Incidence of partial charges on ion selectivity in potassium channels

Philippe Huetz,[a] Céline Boiteux, Mylène Compoint, Christophe Ramseyer, and Claude Girardet
Laboratoire de Physique Moléculaire, UMR CNRS 6624, Faculté des Sciences et Techniques, La Bouloie, Université de Franche-Comté, 25030 Besançon cedex, France

(Received 7 October 2005; accepted 29 November 2005; published online 23 January 2006)

Potassium channels are membrane proteins known to select potassium over sodium ions at a high diffusion rate. We conducted *ab initio* calculations on a filter model of KcsA of about 300 atoms at the Hartree-Fock level of theory. Partial charges were derived from the quantum mechanically determined electrostatic potential either with Merz-Kollman or Hinsen-Roux schemes. Large polarization and/or charge transfer occur on potassium ions located in the filter, while the charges on sodium ions remain closer to unity. As a result, a weaker binding is obtained for K^+ ions. Using a simplified version of a permeation model based on the concerted-motion mechanism for ion translocation within the single-file ion channel [P. H. Nelson, J. Chem. Phys. **117**, 11396 (2002)], we discuss how differences in polarization effects in the adducts with K^+ and Na^+ can play a role as for ionic selectivity and conductance. © *2006 American Institute of Physics*.
[DOI: 10.1063/1.2159483]

I. INTRODUCTION

Potassium channels are ubiquitous membrane proteins found in bacterial as well as in eukaryotic cells. Potassium conduction underlies many different cellular processes such as cell volume regulation, hormone secretion, and electrical impulse formation.[1,2] These channels contain a highly conserved amino acid sequence called the K^+ channel signature (*TVGYG*), forming a structural element known as the selectivity filter, where the backbone carbonyl groups are aligned.[1] Nearly perfect selectivity for K^+ ions over Na^+ ions and high K^+ conduction rates (approaching the diffusion limit) are characteristic of all K^+ channels. For KcsA, the voltage-gated channel from *Streptomyces lividans*,[3] the flow is of ca. 10^7 K^+ ions per second per channel, and only 1 Na^+ ion over at least 10^4 K^+ is transported.[4] In addition to the selectivity filter located at the outermost part of the cell membrane, this tetrameric protein is constituted by a water-filled cavity and the intracellular gate region,[5–7] the correlated behavior of all the parts being crucial for ion conduction.[8] In a very recent review by Roux,[9] the most important experimental and computational findings about ion conduction and selectivity are discussed. In our present paper, we will examine effects more specifically located at the filter region, where K^+ ions alternate with single water molecules.[10]

Merging of both high conduction efficiency and high selectivity could constitute an apparent paradox, which implies for the first property weak binding and damping with the interacting protein groups, while specific interaction energies should prevail for the discrimination of the species. This is quite surprising since it concerns ions of comparable sizes (ionic radii equal to 1.33 Å for K^+ and 0.95 Å for Na^+), with the same classical unit charge attributed to them. Different hypotheses have been proposed to explain the origin of the ionic selectivity of KcsA on the basis of investigations realized on recently determined atomic structures: (i) This could be due to a precise conformational adaptive surrounding of the selectivity filter snugly wrapping K^+ ions in it;[4] however, this condition implies a sub-Ångström arrangement to discriminate two species of such close sizes. (ii) The intrinsic dynamic behavior of the carbonyl groups lining up into the filter could play a determinant role through adjustment of local dipoles.[11] (iii) K^+ ion permeation at the mouth level of the channel is facilitated, for dehydration of K^+ ions is better compensated by reassociation with the protein carbonyls, compared to Na^+ (oxygen coordination numbers are 8 and 6, respectively).[12,13] The dehydration free-energy barrier at the filter entrance is an active current field of research.[14–16]

Based on quantum calculations, we propose in this paper that a different loss of charge between K^+ and Na^+ across the channel could significantly contribute to their specific conduction and/or selectivity in KcsA. Large polarization effects on the K^+ and the oxygen ligands were reported using either density-functional theory (DFT) or *ab initio* methods. These polarization effects were reflected in the values of the partial charges associated with each atom.[13,17,18] These effects were found to be stronger on the carbonyl groups than on the water molecules present in the filter. We here try to precise these results by comparing with Na^+.

In Sec. II we present the methods to extract the electrostatic potential (ESP) derived from *ab initio* approaches that we will compare to AMBER ESP. We limited the study to the filter part of the protein to ensure reasonable computational times, but we are aware that long-range interactions could play a role. Section III is devoted to the comparison of binding energies for K^+ and Na^+, and possible implications for

[a]Author to whom correspondence should be addressed. Fax: +33(0)381666475. Electronic mail: philippe.huetz@univ-fcomte.fr

