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Determinants of K^+ vs Na^+ Selectivity in Potassium Channels

Todor Dudev[†] and Carmay Lim*[,†,‡]

Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, and the Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan

Received January 11, 2009; E-mail: carmay@gate.sinica.edu.tw

Abstract: Ion channels, specialized pore-forming proteins, are an indispensable component of the nervous system and play a crucial role in regulating cardiac, skeletal, and smooth muscle contraction. Potassium ion channels, controlling the action potential of a number of excitable cells, are characterized by a remarkable ability to select K^+ over Na^+. Although the molecular basis for this striking ion selectivity has been a subject of extensive investigations using both experimental and theoretical methods, the following outstanding questions remain: (a) To what extent is the number of water molecules bound to the permeating ion (i.e., the hydration number) important for the K^+/Na^+ competition? (b) Are the chemical type and number of coordinating groups lining the pore critical for the selectivity process? (c) Apart from providing cation-ligating groups, do the channel walls play any other role in the selectivity process? This work reveals that the pore's selectivity for K^+ over Na^+ increases with (i) increasing hydration number of K^+ relative to that of Na^+, (ii) increasing number of K^+-coordinating dipoles, (iii) increasing number of Na^+-coordinating dipoles, and (iv) decreasing magnitude of the coordinating dipoles provided by the pore. Thus, a high K^+/Na^+ selectivity in K^+ channels could be achieved from a combination of several favorable factors involving the native ion, the metal-coordinating ligands, and the protein matrix, viz., (a) an octahydrated permeating K^+, (b) a pore lined with 8 carbonyl ligands, and (c) finely tuned physicomechanical properties of the channel walls providing a low dielectric medium favoring a high hydration number for the permeating K^+ and enough stiffness to force the competing Na^+ to adopt an unfavorable 8-fold coordination. This implies that optimal K^+/Na^+ selectivity in K^+ channels generally does not arise from solely structural or energetic consideration. The factors affecting ion selectivity revealed herein help to rationalize why valinomycin and the KcsA ion channels are highly K^+-selective, whereas the NaK channel is nonselective. The calculations predict that other pores containing a different number/chemical type of coordinating groups from those observed in potassium channels could also select K^+ over Na^+.

Introduction

Ion channels are specialized pore-forming proteins, which, by regulating the ion flow through the cellular membrane, exert control on electrical signals in cells.[1] They are an indispensable component of the nervous system and play a crucial role in regulating cardiac, skeletal, and smooth muscle contraction. Among the ion channels, the monovalent ion channels (conducting K^+ or Na^+) are of particular importance, as they control the action potential of a number of excitable cells by polarizing/depolarizing the cell membrane. Potassium or sodium ion channels are characterized by a remarkable ability to discriminate between cations of the same charge and similar ionic radii in favor of the native ion. Thus, K^+ channels select K^+ over Na^+ by a ratio of $\sim$1000:1,[1] whereas the epithelial Na^+ channel (ENaC) exhibits a Na^+/K^+ selectivity ratio higher than 500:1.[2,3] Both types of channels do not allow anions to enter, and are blocked by *divalent* cations.

The molecular basis for this striking ion selectivity has been a subject of extensive investigations using both experimental and theoretical methods in attempts to unravel the key determinants governing the cation selectivity in monovalent ion channels. The 3D structures of valinomycin, a small, highly K^+-selective ionophore[4,5] and several ion channels such as the potassium KcsA channel[6,7] and the nonselective NaK channel[8] show that the pores are lined with oxygen atoms with partial negative charges that repel anions, explaining the rejection of anions. On the other hand, simulation studies suggest that divalent ions block the K^+ channels because they are bound so tightly that they are unlikely to leave, even with the aid of repulsion from nearby cations.[9,10] In contrast to valence (charge) selectivity, no consensus has so far been reached on the major factors dictating K^+ vs Na^+ size selectivity in monovalent ion

[†] Academia Sinica.
[‡] National Tsing Hua University.

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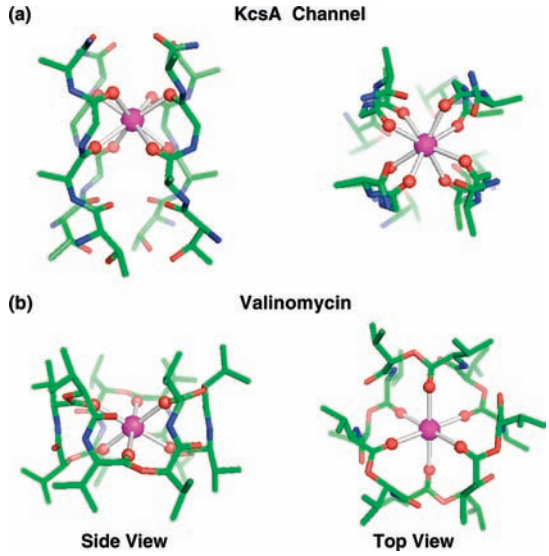

Figure 1. Ball and stick diagrams of K^+ complexes with (a) KcsA channel selectivity filter (PDB entry 1K4C) and (b) valinomycin (CSD entry TEFBAH). Color scheme: green, C; blue, N; red, O; and magenta, K.

channels, although the roles of various factors such as the pore size/flexibility,[11−14] dehydration penalty of the permeating cations,[11,13,15] the electrostatic interactions between the cation and channel dipoles,[9,10,12,15,16] and the architecture of the metal-binding site[13,17−19] have been examined (see below).

Potassium channel selectivity is an outcome of the competition between the bulk solvent and the protein ligands for the native ion and a "rival" cation, e.g., Na^+, and can be assessed by the free energy of the $K^+ \rightarrow Na^+$ exchange reaction,

$$[Na^+\text{-aq}] + [K^+\text{-channel}] \rightarrow [Na^+\text{-channel}] + [K^+\text{-aq}] \quad (1)$$

In eq 1, $[Na^+$-aq] and $[K^+$-aq] represent hydrated Na^+ and K^+ ions outside the selectivity filter, respectively, whereas $[Na^+$-channel] and $[K^+$-channel] denote Na^+ and K^+ bound to the ion channel. The *selectivity filter* is the region that controls the ion selectivity of a given ion channel. It forms the narrowest part of the pore and may consist of one or a few tri-, tetra- or pentameric rings lined with metal-coordinating groups such as backbone amide groups or amino acid side chains (Ser, Thr, Asp or Glu). For example, the potassium KcsA channel has a carbonyl-rich selectivity filter comprised of four tetrameric rings one on top of the other (see Figure 1).[7] For this ion channel, the experimentally established ion exchange free energy for eq 1, $\Delta G(K^+ \rightarrow Na^+)$, is ∼5−6 kcal/mol,[20,21] with a lower bound of 3 kcal/mol.[22] This value has been successfully reproduced by a number of free energy perturbation calculations which, despite differences in the models and simulation protocols used, yield $\Delta G(K^+ \rightarrow Na^+)$ between 4 and 6 kcal/mol.[12,16,17,23−28] The consensus from these simulation studies is that the selectivity filter is quite flexible: the root-mean-square fluctuations of the atoms lining the filter are of the order of 0.5−1.0 Å, consistent with the crystallographic thermal *B*-factors.[7] These structural fluctuations are greater than the ionic radii difference between hexahydrated K^+ and Na^+ (0.36 Å),[29] enabling the carbonyl oxygen atoms to adjust their positions to bind Na^+, precluding size-based selectivity. Hence, the classical "snug-fit" mechanism,[13,30] which assumes a *rigid* pore of *precise geometry* that snugly fits only K^+ but not the smaller Na^+, does not seem to control ion selectivity in KcsA channels.[14]

