvation energies for cations Na⁺ and K⁺ at their crossing from the bulk of water to the selectivity filter of the channel, which is calculated by the Born Eqs. (1) and (2):

$$\Delta \Delta W_{\rm NE} = \frac{\Delta W_{\rm NE}(r_{\rm Na}) - \Delta W_{\rm NE}(r_{\rm K})}{kT}.$$
 (4)

We can introduce the same definition for $\Delta \Delta W_{Bo}$. The dependences of $\Delta\Delta W$ on the dielectric permittivity in the channel are shown in Fig. 2a. The value of $\Delta\Delta W_{\rm Bo}$ is positive for the entire range $\varepsilon_p < 79$ in the case of calculation by the classical Born formula (Eq. (1)). This means that it is not possible to explain the selectivity of Na⁺ channel within the framework of the analysis of ion solvation using Eq. (1). An opposite situation is observed in the case of calculation of $\Delta\Delta W_{\rm NE}$ using Eqs. (2)–(4). The curve $\Delta\Delta W_{\rm NE}$ crosses the zero line at $\varepsilon_{\rm p} \approx 5$. Thus, the channel exhibits the K⁺ selectivity at $\varepsilon_{p}^{*} < 5$ and the Na⁺ selectivity at $\varepsilon_{p} > 5$. The channel exhibits neither K⁺ nor Na⁺ selectivity near the point $\varepsilon_n \approx 5$. The distribution coefficient *n* is an important characteristic for the analysis of the distribution of ion between the bulk of water and the ion channel:

$$n(r) = \exp \frac{\left[-(\Delta W_{\rm NE}(r) + W_{\rm im})\right]}{kT}.$$
 (5)

Let us consider the value $(n_{\rm K}/n_{\rm Na})$ as a measure of K⁺/Na⁺ selectivity of the ion channel. We can write for this ratio the next formula: $(n_{\rm K}/n_{\rm Na}) = \exp[\Delta\Delta W_{\rm NE}]$, if we combine Eqs. (4) and (5). This value is shown in Fig. 2b for the case of the calculation of $\Delta W_{\rm NE}$ using Eqs. (2) and (3). Our analysis confirms the qualitative considerations of Hille (page 332 in [1]): "There we believe that dipolar groups, forming part of the pore

wall, substitute for the H₂O dipole in providing electrostatic stabilization of the permeating ion." Indeed, the modified Born formula allows us to explain the existence of K⁺ or Na⁺ selectivity of the channel by the fact that average ε_p in the channel is either smaller or greater than short-wavelength ε_* for water, as it follows from Fig. 2b.

Applying the Güntelberg charging cycle within the framework of NE [4] to the calculation of the free energy W_{CAV} of monovalent cations located in the center of a spherical water cavity of R_{CAV} radius inside K⁺-channel, we obtain

$$W_{\rm c} = \frac{e^2}{8\pi\varepsilon_0 R_{\rm c}} \left(\frac{1}{\varepsilon_{\rm m}} - \frac{1}{\varepsilon_{\ast}}\right). \tag{6}$$

To obtain Eq. (6), we used the fact that, according to [1, 9, 11], R_{CAV} is equal to approximately 0.5 nm, that is the order of the value Λ in water. A similar formula used in [9] was obtained within the framework of classical electrostatics. However, in this formula, $\varepsilon_w = 80$ was used instead of the short-wavelength value $\varepsilon_* = 5$. The dielectric permittivity of biomembrane ε_m in Eq. (6) is considerably smaller than ε_w . It is evident that the absolute value W_{CAV} , calculated using Eq. (6), is considerably smaller than that calculated using the classical formula. This result is important for the explanation of the mechanism of stabilization of K⁺ cations in the water cavity of the K⁺-channel.

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