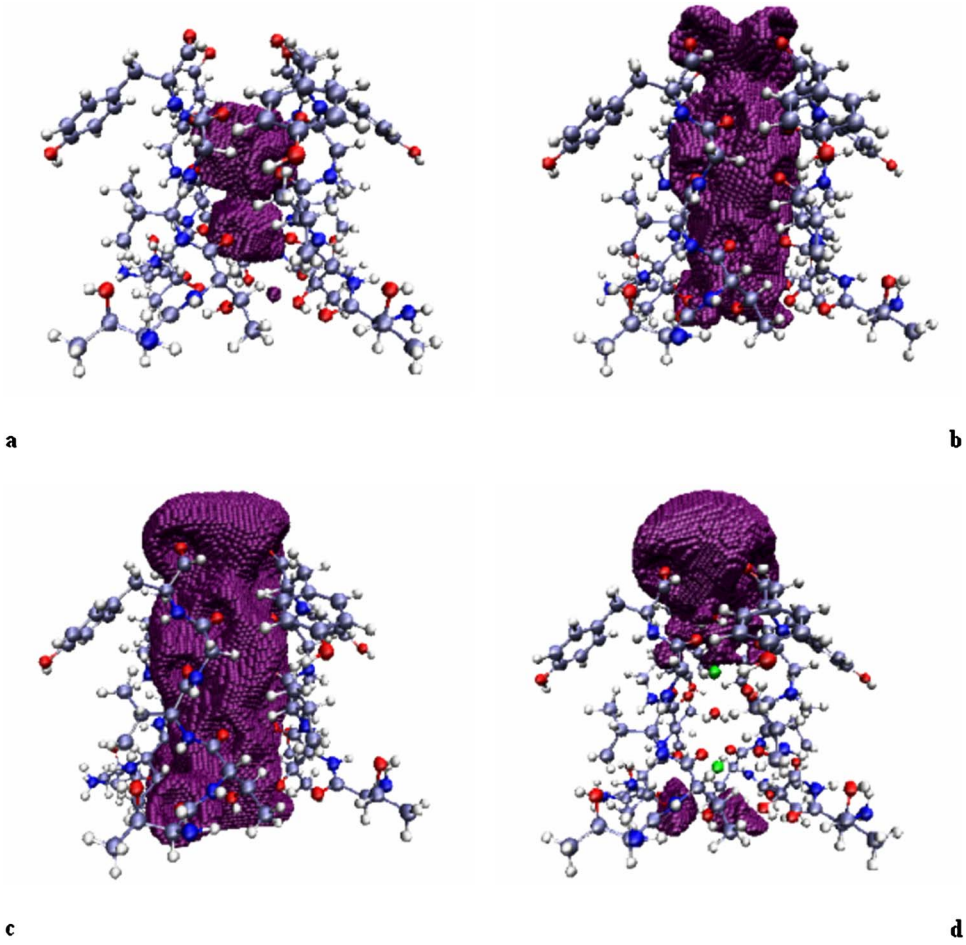

FIG. 1. (Color online) Molecular model of the KcsA selectivity filter (Thr74 to Tyr78 truncated structure), from a configuration averaged over 600 ps MD. In the central vertical axis are present two water molecules and two K^+ ions (d), located respectively in the S_1, S_3 and S_2, S_4 sites (see text). K^+ ions are shown in green, oxygens in red, nitrogens in blue, carbons in dark gray, and hydrogens in light gray. Isopotential-energy surfaces for a unit probe charge exploring the filter are shown in purple. They correspond to energy values equal to -120 kcal mol^{-1} (a), -80 kcal mol^{-1} (b), and -50 kcal mol^{-1} (c). Only the last surface (d) calculated for an energy equal to 20 kcal mol^{-1} takes into account the presence of the whole sequence *WKWK*. For the three others the filter is empty.

conduction and/or selectivity are discussed in Sec. IV, within an available ion permeation model.

II. METHODS

KcsA membrane protein was modeled on the basis of the 2.0 Å resolution closed 1K4C structure.[7] It was equilibrated over several nanoseconds by molecular dynamics (MD) simulations[8] using AMBER6 empirical force fields.[19] The root-mean-square deviations on the filter atoms remained very small (less than 0.5 Å) and, in addition, we showed that the filter structure in the open state remains almost identical to the closed state one.[20] The structure we used in the present study for *ab initio* calculations is displayed in Fig. 1. It corresponds to a truncation in the selectivity filter region of the KcsA structure issued from an average of the positions over 600 ps (equilibrium conditions reached) of the MD production run. For details about the setting up of the MD model we refer the reader to Ref. 8.

To test the accuracy of the ESP usually evaluated in current biomolecular simulations of ionic channels using empirical force fields, we have determined the electrostatic potential, as well as Merz-Kollman (MK) charges, using GAUSSIAN 03 (Refs. 21 and 22) at the Hartree-Fock 6-31G(*d*) level of theory. No further energy minimization of the filter structure was performed in order to be strictly consistent with the MD average snapshot picture. As this level of theory slightly exaggerates the dipole moment, the self-consistent reaction field was switched off.

A number of different methods have been suggested for the evaluation of relevant atomic charges needed to accurately describe electrostatic interactions. Examples of these methods are Mulliken analysis,[23] Löwdin population[24] and natural bond orbital (NBO) population[25] analyses, atoms in molecules (AIM),[26] and ESP-derived charges. In the methods which derive charges from the ESP (or the electrostatic field), a large number of points around the molecule of interest are selected. Atomic charges are calculated so that their resulting ESP fits the quantum-determined one,[27] with the constraint that the total charge of the molecule should be respected. These methods differ mainly in the choice of the points where the ESP is adjusted. The CHELP method[28] selects points symmetrically on spherical shells around each atom (14 points per shell), while in the MK scheme,[29,30] points are selected on embedded Connolly surfaces, with a density of 1 point/Å. A higher density of points selected on 0.3 Å regularly spaced cubic grids is used in the CHELPG method.[27] A random sampling[31] can be also alternatively used in order to avoid the choice of coordinate axes. All these methods include neither points within the van der Waals (VDW) radii of atoms nor points too far from the molecule. Exclusion limits as well as VDW radii themselves vary appreciably among the methods. In the MK procedure, points are sampled at distances of 1.4, 1.6, 1.8, and 2.0 times VDW radii.

The charges derived from the potential generally reproduce the first multipole moments well and optimally also

TABLE I. Lennard-Jones potentials whose parameters were taken from Refs. 36 and 37.

	Lennard-Jones parameters	
	$C_6^{1/2}$ [(kcal mol^{-1} Å^6)$^{1/2}$]	$C_{12}^{1/2}$ [(kcal mol^{-1} Å^{12})$^{1/2}$]
H	0	0
O	23.25	421.0
C	23.65	898.0
N	24.13	636.0
K^+	4.35	522.7
Na^+	4.15	70.87

intermolecular interactions. A number of studies have shown the superiority of such charges compared to those derived from other methods[29,32] and, among them, it is generally assumed that the MK method gives the best values according to electrostatic criteria.[27] They are conformation dependent but do not depend on the choice of the basis set.[27,30,33] 6-31G* is the minimum-quality basis set level, so that *ab initio*-derived charges do not fluctuate significantly when the ESP is close to convergence. However, in the ESP-derived charges, it has been reported that the least-squares fit does not always behave properly due to ill-conditioned matrices. Hinsen and Roux[31] have therefore suggested an improved least-squares procedure which uses pseudoinverse matrices calculated by singular value decomposition (SVD). This method has been successfully applied to the study of proton transfer in acetylacetone[31] and in an attempt to improve ESP charges for sugars.[34] In the following we calculated the charges using the MK procedure and checked with the Hinsen and Roux (HR) method [MMTK.ChargeFit module (Ref. 35)] for possible artefacts that would be eventually revealed. When determining the minimum of ESP energy felt by a $\delta+$ charged ion forced through the filter (Z direction), Lennard-Jones (LJ) parameters were included (Table I).[36,37] We are aware that these LJ parameters are themselves charge dependent, since they represent instantaneous electronic cloud fluctuations. Nevertheless, to our knowledge, the nature of such a dependence has not been precised yet.