However, the theoretical studies disagree on the key factor(s) determining the selectivity of K^+ over Na^+ in K^+ channels. Noskov et al.[12,16,28] proposed a "carbonyl-repulsion" mechanism whereby the increased CO−CO repulsion upon coordinating the smaller Na^+ leads to K^+/Na^+ selectivity in K^+ channels. They emphasized that the *unique carbonyl* ligating properties (especially the magnitude of the C═O dipole moment), but *not* the architectural rigidity and thus size of the pore, leads to selectivity. Several groups have subsequently challenged this view. Bostick and Brooks[27] concluded that "carbonyl moieties are *not* uniquely suited to select K^+ over Na^+", as even water ligands have the potential to discriminate between the two cations; rather, external topological restraints from the protein are needed for selective binding of K^+ by carbonyl ligands. Likewise, Thomas et al.[17] concluded that the intrinsic dipole moment of the carbonyl ligands is not the key factor creating K^+ selectivity in K^+ channels since selectivity is lost when the carbonyl ligands have complete freedom to orient about the ions. They together with Varma and Rempe[18] found that the 8-fold coordination of the permeating ions in the selectivity filter, which favors K^+ over the smaller Na^+, is the key cause of K^+ selectivity in K^+ channels, in accord with earlier density functional theory (DFT) studies by Ban et al.[31] Such an 8-fold coordination is enforced by some form of rigidity provided by the protein matrix, as structural distortions that reduce the Na^+ coordination number from 8 to 6 or 5 in the selectivity filter reversed ion selectivity in favor of Na^+.[17,18] Thus, structural rigidity is needed for K^+ selectivity in K^+ channels like the classical "snug-fit" mechanism,[13,30] but not to maintain specific pore size; instead it is to maintain specific metal coordination numbers.[18,19]

The above summary of the key findings on the cation competition in K^+ channels shows that the major determinants of ion selectivity remain elusive. Notably, although the effect of the cation coordination number in the selectivity filter (the number of filter ligands coordinated to the metal cation) in determining the pore selectivity has been investigated, the effect of the cation *hydration* number in metal−aqua complexes (the

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number of water ligands coordinated to the metal cation) in the process has received little attention. Several outstanding questions remain: (1) To what extent is the permeating ion *hydration* number important for the K^+/Na^+ competition? (2) Are the *number* and the *chemical type* of the coordinating groups lining the selectivity filter critical for the selectivity process? In other words, can *fewer* than 8 coordinating ligands that are *not* carbonyl groups also give rise to K^+/Na^+ selectivity? (3) Apart from providing cation-ligating groups, do the channel walls play any other role in the selectivity process?

Herein, the above questions are addressed by modeling K^+ with a hydration number ranging from 6 to 8, as observed experimentally,[32] and ion channel selectivity filters with coordinating groups differing in number (4, 5, 6 or 8) and type ($-CONH_2$ or $-OH$). Since our aim is to evaluate qualitatively how various factors such as (i) the hydration number of K^+ relative to that of Na^+, (ii) the solvent exposure (dielectric constant) of the channel pore, (iii) the numbers of K^+- and Na^+-coordinating dipoles, and (iv) the magnitude of the metal-coordinating dipoles affect the K^+/Na^+ selectivity *not only* for a specific ion channel but for both selective and nonselective channel proteins, we will employ a combined DFT/continuum dielectric approach, as in previous works.[33−35] Furthermore, since the first-shell ligands play a key role in the K^+/Na^+ competition,[25] they and the metal cations were treated explicitly using DFT to account for electronic effects such as polarization of the participating entities and charge transfer from the ligands to the metal cation, whereas the rest of the protein was represented by a continuum dielectric varying from 4 to 20 (see Methods). The DFT and continuum dielectric calculations were rigorously calibrated against all available experimental data and used to compute the ion exchange free energies, $\Delta G(K^+ \rightarrow Na^+)$, in the gas phase and in a protein environment, as described in the next section.

Methods

Models Used. (1) Aqueous Solution. The number of water molecules bound to the metal ion was assigned according to the experimental first-shell hydration number of the ion in aqueous solution. For Na^+, this number is predominantly 6,[18,27,32,36] so hexahydrated $[Na(H_2O)_6]^+$ was modeled. On the other hand, the first-shell hydration number of K^+ in aqueous solution is more variable, ranging from 6 to 8,[18,27,32,37−39] whereas inside the KcsA central cavity, K^+ is found bound to eight water molecules.[7] In accord with the experimental and theoretical findings, K^+ hydrates were modeled as $[K(H_2O)_6]^+$, $[K(H_2O)_7]^+$, and $[K(H_2O)_8]^+$.

(2) Ion Channel. The peptide backbone groups in K^+ channels were modeled by $-CONH_2$, while Ser/Thr side chains that have been found to line some ion channel selectivity filters were modeled by $-OH$.[40−42] Different numbers (3, 4 or 5) of these amide or hydroxyl groups were coordinated to the permeating ion (Na^+ or K^+) and attached to a carbon−hydrogen ring scaffold via methylene spacers, as shown in Figure 2. To mimic the selectivity filters in valinomycin[4] and the KcsA K^+ channels,[7] which provide 6 and 8 ligating carbonyl groups, respectively (see Figure 1), six-coordinated (Figure 3a) and eight-coordinated (Figure 3b) metal-binding sites were created by doubling the monolayered trimeric and tetrameric (Figure 2a) selectivity filters, respectively. The $-CONH_2$ groups in the monolayer filters (Figure 2) were methylated to $-CONH(CH_3)$ in the bilayer filters to avoid interlayer $CO\cdots NH$ hydrogen bond formation and distortion of the overall structure. In line with experimental observations,[7,13] the metal ion (K^+ or Na^+) was fully dehydrated (unless stated otherwise) upon binding to ligands lining the selectivity filter. All models were built using GaussView version 3.09.[43]

Justification of the Model Structures. To evaluate the extent to which the ring's structure affects the flexibility of the attached ligating groups during metal binding, the optimized metal−oxygen distances in the $[Na/K-filter4\text{-}OH]^+$ and $[Na/K-filter4\text{-}CONH_2]^+$ complexes were compared with those in Na^+/K^+ complexes containing four free ethanol or formamide molecules, respectively. The bond distances calculated at the B3-LYP/6-31+G(3d,p) level (see next section) are similar, indicating that the carbon−hydrogen ring in the model system does not impede the ligand dipoles from coordinating to the monocation. The Na/K−O distance in $[Na/K-filter4\text{-}OH]^+$ (2.36/2.76 Å) is similar to that in $[Na/K(ethanol)_4]^+$ (2.34/2.74 Å). Likewise, the Na/K−O distance in $[Na/K-filter4\text{-}CONH_2]^+$ (2.28/2.66 Å) is similar to that in $[Na/K(formamide)_4]^+$ (2.27/2.65 Å).

The complexes modeling the selectivity filter (Figures 2 and 3) were constructed on the basis of the following considerations: (1) The ring, which mimics the oligomeric state of the ion channel protein, prevents the ligands from leaving the metal center during geometry optimization. (2) The entire structure (ring, metal-ligating groups and their connection to the ring) is flexible enough to allow the ligating groups to optimize their positions upon metal binding (see above). (3) The ring's shape and C−H bond directions are such that they do not obstruct the pore lumen. (4) As the number of carbon atoms in the tetrameric ring (16 for Figure 2a or 2c) is similar to that in the pentameric ring (15 for Figure 2b), the ring's C−H chain length would not bias the results.

Choice of Method/Basis. As the dipole moment of the metal ligand is critical in determining the strength of the interactions between the permeating ion and the pore wall, an ab initio method/basis set combination that correctly reproduces the dipole moments of the metal ligands is needed. Hence, a series of ab initio/DFT methods (HF, MP2, S-VWN, and B3-LYP) in combination with Pople's basis sets increasing in size from 6-31+G(d,p) to 6-311++G(3df,3pd) were examined for their ability to reproduce the gas-phase dipole moments of water, methanol (modeling the Ser/Thr side chains) and formamide (modeling the backbone carbonyl groups). Among the various combinations, the B3-LYP functional and the 6-31+G(3d,p) basis set combination was most efficient in yielding dipole moments of the metal ligands that are closest to the respective experimental values (see Table S1 in the Supporting Information). It also reproduced the Na/K−O distances in crown-ether complexes (Figure S1 in the Supporting Information), which resemble metal-occupied ion channel pores, within experimental error (Table 1).

Gas-Phase Free Energy Calculations. On the basis of the results in Table S1 in the Supporting Information and Table 1, the B3-LYP/6-31+G(3d,p) method was used to optimize the *geometry* of each complex without any constraints and to compute the electronic energies, E_{el}, using the Gaussian 03 program.[46] Due to computer memory limitations, the smaller 6-31+G(d,p) basis set was used

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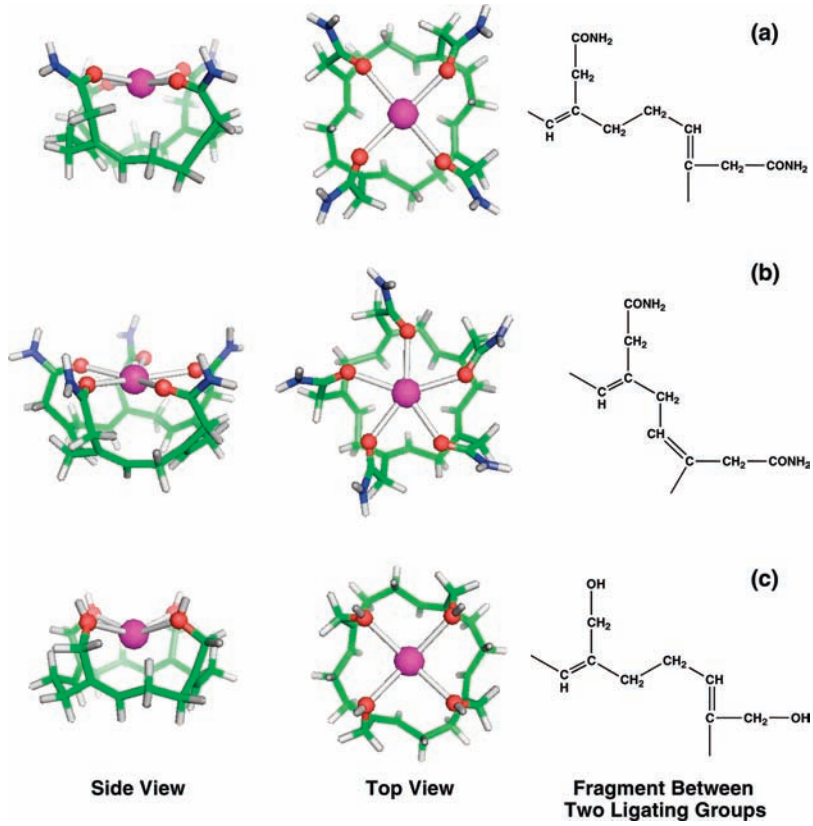

Figure 2. Ball and stick diagrams of B3LYP/6-31+G(3d,p) fully optimized structures of K^+ complexes with monolayered (a) tetrameric filter with $-CONH_2$ ligating groups (filter4-$CONH_2$), (b) pentameric filter with $-CONH_2$ ligating groups (filter5-$CONH_2$), and (c) tetrameric filter with $-OH$ coordinating groups (filter4-OH). Color scheme: green, C; blue, N; red, O; gray, H; and magenta, K.

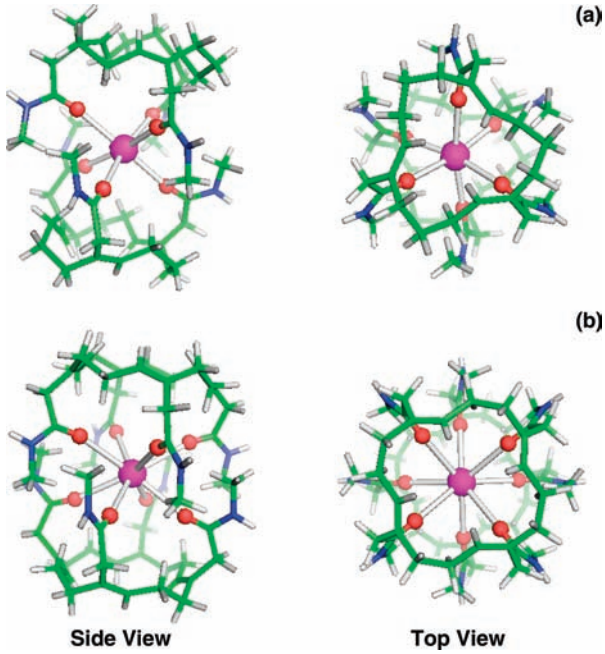

Figure 3. Ball and stick diagrams of B3LYP/6-31+G(3d,p) fully optimized structures of K^+ complexes with amide-containing two-layered (a) trimeric filter (filter6-$CONH_2$), and (b) tetrameric filter (filter8-$CONH_2$). Color scheme: green, C; blue, N; red, O; gray, H; and magenta, K.