III. RESULTS

Together with the filter model structure, we have plotted in Figs. 1(a), 1(b), and 1(c) typical isopotential-energy surfaces issued from quantum calculations, corresponding to energies equal to −120, −80, and −50 kcal mol^{-1}, respectively. These energies reflect directly the potentials for a unit probe charge (here +1, as in standard biomolecular packages, e.g., AMBER) which explores the empty filter. For the potential map at −120 kcal mol^{-1} [Fig. 1(a)] two regions occur which will be assigned to sites S_2 and S_3 in the following. At higher values of the energy [Fig. 1(b)], the whole internal part of the filter is shown up by the probe charge, with four sites $S_1, \ldots, S_4$ being revealed but not fully resolved due to strong overlap zones which do not allow to discriminate these sites. At still higher energy [Fig. 1(c)], a larger attractive zone for the cation appears at the outermembrane entrance, with a similar region, though not as pronounced, at the opposite site (i.e., close to, but not inside, the cavity). This could partially explain some recent experimental and numerical observations. Indeed, in the highest resolution X-ray structure of KcsA,[7] seven ion binding sites were identified within or close to the selectivity filter. In addition to the four positions determined inside the filter (S_1, S_2, S_3, and S_4), one was found in the cavity (S_{cav}), one at the extracellular side of the membrane (S_{ext}), and the last one between S_{ext} and S_1 at the outer mouth of the channel (S_0). The large protuberance observed here certainly designs S_0, while the S_{ext} site cannot be seen in this figure due to the truncation of the protein structure in this quantum approach. Note that the S_{cav} site, which is intrinsically due to the water-filled cavity, does not occur for the same reason.

The filter part of the protein, by itself, is thus responsible for the occurrence of five sites, including S_0. It is now well established[8,14,38] that S_1 to S_4 sites are occupied according to an alternate sequence of *W* and K, with water molecules (*W*) screening the repulsive interaction between the cations (K). Åqvist and Luzhkov especially[38] provided the first demonstration that the single-file configurations of K and *W* in the four sites along the narrow pore (K*W*K*W* and *W*K*W*K) were energetically possible, what was further confirmed by Zhou and MacKinnon.[39] We can therefore draw the potential surface experienced by the same probe charge when the filter is now occupied by a sequence of *W*K*W*K. Due to large repulsive interactions (both electrostatic for the K^+ ions of the sequence and steric for the K^+ and *W*), the probe charge can only explore very limited regions of the filter. This is shown in Fig. 1(d), where the potential surface with energy value of 20 kcal mol^{-1} is drawn. The outermost protuberance already mentioned for the energy map corresponding to the empty filter [Fig. 1(c)] is recovered at higher energy. This indicates that the filter mouth could still be occupied by a K^+ ion, corroborating the existence of the S_0 site, even though the inner part of the filter is filled. This latter feature was first characterized by the computations of Bernèche and Roux:[14] three K^+ ions can be present, their movements being strongly coupled.

Figure 2 represents the compared ESP energy shapes viewed by a K^+ ion (elementary unit charge and characteristic Lennard-Jones parameters included) along the channel axis Z in the filter of KcsA when the ions and water molecules have been removed, all positions elsewhere equal. Minimization of the K^+ position along Z with respect to X and Y directions was performed. The *ab initio* curve allows us to assign the sites discussed in Fig. 1 for a K^+ ion exploring the filter. The other curves are drawn from the partial charges of the filter issued from MK and HR calculations or from those given by the AMBER force field. Since this fit is done on numerous points of space extending around this restricted area of the protein, our comparison is a first test of the quality of the results. All the calculations exhibit the same overall features, i.e., the filter behaves as a strong trap for the K^+ ion, with a deep structured well (about −130 kcal mol^{-1}). Four minima can be observed at the bottom of the well, two of which correspond to well-defined secondary wells while the other two rather appear as shoulders with energy values around −110 and −90 kcal mol^{-1}. While MK and AMBER minimized potential energies are in

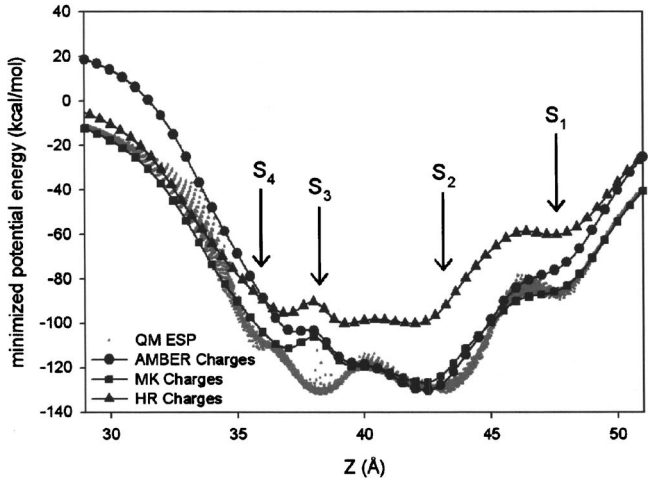

FIG. 2. Potential energy (kcal mol^{-1}) experienced by a K^+ ion of unit charge moving adiabatically in the empty filter. The position of the K^+ is along the *Z* axis and minimized with respect to the directions *X* and *Y*. Gray points are obtained from the *ab initio* quantum mechanically determined ESP, while curves with black squares and triangles are calculated with Merz-Kollman (MK) and Hinsen and Roux (HR) methods, respectively. The curve with circles is determined on the basis of an AMBER empirical force-field calculation.