to compute the vibrational frequencies for the monolayered structures (Figure 2). This would not be expected to affect the trends in the computed free energy changes, $\Delta G(K^+ \rightarrow Na^+)$: benchmark calculations on a trimeric analogue of the model filters with three

Table 1. Calculated and Experimental Gas-Phase Dipole Moments of Metal Ligands and Average Metal−Oxygen Bond Distances in Metal-Bound Crown Ethers

molecule	B3-LYP/6-31+G(3d,p)	experiment
	μ (D)	
H_2O	1.88	1.85 ± 0.02[a]
CH_3OH	1.67	1.70 ± 0.02[a]
$HCONH_2$	3.99	3.73 ± 0.07[a]
	Metal−O (Å)	
$[Na(18\text{-crown-}6)]^+$ [b]	2.75	2.77 ± 0.07[c]
$[K(18\text{-crown-}6)]^+$ [b]	2.82	2.80 ± 0.04[c]

[a] From Lide, 2006.[44] [b] Structures are depicted in Figure S1 in the Supporting Information. [c] From Cambridge Structure Database[45] analysis (this work).

−OH ligating groups (see Figure S2 in the Supporting Information) revealed very little change (<0.2 kcal/mol) in the thermal energy and entropy differences between Na−filter3-OH and K−filter3-OH in going from the 6-31+G(3d,p) basis set ($\Delta E_{th} = 0.17$ kcal/mol, $T\Delta S = -1.28$ kcal/mol) to the smaller 6-31+G(d,p) basis set ($\Delta E_{th} = 0.07$ kcal/mol, $T\Delta S = -1.09$ kcal/mol). No imaginary frequency was found in any of the complexes. The B3-LYP/6-31+G(d,p) frequencies were scaled by an empirical factor of 0.9613[47] and used to compute the thermal energies including zero-point energy (E_{th}) and entropies (S) employing standard statistical mechanical formulas.[48] The differences in ΔE_{el}, ΔE_{th}, ΔPV (work term) and ΔS between the products and reactants in eq 1 were used to calculate $\Delta G(K^+ \rightarrow Na^+)$ in the gas phase (denoted by superscript 1) at room temperature, $T = 298.15$ K, according to the following expression:

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$$\Delta G^1 = \Delta E_{el} + \Delta E_{th} + \Delta PV - T\Delta S \quad (2)$$

For the two-layered complexes (Figure 3), frequency calculations were computationally prohibitive due to the large number of basis functions used to optimize the $[Na/K-filter6\text{-}CONH_2]^+$ and $[Na/K-filter8\text{-}CONH_2]^+$ complexes (2285(Na)/2289(K) and 2707(Na)/2711(K), respectively). Hence, the corresponding thermal energies and entropies for these structures could not be evaluated. However, close inspection of the thermodynamic parameters of the pentameric filter, which is nearest in size to filter6-$CONH_2$ and filter8-$CONH_2$, revealed relatively small thermal energy and entropy differences between Na−filter5-$CONH_2$ and K−filter5-$CONH_2$ ($\Delta E_{th} = -0.1$ kcal/mol and $T\Delta S = 0.7$ kcal/mol). As these differences fall within the error limit (~1 kcal/mol) of the present calculations, the ΔE_{th} and $T\Delta S$ between $[Na-filterX\text{-}CONH_2]^+$ and $[K-filterX\text{-}CONH_2]^+$ ($X = 6$ or 8) were neglected in evaluating the ΔG^1 for ion exchange involving the two-layered complexes.

To determine if it was necessary to add counterpoise correction, the ΔE_{el} for $[NaW_6]^+ + [K-filter4\text{-}CONH_2]^+ \rightarrow [Na-filter4\text{-}CONH_2]^+ + [KW_6]^+$ was computed with and without adding such a correction. The basis set superposition error (BSSE) for this ion exchange reaction was found to be negligible (ΔBSSE = −0.1 kcal/mol). Hence, BSSE was not considered in the present calculations.

Solution Free Energy Calculations. The ion exchange free energy for eq 1 in a given environment characterized by a dielectric constant $\varepsilon = x$ can be computed according to Scheme 1. ΔG^1, the gas-phase free energy, was computed as described above. $\Delta G_{solv}{}^x$, the free energy for transferring a molecule in the gas phase to a medium characterized by a dielectric constant, x, was estimated by solving Poisson's equation using finite difference methods.[49,50] Thus, the ion exchange free energy in an environment modeled by dielectric constant x, ΔG^x, can be computed from

$$\Delta G^x = \Delta G^1 + \Delta G_{solv}{}^x(\text{products}) - \Delta G_{solv}{}^x(\text{reactants}) \quad (3)$$

Recent studies[18,19] have shown that the ion channel selectivity filter and its immediate surroundings are distinctly not in a bulk water environment. Thus, the Poisson's equation was solved for $\varepsilon = 4$, 10, and 20 to mimic binding sites of varying degrees of solvent exposure. Furthermore, the ion exchange (eq 1) was modeled to occur in proximity of the selectivity filter so that the dielectric environment was assumed to be uniform for all participating entities.

The solvation free energies were evaluated using the MEAD (Macroscopic Electrostatics with Atomic Detail) program.[51] The effective solute radii, which were obtained by adjusting the CHARMM (version 22)[52] van der Waals radii to reproduce the experimental hydration free energies of Na^+, K^+, and model ligand molecules, are as follows (in Å): $R_{Na} = 1.72$, $R_K = 1.90$, $R_C = 1.95$, $R_N = 1.75$, $R_O(HCONH_2) = 1.72$, $R_O(H_2O) = 1.85$, $R_O(CH_3OH) = 1.90$, $R_H = 1.50$, $R_H(H_2O-Na) = 1.26$, $R_H(H_2O-K) = 1.20$. These effective solute radii reproduce the experimental hydration free energies of Na^+, K^+, and model ligands within 1 kcal/mol (Table 2).

Scheme 1

$$\begin{array}{ccc} & \xrightarrow{\Delta G^1} & \\ \text{Reactants } (\varepsilon = 1) & \rightarrow & \text{Products } (\varepsilon = 1) \\ \Delta G_{solv}{}^x(\text{Reactants}) \downarrow & & \downarrow \Delta G_{solv}{}^x(\text{Products}) \\ \text{Reactants } (\varepsilon = x) & \rightarrow & \text{Products } (\varepsilon = x) \\ & \xrightarrow{\Delta G^x} & \end{array}$$

Table 2. Comparison between Computed and Experimental Hydration Free Energies $\Delta G_{solv}{}^{80}$ of Metal Cations and Ligands (in kcal/mol)

	$\Delta G_{solv}{}^{80}$ (kcal/mol)		
metal/ligand	expt	calc	error[a]
Na^+	−98.3[b]	−98.7	−0.4
K^+	−80.8[b]	−81.0[c]	−0.2[c]
		−80.9[d]	−0.1[d]
		−81.2[e]	−0.4[e]
H_2O	−6.3[f]	−6.7	−0.4
CH_3OH	−5.1[g]	−6.1	−1.0
$HCONH_2$	−10.0[h]	−10.6	−0.6

[a] Error = $\Delta G_{solv}{}^{80}$(calc) − $\Delta G_{solv}{}^{80}$(expt). [b] From Friedman, 1973.[53] [c] Hexahydrated K^+. [d] Heptahydrated K^+. [e] Octahydrated K^+. [f] From Ben-Naim and Marcus, 1984.[54] [g] From Chambers et al., 1996.[55] [h] Experimental solvation free energy of $HCONH(CH_3)$ from Wolfenden et al., 1978.[56]

Results

Validation against Experimental Ion Exchange Free Energies. To assess the accuracy of the ion exchange free energy calculations (see Methods) in revealing reliable trends, we compared the experimental $\Delta G(K^+ \rightarrow Na^+)$ of 2.0 ± 0.1 kcal/mol[57] for replacing K^+ with Na^+ in 18-crown-6 ether in aqueous solution with the computed $\Delta G(K^+ \rightarrow Na^+)$ for the following reactions, which differ in the K^+ hydration number:

$$[Na(H_2O)_6]^+ + [K(18\text{-crown-}6)]^+ \rightarrow [Na(18\text{-crown-}6)]^+ + [K(H_2O)_6]^+ \quad (4a)$$

$$[Na(H_2O)_6]^+ + H_2O + [K(18\text{-crown-}6)]^+ \rightarrow [Na(18\text{-crown-}6)]^+ + [K(H_2O)_7]^+ \quad (4b)$$

$$[Na(H_2O)_6]^+ + 2H_2O + [K(18\text{-crown-}6)]^+ \rightarrow [Na(18\text{-crown-}6)]^+ + [K(H_2O)_8]^+ \quad (4c)$$

The calculations reproduced the trend in ion selectivity of the crown ether: the computed $\Delta G(K^+ \rightarrow Na^+)$ for eq 4a (1.4 kcal/mol), eq 4b (6.7 kcal/mol) and eq 4c (13.5 kcal/mol) are all positive, as observed experimentally. Furthermore, the $\Delta G(K^+ \rightarrow Na^+)$ for reaction 4a, where K^+ is hexahydrated, is in *accord* with the experimental value, and is consistent with recent experimental observations indicating that $[K(H_2O)_6]^+$ is the dominant species in aqueous solution.[37]

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Table 3. Calculated Electronic Energies, Entropies and Free Energies of Ion Selectivity for Carbonyl-Containing Selectivity Filters in Media with Different Dielectric Constants (in kcal/mol)

reaction[a]	ΔE_{el}	$T\Delta S^1$	ΔG^1	ΔG^4	ΔG^{10}	ΔG^{20}
1. $[NaW_6]^+ + [K-filter4\text{-}CONH_2]^+ \rightarrow [Na-filter4\text{-}CONH_2]^+ + [KW_6]^+$	−3.1	−1.2	−1.5	−1.0	−1.3	−1.5
2. $[NaW_6]^+ + [K-filter4\text{-}CONH_2]^+ + W \rightarrow [Na-filter4\text{-}CONH_2]^+ + [KW_7]^+$	−15.2	−10.7	−2.5	2.5	3.6	3.9
3. $[NaW_6]^+ + [K-filter4\text{-}CONH_2]^+ + 2W \rightarrow [Na-filter4\text{-}CONH_2]^+ + [KW_8]^+$	−27.1	−19.8	−3.9	6.0	9.0	10.2
4. $[NaW_6]^+ + [KW-filter4\text{-}CONH_2]^+ \rightarrow [NaW-filter4\text{-}CONH_2]^+ + [KW_6]^+$	−2.6	−1.0	−1.0	−0.7	−0.9	−1.1
5. $[NaW_6]^+ + [KW-filter4\text{-}CONH_2]^+ + W \rightarrow [NaW-filter4\text{-}CONH_2]^+ + [KW_7]^+$	−14.7	−10.5	−2.0	2.8	4.0	4.3
6. $[NaW_6]^+ + [KW-filter4\text{-}CONH_2]^+ + 2W \rightarrow [NaW-filter4\text{-}CONH_2]^+ + [KW_8]^+$	−26.6	−19.6	−3.4	6.3	9.4	10.6
7. $[NaW_6]^+ + [K-filter5\text{-}CONH_2]^+ \rightarrow [Na-filter5\text{-}CONH_2]^+ + [KW_6]^+$	3.9	1.0	3.1	4.4	4.5	4.5
8. $[NaW_6]^+ + [K-filter5\text{-}CONH_2]^+ + W \rightarrow [Na-filter5\text{-}CONH_2]^+ + [KW_7]^+$	−8.2	−8.6	2.1	7.9	9.5	9.9
9. $[NaW_6]^+ + [K-filter5\text{-}CONH_2]^+ + 2W \rightarrow [Na-filter5\text{-}CONH_2]^+ + [KW_8]^+$	−20.1	−17.6	0.7	11.4	14.9	16.2
10. $[NaW_6]^+ + [K-filter6\text{-}CONH_2]^+ \rightarrow [Na-filter6\text{-}CONH_2]^+ + [KW_6]^+$	6.5	0.3[b]	6.6[c]	6.4	5.9	5.5
11. $[NaW_6]^+ + [K-filter6\text{-}CONH_2]^+ + W \rightarrow [Na-filter6\text{-}CONH_2]^+ + [KW_7]^+$	−5.6	−9.3[b]	5.5[c]	9.8	10.7	10.8
12. $[NaW_6]^+ + [K-filter6\text{-}CONH_2]^+ + 2W \rightarrow [Na-filter6\text{-}CONH_2]^+ + [KW_8]^+$	−17.5	−18.3[b]	4.2[c]	13.4	16.2	17.2
13. $[NaW_6]^+ + [K-filter8\text{-}CONH_2]^+ \rightarrow [Na-filter8\text{-}CONH_2]^+ + [KW_6]^+$	16.2	0.3[b]	16.3[c]	16.1	15.6	15.3
14. $[NaW_6]^+ + [K-filter8\text{-}CONH_2]^+ + W \rightarrow [Na-filter8\text{-}CONH_2]^+ + [KW_7]^+$	4.1	−9.3[b]	15.2[c]	19.5	20.4	20.6
15. $[NaW_6]^+ + [K-filter8\text{-}CONH_2]^+ + 2W \rightarrow [Na-filter8\text{-}CONH_2]^+ + [KW_8]^+$	−7.8	−18.3[b]	13.9[c]	23.1	25.9	27.0