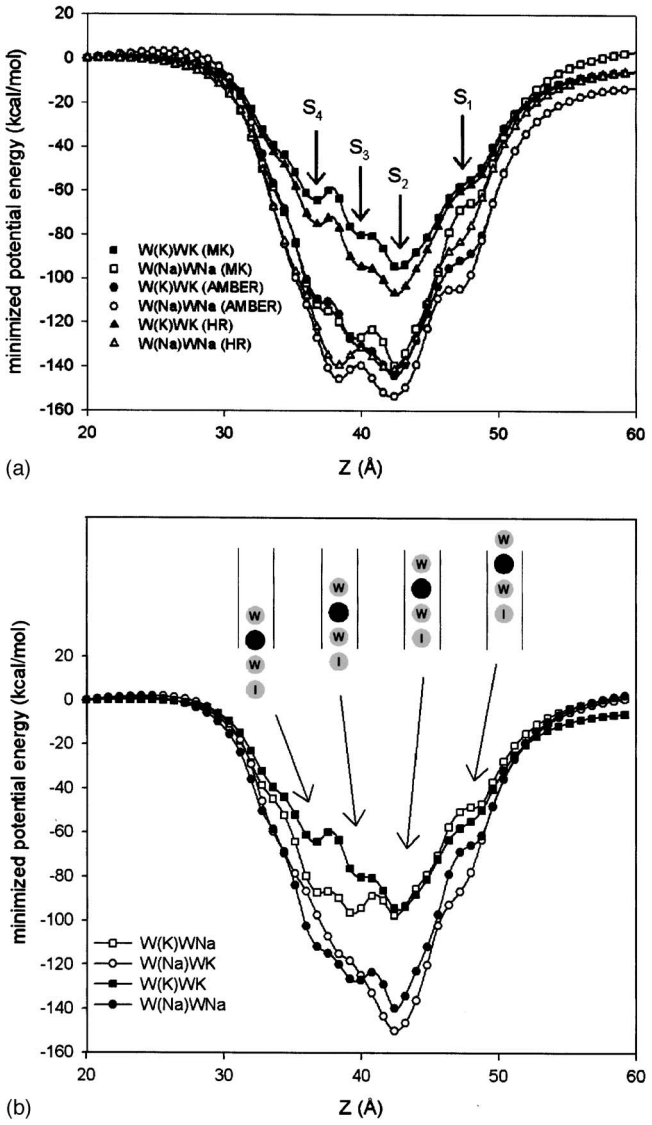

FIG. 3. (a) Potential energy (kcal mol^{-1}) experienced by $K^{\delta+}$ or $Na^{\delta+}$ ions at position 2 in *WKWK* or *W*Na*W*Na rigid sequences (see text) for MK and HR methods, with the average values of ion and water oxygen charges given in Table II. AMBER corresponding curves are shown as references. The four sites corresponding to the *WKWK* ESP curves are indicated. (b) Comparison of this potential for the four sequences *WKWK*, *W*Na*WK*, *WKW*Na, and *W*Na*W*Na within the MK model. In the upper part we schematized the path of the ion (black circle) we chose to experience the potential along the filter. This ion can be K or Na, and I refers to the other K or Na ion according to the sequence.

good agreement with the quantum mechanically (QM) determined ESP results, HR method tends to underestimate the charges and thus decreases the strength of intermolecular interactions. It should be noted that HR method is very sensitive to the choice of the minimum exclusion radii. Here, we have chosen values identical to those used in the MK method, i.e., VDW radii [1.20 Å (H), 1.50 Å (C and N), and 1.40 Å (O)] and for the ions 1.5 times ionic radii [equal to 1.33 Å (K^+) and 0.95 Å (Na^+)]. However, the shape obtained within the HR scheme is consistent with the other methods, confirming that nonsense charges have not been assigned to certain (or buried) atoms of interest.

The same MK and HR partial charge calculations we performed on the empty filter have been applied to the filter filled with the *WKWK* sequence, the *W* and K species occupying the odd- and even-numbered sites, respectively (S_1-S_4 sites, see Figs. 2 and 3). This average configuration issued from our MD simulations[8] is consistent with the experimental data.[7] Moreover, we have considered three other sequences *W*Na*WK*, *WKW*Na, and *W*Na*W*Na by replacing one or two K^+ ions by Na^+ at exactly the same positions to allow for direct comparison and get an idea of what would be the charges in the presence of one or two sodium ions in the same configuration.

In Table II, some charges of interest, namely, those located on the ions and on the oxygens of the two water molecules, are reported. We see that a strong charge transfer especially occurs at the level of the potassium ion. Its charge is not +1, which is the fixed value usually taken in MD simulations, but can get as low as +0.68 in the S_4 site and slightly higher in the S_2 one. By contrast, the sodium ion undergoes smaller deviations with respect to the elementary charge, in the MK as well as in the HR approaches. This is consistent with the fact that sodium is less polarizable than potassium. Concerning the two water molecules, the MK and HR charges obtained with potassium are rather close to the values used in the TIP3P or SPC models of charge commonly used in molecular dynamics force fields [−0.834 (Ref. 36) or −0.820 (Ref. 40) for the oxygen atom, respectively]. In the $Na^{\delta+}$-filled situation, the $O(W_3)$ charge might be slightly overestimated. It may be mentioned that these methods are very sensitive to point-charge sampling. HR method seems here to attenuate this problem. In the mixed sequences, where a single K ion is substituted by a Na ion at either the S_2 or the S_4 site, we obtain very similar charge values for the ions at corresponding positions. Note that less important changes on the oxygen charges of water molecules occur in these mixed situations.

Since the peculiar charge behavior of the cations should

TABLE II. MK, HR, and partial charges in units of electron charge (e^-) for O(*W*) and K or for O(*W*) and Na located in the corresponding 1 to 4 sites of the KcsA selectivity filter for the four sequences *WKWK* (upper part), *W*Na*W*Na (lower part), and mixed *W*Na*W*K and *WKW*Na (middle part). Water molecules and cations are indexed by the site number where they are located. In the mixed situations, values in parentheses are averages between charges at positions S_2 of one mixed situation and S_4 of the other for each ion.

	Water			Potassium		
WKWK	$O(W_1)$	$O(W_3)$	O_{mean}	K_2	K_4	K_{mean}
AMBER	−0.834	−0.834	−0.834	1	1	1
MK	−0.767	−0.816	−0.791	0.755	0.687	0.721
HR	−0.840	−0.953	−0.896	0.790	0.676	0.733
	Water			Ions		
*W*Na*WK*	$O(W_1)$	$O(W_3)$	O_{mean}	Na_2 (Na_{mean})		K_4 (K_{mean})
AMBER	−0.834	−0.834	−0.834	1		1
MK	−0.685	−1.054	−0.869	0.939 (0.921)		0.687 (0.723)
HR	−0.790	−0.928	−0.859	0.846 (0.829)		0.688 (0.739)
	Water			Ions		
*WKW*Na	$O(W_1)$	$O(W_3)$	O_{mean}	K_2 (K_{mean})		Na_4 (Na_{mean})
AMBER	−0.834	−0.834	−0.834	1		1
MK	−0.776	−0.983	−0.879	0.758 (0.723)		0.903 (0.921)
HR	−0.816	−0.991	−0.903	0.791 (0.739)		0.813 (0.829)
	Water			Sodium		
*W*Na*W*Na	$O(W_1)$	$O(W_3)$	O_{mean}	Na_2	Na_4	Na_{mean}
AMBER	−0.834	−0.834	−0.834	1	1	1
MK	−0.681	−1.225	−0.953	0.955	0.890	0.923
HR	−0.786	−0.969	−0.877	0.850	0.801	0.826

have an incidence on their binding energies in the filter, we have recalculated these energies for $K^{\delta+}$ and $Na^{\delta+}$, with δ^+ being the average over S_2 and S_4 MK or HR charges (Table II), using the respective LJ parameters. For all the curves in Fig. 3, the Na or K ion in position 2 explores the empty filter (charges corresponding to each sequence), being accompanied in its motion by the rest of the sequence, the internal configuration of the whole sequence being kept rigid. This situation is different from the one shown in Fig. 2, where a single K^+ ion was adiabatically moved through the empty filter. Figure 3(a) displays the potential energy curves experienced by a potassium or sodium ion in the sequences *WKWK* and *W*Na*W*Na for both methods. The ions bear either their elementary unit charge (AMBER), curves plotted as a reference, or their partial mean charge (MK or HR). In Fig. 3(b) we compare these energy curves calculated in the MK scheme for the four sequences *WKWK*, *W*Na*WK*, *WKW*Na, and *W*Na*W*Na. Several features can be commented in Fig. 3.