[a] Water ligands are denoted as W. Structures of the monolayered metal-bound filters $[K-filter4\text{-}CONH_2]^+$ and $[K-filter5\text{-}CONH_2]^+$ are shown in Figures 2a and 2b, respectively, while those of the two-layered $[K-filter6\text{-}CONH_2]^+$ and $[K-filter8\text{-}CONH_2]^+$ are given in Figures 3a and 3b, respectively. [b] Calculated by neglecting $T\Delta S$ between $[Na-filter6/8\text{-}CONH_2]^+$ and $[K-filter6/8\text{-}CONH_2]^+$ (see text). [c] Calculated by neglecting ΔE_{th} and $T\Delta S$ between $[Na-filter6/8\text{-}CONH_2]^+$ and $[K-filter6/8\text{-}CONH_2]^+$ (see text), but including ΔE_{th} and $T\Delta S$ between other reactant and product molecules.

Selectivity Filters Lined with Carbonyl Groups. The electronic energies, entropies and free energies of ion exchange in selectivity filters containing different numbers of carbonyl ligands are listed in Table 3. These free energy differences should be considered qualitative, and not quantitative, as they were estimated using gas-phase free energies combined with an implicit solvent model, but their trends are expected to be reliable (see above). Below, we first describe how the filter/pore's ion selectivity depends on (i) the hydration number of K^+ relative to that of Na^+, (ii) the partial hydration of the metal ion bound to the selectivity filter ligands, (iii) the solvent exposure of the selectivity filter, and (iv) the number of ligating carbonyl groups, assuming each type of filter to be relatively rigid so that Na^+ is forced to adopt the same coordination number as K^+ in the filter. Then, we assess the effect on the pore's ion selectivity if the filter were sufficiently flexible to allow conformational changes enabling Na^+ to adopt its preferred coordination number. Finally, we evaluate if the pore's ion selectivity is a unique property of the ligating carbonyl ligands. In each case, we compare the computed trends with available experimental findings.

(1) Dependence on the K^+ Hydration Number. To evaluate the effect of the *K^+ hydration number* in determining the pore's ion selectivity, ion exchange free energies were computed with K^+ bound to 6, 7, or 8 water molecules, in line with experimental observations (see Methods). Comparison of the ΔG^x ($x = 4$, 10, 20) free energies for each triad of reactions in Table 3 shows that, for a given type of selectivity filter, increasing the hydration number of K^+ relative to that of Na^+ favors K^+ over Na^+ (more positive ΔG^x); e.g., the ΔG^4 for reactions 1, 2, and 3 are −1.0, 2.5, and 6.0 kcal/mol, respectively. This is mainly because when K^+ replaces Na^+ in the filter with the same coordination geometry (reverse of the reactions in Table 3), water molecules are released and become favorably solvated if the hydration number of K^+ exceeds that of Na^+.

The trend observed in the selectivity filters, however, is reversed in the absence of the protein solution environment. In the gas phase, increasing the hydration number of K^+ relative to that of Na^+ favors the substitution of K^+ in a given type of selectivity filter for Na^+ (Table 3, more negative/less positive ΔG^1). For example, for the first three reactions in Table 3, increasing the K^+ hydration number from six to eight decreases ΔE_{el} (from −3.1 to −27.1 kcal/mol) more than $T\Delta S^1$ (from −1.2 to −19.8 kcal/mol), thus the resultant ΔG^1 also decreases slightly (from −1.5 to −3.9 kcal/mol). This implies that in the absence of the protein matrix, the enthalpic gain upon binding an additional water molecule to K^+ outweighs the respective entropic loss in restricting the water oxygen atoms to orient toward the cation. Thus, *both* the ion hydration number and the ion channel protein matrix, which controls the solvent accessibility and thus dielectric constant of the selectivity filter/pore, affect ion selectivity: a hydration number of K^+ *larger* than that of Na^+ in the *pore* favors K^+ over Na^+.

(2) Dependence on the Ion's Partial Hydration inside the Selectivity Filter. Although the X-ray structures show that the metal cation is completely dehydrated inside the *multilayered* selectivity filter of K^+ channels,[7,8,13] it may be partially hydrated in *monolayered* selectivity filters (e.g., filter4-$CONH_2$), whose ligating groups have not saturated the metal-coordinating positions. To assess the effect of intrapore partial hydration of the ions on the pore's ion selectivity, the free energies for replacing K^+ bound to a water molecule and carbonyl groups from the filter4-$CONH_2$ with Na^+ were computed (Table 3, reactions 4−6). Comparison between the free energies for the fully (reactions 1−3) and partially (reactions 4−6) dehydrated metal complexes shows that the differences in the respective ΔG^x ($x = 1-20$) values are only 0.3−0.5 kcal/mol. Thus, when the metal coordination number upon ion exchange remains the same, intrapore partial hydration of the metal ion has negligible effect on the pore's ion selectivity.

(3) Dependence on the Filter's Solvent Exposure. To assess the effect of the pore's *solvent exposure* in determining its ion selectivity, the ion exchange free energies computed using a dielectric constant of 4, 10, and 20 (Scheme 1) were compared. Comparison of the ΔG^4, ΔG^{10}, and ΔG^{20} values in Table 3 show that increasing the pore's *solvent exposure* does not alter ion

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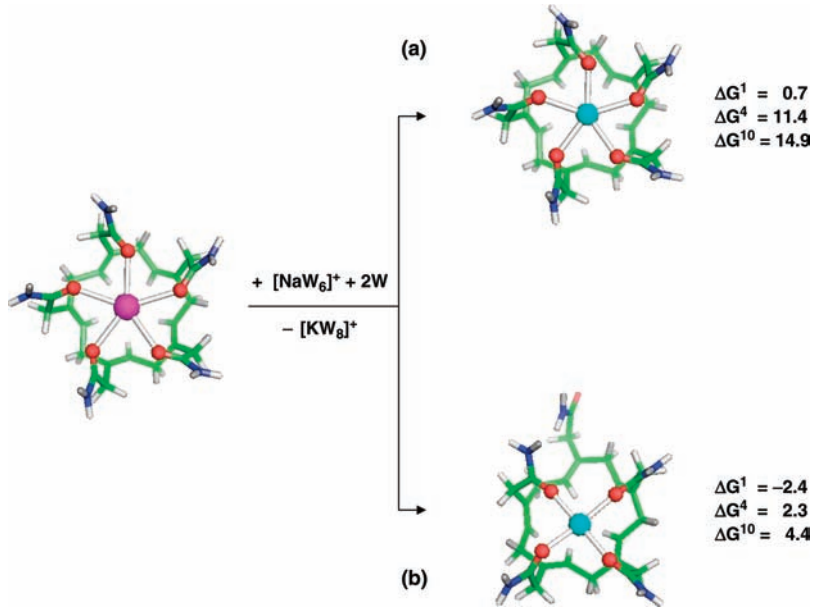

Figure 4. Free energies of $K^+ \rightarrow Na^+$ exchange in a pentameric amide-containing selectivity filter where (a) Na^+ binds to 5 carbonyl dipoles and (b) 4 carbonyl dipoles (in kcal/mol). Color scheme: green, C; blue, N; red, O; gray, H; magenta, K; and cyan, Na.

selectivity: the ΔG^x values ($x = 4-20$) for all the reactions in Table 3 have the same sign. In particular, the ΔG^4 and ΔG^{20} for the reactions involving KW_6 and KW_7 in Table 3 differ by ≤ 2 kcal/mol. Notably, the ΔG^x ($x = 4-20$) for reactions 10−12 in Table 3 are in accord with several independent experiments showing that valinomycin, which provides 6 carbonyl ligands (Figure 1), selects K^+ over Na^+ in solvents with dielectric constants ranging from 2 (hexane), 9 (dichloromethane), 33 (methanol) to 80 (water).[19]

(4) Dependence on the Filter's Number of Carbonyl Groups. To determine the effect of *the number of carbonyl groups* lining the filters on ion selectivity, the ion exchange free energies for filters containing 4, 5, 6, and 8 amide groups were compared. Increasing the number of metal-coordinating carbonyl groups in the pore increases the selectivity for K^+ over Na^+ (the ΔG^x for a given K^+ hydration number becomes more positive), thus rendering filters containing 8 carbonyl groups the most selective within the series. For example, the ΔG^4 and ΔG^{20} values for the last reaction in each triad are 6.0 and 10.2 kcal/mol for filter4 (reaction 3), 11.4 and 16.2 kcal/mol for filter5 (reaction 9), 13.4 and 17.2 kcal/mol for filter6 (reaction 12), and 23.1 and 27.0 kcal/mol for filter8 (reaction 15). Thus, the last reaction in Table 3 has the most positive free energies in the series, implying that a filter that provides 8 carbonyl ligands for K^+ and an environment that favors an octahydrated K^+ (see above and Discussion), as observed experimentally in KcsA channels,[7] secures the best conditions for K^+ selectivity.

To explain the effect of the number of coordinating carbonyls lining the filter on the ion exchange free energies, the incremental binding free energy, $\Delta G^M(n \rightarrow n+1)$, for $[M(HCONH_2)_n]^+ + HCONH_2 \rightarrow [M(HCONH_2)_{n+1}]^+$ (M = Na or K, $n = 0-4$) in the gas phase was calculated at the B3-LYP/6-31+G(3d,p) level. The incremental binding free energy *difference*, $\Delta\Delta G(n \rightarrow n+1) = \Delta G^{Na}(n \rightarrow n+1) - \Delta G^K(n \rightarrow n+1)$, gradually decreases in magnitude with an increasing number of formamides coordinated to the metal cation: $\Delta\Delta G(0 \rightarrow 1) = -8.7$ kcal/mol, $\Delta\Delta G(1 \rightarrow 2) = -6.8$ kcal/mol, $\Delta\Delta G(2 \rightarrow 3) = -3.0$ kcal/mol, $\Delta\Delta G(3 \rightarrow 4) = -1.1$ kcal/mol, and $\Delta\Delta G(4 \rightarrow 5) = -0.2$ kcal/mol. This trend indicates that when the number of ligands is small, Na^+ binds to formamides more favorably than K^+, as it has a higher charge density than the competing K^+. However, since the ionic radius of Na^+ is smaller than that of K^+ for the *same* coordination number, increasing the number of formamides around the smaller Na^+ increases the ligand−ligand repulsion more than that in the respective K^+ complexes, thus diminishing the free energy gain resulting from the more favorable Na^+−ligand interactions. As a result, binding of a fifth formamide ligand to Na^+ or K^+ already bound to four formamides is equally favorable (see above).

The gas-phase ion exchange free energies for the model filters (Table 3) follow the aforementioned trend in the changes: For the smallest tetrameric filter and hexahydrated K^+, the ΔG^1 in Table 3 is negative as favorable charge−dipole interactions outweigh unfavorable dipole−dipole repulsion, stabilizing the Na^+ complex relative to the respective K^+ complex in the filter more than that in aqueous solution. This results in a net negative ΔE_{el} (Table 3, reaction 1, −3.1 kcal/mol). However, for the larger filter5, filter6 and filter8, the ΔG^1 values in Table 3 become positive as the increasing number of coordinating dipoles and dipole−dipole repulsion diminish the stability of the Na^+-bound filters relative to the respective K^+-bound ones, yielding net positive ΔE_{el} (Table 3, reaction 7, 10, and 13). Solvation does not alter this tendency.

(5) Dependence on the Filter's Flexibility. The trends deduced so far are based on the assumption that the metal coordination number in the selectivity filter does not change upon $K^+ \rightarrow Na^+$ substitution, i.e. the incoming Na^+ binds to the same number of channel coordinating groups as the outgoing K^+, implying a rigid pore. For pores lined with more than four ligating groups, Na^+ binding is less favorable due to the increased repulsive interactions among the channel ligands (see above), so Na^+ may prefer to bind to fewer ligands than K^+. To evaluate the effect on the channel's ion selectivity if its walls were flexible enough to allow Na^+ to adopt its preferred coordination geometry, a Na^+-bound pentameric filter was modeled with Na^+ coordinated to only four of the five carbonyl groups (Figure 4b). Comparison of the resulting $K^+ \rightarrow Na^+$ free energies with those for the "rigid" counterpart, where Na^+ is