Firstly, for all four sequences, the overall scheme of the site locations is not fundamentally changed, as expected with just a difference in the charge values. By contrast and with ESP charges, large energy shifts are obtained when compared to curves drawn with AMBER charges [Fig. 3(a)]. The depth of the filter trapping well is reduced by about 62 (resp. 50) kcal mol^{-1} for the *WKWK* sequence in the MK (resp. HR) approach. The curves obtained for the *W*Na*W*Na sequence display nearly the same behavior with however a less pronounced energy decrease with MK and HR. Replacing K by Na ion (and vice versa) in position 4 of the sequence does not strongly modify the depth of the minimum well, as shown in Fig. 3(b). Nevertheless, a marked difference can be noticed when exchanging K and Na with K in position 2 (sites S_3 and S_4).

Secondly, different binding energies are obtained in the MK and HR approaches, while the AMBER force field does not allow clear energy discrimination between the two species behavior. S_2 appears to be the most stable site, in agreement with experimental data[6] and other computational results.[41] For $K^{\delta+}$ in *WKWK*, the energy shift found when comparing MK and HR results is mainly due to the variation of the mean charges on the oxygen atom of water, since the cation mean charge is the same in the two approaches, as well as to the different overall MK and HR charge distributions of the protein environment. For $Na^{\delta+}$ in *W*Na*W*Na, this shift is due to a superimposition of both the water oxygen and cation mean charge changes. The minimum well (ESP charges) is obtained [Fig. 3(a)] for a value of −135 kcal mol^{-1} for $Na^{\delta+}$ in the MK approach (−142 kcal mol^{-1} in the HR one) and of −95 kcal mol^{-1} for $K^{\delta+}$ in MK (−105 kcal mol^{-1} in HR). The energy difference between $K^{\delta+}$ and $Na^{\delta+}$ deepest wells is thus around 40 kcal mol^{-1} in both approaches, while it is in average only 10 kcal mol^{-1} when the unit ionic charges are considered. Changing the ion in position 4 of the sequence increases this difference by about 5 kcal mol^{-1} [Fig. 3(b)].

Thirdly, while the location of the S_1 and S_2 sites is nearly the same for Na and K, the position of the sites S_3 and S_4 is

significantly shifted by about 1.5 Å for Na with respect to K (*W*K*W*K versus *W*Na*W*Na). Such a feature does not depend on the value of the ionic charge but is rather associated with the size of the two ions in the K-adapted channel. Indeed, while for Na^+ and $Na^{\delta+}$ there is a well at ~38 Å, a bump in the curves occurs for potassium ion. The reverse situation is observed at ~41 Å with a bump for Na. This might be due to the fact that the Na^+ radius being slightly smaller than the K^+ one, it does not fit well in the coordination cage provided by the filter and it must adapt its location when compared to K^+.

Fourthly, examination of Fig. 3(b) curves shows that the energy required by the Na ion at position 2 in the sequence to escape from the filter is always larger than for the K ion at the same position, whatever the second ion is in the sequence. The energy difference is around 44 kcal mol^{-1}.

Fifthly, the passing through of the rigid sequence inside the filter presents barrier heights of at least 10 kcal mol^{-1} for $K^{\delta+}$ and $Na^{\delta+}$. For $K^{\delta+}$ this value is clearly too high when compared to data in the literature. In fact, it is artificially enhanced by the fact that we only discuss the shape of the potential energy and disregard the entropic contribution.[42]

The main conclusion emanating from these results is the weakest binding within the filter obtained for $K^{\delta+}$ with respect to $Na^{\delta+}$, which leads to the question: how may lower binding energy observed for $K^{\delta+}$ affect the property of selectivity in the filter?

IV. DISCUSSION

The fundamental atomic insight for ion conduction in the KcsA channel is the concept of single-file *W*K*W*K moving through the filter in a highly correlated fashion. The transport cycle process has been extensively used in kinetic theories to describe permeation in ion channels.[43–46] Based on the assumption that translocation throughout the filter is not rate limiting, Nelson[43] has built a sorption-limited permeation model from an occupancy-states explanation. In this model, except the influence of the transmembrane potential acting on the cation charges, no dynamical concept is considered and, in particular, the interactions between the cations, the water molecules, and between the two species are disregarded. The only reference to potential is via the global well or the sites created by the filter locating the single-file species positions.

The permeation rate J is then determined as a function of the concentration $[S]$ of cations, assumed to be the same at both sides of the filter, of the association rate $\nu_a\,[S]$ corresponding to the inclusion of a cation in the file at one side of the filter, and of the dissociation rate $k_d \exp(-\phi)$ at the other side, corresponding to the escape of a cation from the file at the other side of the filter. Association for a cation is the transfer from bulk or confined water medium to the filter (organic medium), while dissociation is the reverse process. $\phi = \delta^+ LV/kT$ defines the electrical work over the filter length L for the cation with charge δ^+, reduced by the thermal energy.[43] V is the transmembrane potential. The selectivity ratio $P_{\mathrm{Na/K}}$, defined as the ratio of the fluxes $J_{\mathrm{Na}}/J_{\mathrm{K}}$, can be expressed as

$$P_{\mathrm{Na/K}}(V) = e^{(\delta^+_{\mathrm{Na}}-\delta^+_{\mathrm{K}})(LV/kT)} \frac{\mathrm{th}(\delta^+_{\mathrm{Na}}(LV/2kT))}{\mathrm{th}(\delta^+_{\mathrm{K}}(LV/2kT))} \times \frac{1+\alpha_{\mathrm{Na}}\mathrm{ch}(\delta^+_{\mathrm{Na}}(LV/kT))}{1+\alpha_{\mathrm{K}}\mathrm{ch}(\delta^+_{K}(LV/kT))} \frac{\nu_d(\mathrm{Na})}{\nu_d(\mathrm{K})} e^{-\Delta\Delta E/kT}, \quad (1)$$

where $\Delta\Delta E$ is the energy difference for the dissociation of Na and K ions and $\alpha = k_d/(\nu_a[S])$ defines the ratio between dissociation and association rates for each cation. An Arrhenius law $k_d = \nu_d \exp(-\Delta E/kT)$ is used to describe the escape from the filter. ΔE represents the energy required for this escape and for the concomitant rehydration. At equilibrium ($V=0$), Eq. (1) reduces to

$$P_{\mathrm{Na/K}}(0) = \frac{\nu_d(\mathrm{Na})}{\nu_d(\mathrm{K})} e^{-\Delta\Delta E/kT} \frac{1+2\alpha_{\mathrm{K}}}{1+2\alpha_{\mathrm{Na}}}. \quad (2)$$

While the ratio $\nu_d(\mathrm{Na})/\nu_d(\mathrm{K})$ can be assimilated to the inverse square-root ratio of the cation masses, the α_{K} and α_{Na} parameters are more difficult to estimate. If we take the same value for these latter parameters,[43] the selectivity ratio becomes directly proportional to $\exp(-\Delta\Delta E/kT)$. In fact, α_{K} and α_{Na} values are directly related to the association/dissociation processes in the filter, which should be *a priori* different for the two ions, but information on this feature is missing. Nevertheless, an error on this ratio would be less dramatic than one that would be directly manifested in the exponential term [Eq. (2)].

The energy difference $\Delta\Delta E$ at the filter level can be viewed as the free energy difference when a Na or a K ion leaves the binding site it occupies at one end of the filter to enter either the cavity or the extracellular medium (depending on the sense of the ion current) and becomes hydrated. The dissociation from a binding site in the filter followed by the entry in one or the other hydrated medium is not at all a symmetric process due to the asymmetry of S_1 and S_4 sites and to the water density differences between bulk (extracellular) and confined (cavity) media. Both neglecting the influence of the entropic term difference in $\Delta\Delta E$ when comparing Na to K and assuming similar hydration/dehydration process for the two cations (which is a rough approximation), the value of the selectivity ratio $P_{\mathrm{Na/K}}$ corresponding to a potential barrier of about 44 kcal mol^{-1} (Fig. 3) is totally unrealistic (10^{-32}). Indeed, within the present permeation model, an energy $\Delta\Delta E < 10$ kcal mol^{-1} would be required to be consistent with experimental findings.[4] The different behaviors of the two Na and K cations with regard to hydration/dehydration seem thus to be fundamental to explain the filter selectivity. According to literature data,[47] calculated or observed bulk hydration free energy difference between Na and K ions is ~18 kcal mol^{-1}. Kebarle[48] mentions also that the entropic contribution for hydration looks to be of the same magnitude for Na and K. Thus, if one subtracts the hydration contribution from the calculated $\Delta\Delta E \approx 44$ kcal mol^{-1}, the effective energy difference would be reduced to 26 kcal mol^{-1}, a value which is still overestimated by a factor of about 2 when compared to the expected one. Of course, this is purely indicative in the sense that this model is based on a static analysis of the system through information obtained from the potential wells. Note that we should con-

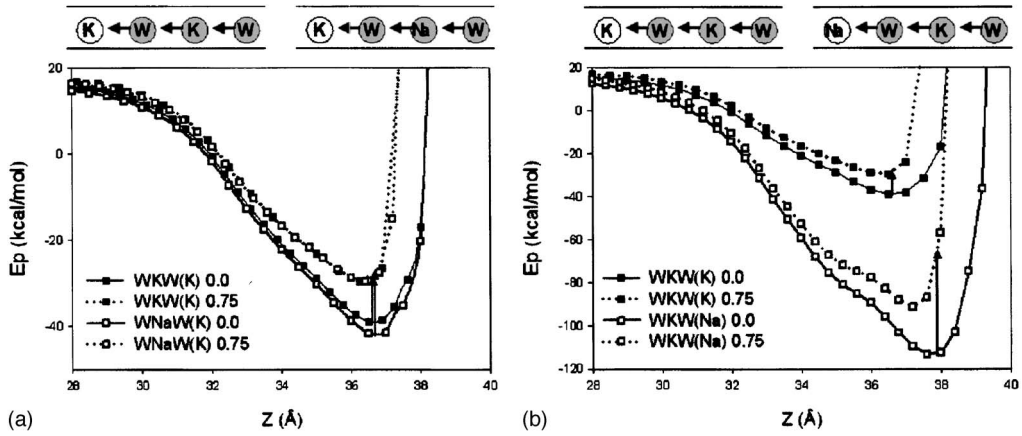

FIG. 4. Influence on the potential, observed in site S_4, of a decrease of the distance between the ion located in this site (empty circle in the upper part of the figure) and the rest of the sequence, simulating the knock-on mechanism in the filter, within the MK approach. (a) The ion in the S_4 site is a potassium, with either K or Na in position 2. (b) The ion in the S_4 site is either a sodium or a potassium, with K in position 2. Initial sequences: full curves; sequences contracted by 0.75 Å: dotted curves. The arrows describe the energies gained by the ion in the S_4 site when the sequence is contracted within the sudden approximation approach.

sider that hydration takes place in confined water (cavity) instead of bulk water, but no information on the hydration energy difference between K^+ and Na^+ in such a confined medium is presently available.

Another way to describe ionic conductivity and to introduce selectivity in potassium membrane pores is more dynamical and involves the knock-on mechanism[49,50] (cf. snapshot given in Fig. 5 of Ref. 8) when repulsion interactions take place between the cations. To simulate the possibility for the four sequences *W*K*W*K, *W*Na*W*K, *W*K*W*Na, and *W*Na*W*Na to be nonrigid in their translocation motion through the filter, we have varied the distance between the water molecule and the cation in positions 3 and 4 of the sequence, the elements of positions 1 to 3 following in a rigid way. We decreased this distance for the ion closest to the cavity up to 1 Å by steps of 0.25 Å. In Fig. 4, we plot the behavior of the potential seen by the ion in site S_4 when placed in a knock-on condition corresponding to the distance contraction of 0.75 Å that we found in Ref. 8. In Fig. 4(a), we see that the influence of the ion in position 2 of the sequence on the escape of the K_4 ion is small, since the energy difference is about 3 kcal mol^{-1}. By contrast, in Fig. 4(b), significant changes in the potential energy difference between Na_4 and K_4 escapes from the filter can be observed, since it decreases from 75 kcal mol^{-1} in the equilibrium situation (no distance contraction) to 39 kcal mol^{-1} in the knock-on situation (distance contraction of 0.75 Å considered). These changes are estimated within a Franck-Condon-type approach, i.e., assuming that the energy excess due to the distance contraction is completely used by each cation to reach the final state (escape from the filter). Note that this difference is very close to the energy $\Delta\Delta E$ determined in the static approach, and correcting it by the hydration contribution would still lead to an effective energy difference of about 21 kcal mol^{-1}.

Although the values obtained here for $\Delta\Delta E$ in both situations correspond to a gross estimate, this demonstrates in a qualitative way that considering partial charge (and not unitary charge) for the cations, together with fluctuations of their positions in the sequence (i.e., differences in repulsion strengths), could be an important phenomenon to discriminate the behavior of the two cation species in the filter. More accurate calculations, which would lead to quantitative agreement with experiments, should include in a continuous dynamical approach the different behaviors of the two cations Na and K in the transfer from an organic medium (the filter) to water with appropriate density, and vice versa. Especially the static and dynamic effects discussed before should in principle be envisaged as tightly coupled. Calculations should moreover take into account the entropic effects and consider the influence of the translocation rate constant differences between K and Na. The neglect of these processes is probably at the origin of the too large $\Delta\Delta E$ value. Indeed, molecular flexibility of the channel structure[11] should be more extensively taken into consideration in a quantum calculation of the polarization and charge transfer phenomena. This would require to study variations of the K^+ and Na^+ cations and carbonyl charges when a set of channel configurations are considered to characterize the flexibility of the protein structure, instead of a single average configuration as done here (see Sec. II). This would also imply following the behavior of these charges when the cations move along the pore. Significant charge fluctuations could occur when the cations move from one side to an adjacent one, which would greatly reduce the value of the $\Delta\Delta E$ barrier determined in the average configuration of the pore. For the comparison with a K*W*K*W* situation (*W*K*W*K in the present study), as well as with the corresponding sequence with one Na substituting S_2 or S_4 K, work is in progress. Regarding the translocation process, it was recently analyzed[51] on the basis of Nelson's approach and was shown to have important effects on the Na^+ block of K^+ current through the KcsA channel. Nevertheless, the limited number of microscopic characteristics included in this latter model precludes its application to an accurate quantitative analysis.

Another interesting feature, which is linked with the importance of considering partial charges in apprehending selectivity, deals with the behavior of the dipoles associated to

TABLE III. Average dipoles (debye units) for the two times four carbonyl groups of the Val76 and Gly77 residues located around S_2. The dipoles are calculated with either AMBER charges or MK partial charges. In the latter case, three situations have been investigated, namely, when the filter is empty or filled with either *WKWK* or *WNaWNa* sequences (charges calculated for each situation).

	Val76 C=O	Gly77 C=O
AMBER	3.41	3.43
MK (empty)	4.12	3.15
MK (*WKWK*)	4.23	3.45
MK (*WNaWNa*)	4.48	3.54

the carbonyl groups. In a recent paper, Noskov *et al.*[11] mentioned that the carbonyl groups coordinating the ion in the S_2 site are very dynamic and that their intrinsic electrostatic properties control ion selectivity. More precisely, on the basis of a simple model and free energy calculations, the authors showed that, as long as the coordinating ligands have a dipole between 2.5 and 4.5 D, the filter selects potassium ions rather than sodium ions. It would, however, become selective for Na^+ if the magnitude of the dipoles would reach about 7 D. It is thus interesting to analyze intrinsic dipoles of the carbonyl groups obtained in the different situations with the MK method and in comparison with the AMBER method. The dipoles averaged over four in each ring and located on the eight C=O carbonyl groups of Val76 and Gly77 residues around S_2 are listed in Table III. They are calculated with the charges issued either from AMBER or MK calculations in the presence or absence of the *WKWK* or *WNaWNa* sequences.

These results show that the dipoles of the central ligands are still carbonyl-like since on the average they range from 4.12 to 4.48 D for Val76 and from 3.15 to 3.54 D for Gly77 in the MK approach. It is to notice that these values are centered around the dipole moment value determined for a carbonyl belonging to a N–C=O group in gas phase, i.e., 3.7 D,[52] which gives an idea of the dielectric constant around these carbonyls. Whereas AMBER charges do not lead to any difference between Val76 and Gly77, there is about 1 D difference with MK charges, whatever the configuration is, Gly77 MK-determined C=O dipoles being consistent with AMBER. The filter filling state leads only to small deviations. The 0.2 difference for K versus Na charge in MK (see Table II) does not induce a noticeable difference in the dipole moment. Nor does the empty situation. This is quite astonishing, but one has to recall that the protein surrounding in the filter is the one optimized for the sequence *WKWK*. All in all, these results are consistent with the observations made by the group of Noskov *et al.*,[11] mentioning dipoles ranging between 2.5 and 4.5 D for selectivity of potassium to be ensured.

V. CONCLUSION

The aim of this paper was devoted to get some insight into the microscopic origins of selectivity and high conduction rates observed for potassium ions in the KcsA channel and thus, to a larger extent, in potassium channels. These two features are apparently antagonist since high diffusion requires weak binding, while selectivity is normally ensured by strong interactions. We have investigated the role of possible polarization and/or charge transfer consequences on the binding of potassium and sodium ions into the selectivity filter. Partial charges have been calculated from Merz-Kollman and Hinsen-Roux schemes. Usually, calculations of that kind in confined media lead to "poorly determined" charges, but a number of reasons allow to think that MK and HR charges determined here may be satisfactory to describe the system. Indeed, *ab initio* potentials are well reproduced in the filter; correct charges on water molecules and correct values for the carbonyls' moments are determined. As a general trend, a larger charge effect occurs on potassium in comparison to sodium, leading to a strong difference in binding of these ions inside the KcsA filter. This feature means that even though sodium ions have a slightly smaller ionic radius and are believed to possess about the same unity charge as potassium ions in the free filter entrance solvent medium, they are much more strongly trapped when inside the filter. On the basis of a simple qualitative approach, we have estimated the influence of this partial charge change on the sequence translocation inside the filter, including both the hydration/dehydration differences (as far as they are known) between K^+ and Na^+ ions and the different behaviors in the knock-on mechanism. We will investigate in a forthcoming study the partial charge evolution of the ions along the whole filter, dependent on relevant MD-retrieved configurations, in order to better understand the charge effect at this nanoscale dimension. We will also focus on the tight correlations which exist between the charges on the ions and the filter atoms and on the transfer from the water (bulk or confined) to the filter, and inversely. Better evaluating free energy differences between potassium and sodium remains an approach of choice for understanding ionic channel selectivity (e.g., potential of mean force calculations).

ACKNOWLEDGMENTS

The authors would like to thank K. Hinsen for fruitful discussion and for providing the MMTK.ChargeFit module. One author (P.H.) wishes to thank the Ligue du Doubs Contre le Cancer of France, Montbéliard Committee, for financial support.

[1] B. Hille, *Ionic Channels of Excitable Membranes*, 2nd ed. (Sinauer Associates, Sunderland, MA, 1992).
[2] F. M. Ashcroft, *Ion Channels and Diseases* (Academic, San Diego, 2000).
[3] H. Schrempf, O. Schmidt, R. Kummerlen, S. Hinnah, D. Muller, M. Betzler, T. Steinkamp, and R. Wagner, EMBO J. **14**, 5170 (1995).
[4] R. MacKinnon, FEBS Lett. **555**, 62 (2003).
[5] D. A. Doyle, J. M. Cabral, A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. MacKinnon, Science **280**, 69 (1998).
[6] J. H. Morais-Cabral, Y. Zhou, and R. MacKinnon, Nature (London) **414**, 37 (2001).
[7] Y. Zhou, J. H. Morais-Cabral, A. Kaufman, and R. MacKinnon, Nature (London) **414**, 43 (2001).
[8] M. Compoint, P. Carloni, C. Ramseyer, and C. Girardet, Biochim. Biophys. Acta **1661**, 26 (2004).
[9] B. Roux, Annu. Rev. Biophys. Biomol. Struct. **34**, 153 (2005).
[10] S. Bernèche and B. Roux, Biophys. J. **78**, 2900 (2000).
[11] S. Y. Noskov, S. Bernèche, and B. Roux, Nature (London) **431**, 830

(2004).
[12] L. Guidoni, V. Torre, and P. Carloni, Biochemistry **38**, 8599 (1999).
[13] L. Guidoni and P. Carloni, Biochim. Biophys. Acta **1563**, 1 (2002).
[14] S. Bernèche and B. Roux, Nature (London) **414**, 73 (2001).
[15] A. Warshel and W. W. Parson, Q. Rev. Biophys. **34**, 563 (2001).
[16] A. Burykin, M. Kato, and A. Warshel, Proteins **52**, 412 (2003).
[17] M. Compoint, C. Ramseyer, and P. Huetz, Chem. Phys. Lett. **397**, 510 (2004).
[18] A. A. Bliznyuk and A. P. Rendell, J. Phys. Chem. **108**, 13866 (2004).
[19] D. A. Case, D. A. Pearlman, J. W. Caldwell *et al.*, AMBER6, University of California, San Francisco, 1999.
[20] M. Compoint, F. Picaud, C. Ramseyer, and C. Girardet, J. Chem. Phys. **122**, 134707 (2005).
[21] J. B. Foresman and A. Frisch, *Exploring Chemistry with Electronic Structure Methods*, 2nd ed. (Gaussian Inc., Pittsburgh, PA, 1996).
[22] M. J. Frisch, G. W. Trucks, H. B. Schlegel *et al.*, GAUSSIAN 03, Revision C.02, Gaussian, Inc., Wallingford, CT, 2004.
[23] R. S. Mulliken and P. Politzer, J. Chem. Phys. **55**, 5135 (1971).
[24] P. O. Löwdin, Adv. Quantum Chem. **83**, 735 (1970).
[25] A. E. Reed, R. B. Weinstock, and F. A. Weinhold, J. Chem. Phys. **83**, 735 (1985).
[26] R. F. W. Bader, *Atoms in Molecules: A Quantum Theory* (Clarendon, Oxford, 1990).
[27] E. Sigfridsson and U. Ryde, J. Comput. Chem. **19**, 377 (1998).
[28] L. E. Chirlain and M. M. Francl, J. Comput. Chem. **6**, 894 (1987).
[29] U. C. Singh and P. A. Kollman, J. Comput. Chem. **5**, 129 (1984).
[30] B. H. Besler, K. M. Merz, Jr., and P. A. Kollman, J. Comput. Chem. **11**, 431 (1990).
[31] K. Hinsen and B. Roux, J. Comput. Chem. **18**, 368 (1997).
[32] C. I. Bayly, P. Cieplak, W. D. Cornell, and P. A. Kollmann, J. Phys. Chem. **97**, 10269 (1993).
[33] Besler *et al.* and Bayly *et al.* have also argued that for optimal reproduction of biomolecular properties in aqueous solution using additive potentials, 6-31G* basis set is indeed an excellent choice (see Refs. 30 and 32).
[34] C. Carey, L. E. Chirlian, M. M. Francl, and D. M. Gange, Glycoconjugate J. **14**, 501 (1997).
[35] K. Hinsen, http://dirac.cnrs-orleans.fr/MMTK/
[36] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, and J. Hermans, in *Intermolecular Forces*, edited by B. Pullman (Reidel, Dordrecht, 1981).
[37] J. Åqvist, J. Phys. Chem. **94**, 8021 (1990).
[38] J. Åqvist and V. Luzhkov, Nature (London) **404**, 881 (2000).
[39] M. Zhou and R. MacKinnon, J. Mol. Biol. **338**, 839 (2004).
[40] W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein, J. Chem. Phys. **79**, 926 (1983).
[41] B. Luzhkov and J. Åqvist, Biochim. Biophys. Acta **1481**, 360 (2000).
[42] B. Roux, S. Bernèche, and W. Im, Biochemistry **39**, 13295 (2000).
[43] P. H. Nelson, J. Chem. Phys. **117**, 11396 (2002).
[44] S. Mafé and J. Pellicer, Phys. Rev. E **71**, 021901 (2005).
[45] S. O. Yesylevskyy and V. N. Kharkyanen, Phys. Chem. Chem. Phys. **6**, 3111 (2004).
[46] I. S. Tolokh, I. I. Tolokh, H. C. Cho, N. D'Avanzo, P. H. Backx, S. Goldman, and C. G. Gray, Phys. Rev. E **71**, 021912 (2005).
[47] V. B. Luzhkov and J. Åqvist, Biochim. Biophys. Acta **1548**, 194 (2001).
[48] P. Kebarle, Annu. Rev. Phys. Chem. **28**, 445 (1977).
[49] A. L. Hodgkin and R. D. Keynes, J. Physiol. (London) **128**, 61 (1955).
[50] S. Bernèche and B. Roux, PNAS **100**, 8644 (2003).
[51] S. Mafé, J. Pellicer, and J. Cervera, J. Chem. Phys. **122**, 204712 (2005).
[52] C. P. Smyth, *Dielectric Behavior and Structure* (McGraw-Hill, New York, 1955).