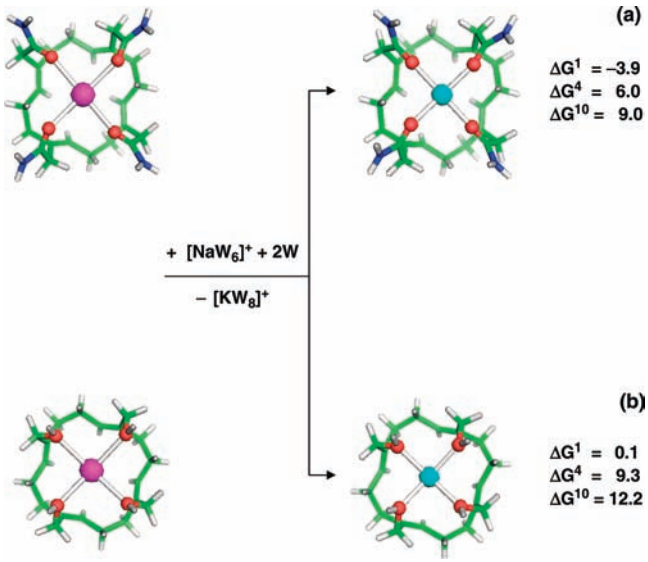

Figure 5. Free energies of $K^+ \rightarrow Na^+$ exchange in selectivity filters with (a) 4 $-CONH_2$ coordinating dipoles and (b) 4 $-OH$ ligating groups (in kcal/mol). Color scheme: green, C; blue, N; red, O; gray, H; magenta, K; and cyan, Na.

bound to all five carbonyl groups (Figure 4a), shows that decreasing the number of filter ligands that directly coordinate to Na^+ upon metal substitution *lowers* the selectivity of the channel for K^+. This is evidenced by the less positive ΔG^x for tetracoordinated Na^+ (Figure 4b, $\Delta G^4 = 2.3$ kcal/mol) compared to pentacoordinated Na^+ (Figure 4a, $\Delta G^4 = 11.4$ kcal/mol). This result is consistent with previous DFT/B3-LYP calculations showing that decreasing the number of metal-bound ligands to 6 or 5 upon $K^+ \rightarrow Na^+$ substitution reversed ion selectivity in favor of Na^+.[17,18]

Selectivity Filters Lined with Hydroxyl Groups. Although the selectivity filters of K^+ channels are lined with carbonyl ligands only, it is not clear whether the K^+/Na^+ selectivity is a unique property of these carbonyl groups or if it could be achieved with other ligating groups lining the pore. To shed light on this issue, a tetrameric filter lined with 4 hydroxyl groups mimicking Ser/Thr side chains (Figure 2c) was modeled and its selectivity was compared with that of its carbonyl-containing analogue (Figure 2a). The results in Figure 5 reveal that the filter with hydroxyl ligating groups not only is selective for K^+ but is even more selective for K^+ over Na^+ than its carbonyl-containing counterpart: The ion exchange free energies for the filter with 4 $-OH$ groups are all positive and larger than those for the filter with 4 $-CONH_2$ groups. This is because the smaller dipole moment of the $-OH$ moiety compared to that of $-CONH_2$[35] attenuates the binding strength in Na^+ complexes to a greater extent than the respective K^+ complexes. These results are in line with those of Noskov et al. who found that decreasing the dipole moment of the coordinating group (weaker field strength[15]) increases the K^+/Na^+ selectivity of the channel.[12] Thus, selectivity filters lined with hydroxyl groups are also suited for selecting K^+ over Na^+, like their carbonyl-containing counterparts.

Dependence on the Incoming/Outgoing Ion's Environment. Since recent studies[18,19] showed that "the binding-site environment is distinctly not a liquid environment", the above free energies (in Table 3 and Figures 4 and 5) were computed for ion exchange in the vicinity of the selectivity filter with the same dielectric environment for all participating entities (see Methods); viz.,

$$[Na^+\text{-aq}](\varepsilon{=}x) + [K^+\text{-channel}](\varepsilon{=}x) \rightarrow [Na^+\text{-channel}](\varepsilon{=}x) + [K^+\text{-aq}](\varepsilon{=}x) \quad (5a)$$

To determine if the above trends and conclusions would change if the incoming/outgoing metal ion and water molecule(s) were in bulk water, rather than in the same environment as the ion-binding site complexes, we computed the free energies for

$$[Na^+\text{-aq}](\varepsilon{=}80) + [K^+\text{-channel}](\varepsilon{=}x) \rightarrow [Na^+\text{-channel}](\varepsilon{=}x) + [K^+\text{-aq}](\varepsilon{=}80) \quad (5b)$$

The free energies for reaction 5b (Supporting Information Table S2) show the same trends as those for reaction 5a (Table 3). For the filter with 4 $CONH_2$ groups, the free energy difference between reactions 5b and 5a for $x = 4$ is -1.5, 0.4, and 3.6 kcal/mol for hexa-, hepta-, and octahydrated K^+, respectively (compare first 3 reactions in Supporting Information Table S2 with those in Table 3). It decreases as x increases and becomes negligible (<1 kcal/mol) for $x = 20$.

Discussion

In previous works, different model systems varying in size and composition and methods (molecular dynamics, Brownian dynamics, quantum mechanics) were used to evaluate the roles of various factors in the selectivity of K^+ over Na^+ in monovalent ion channels (see Introduction).[9−12,14,16−19,24,27] In contrast, the same methodology was used herein to evaluate how the competition between K^+ and Na^+ for the channel depends on (i) the cation hydration number, (ii) the selectivity filter coordination number; i.e., the number of coordinating dipoles provided by the filter, (iii) the chemical type of the ligating groups, and (iv) the dielectric constant of the medium. Furthermore, most previous works propose a key factor dominating the K^+/Na^+ selectivity in monovalent ion channels (see Introduction); e.g., the rigidity of the selectivity filter in the "snug-fit" mechanism,[6,13,30] the type/chemistry of coordinating ligands in the "carbonyl-repulsion" mechanism,[12,16,28] or the number of coordinating ligands.[14,17,18] In contrast, the present analysis indicates that there appears to be no single universal determinant of the K^+/Na^+ selectivity in K^+ channels in general.

Instead, a well-balanced combination of several favorable factors determines the metal ion selectivity in these structures, as outlined below. However, one of these factors may dominate for a specific K^+ channel such as the KcsA channel from *Streptomyces lividans* whose structure has been solved. Thus, we have also performed detailed all-atom molecular dynamics and free energy perturbation simulations for the KcsA channel to evaluate *quantitatively* the magnitude of the various contributions (e.g., attractive ion−dipole interactions vs repulsive dipole−dipole and ion−ion repulsion, and rigidity vs flexibility of the channel protein) to the observed K^+/Na^+ selectivity of this particular K^+ channel; this work will be reported elsewhere. Below, we summarize how each of the aforementioned factors *qualitatively* affects the K^+/Na^+ selectivity in monovalent channels.

K^+ Hydration Number and Pore's Dielectric Constant. Relative to hexahydrated Na^+, an increase in the K^+ hydration number inside the pore favors K^+ over Na^+ (Table 3). Thus, an octahydrated K^+ in the pore optimizes K^+ selectivity, consistent with the experimental observation of an octahydrated K^+ inside the KcsA cavity.[7] Recent studies suggest a correlation between

the K^+ hydration number and the dielectric constant ε of the medium: a lower dielectric constant, characteristic of metal-binding sites with *lower* solvent accessibility, favors a *higher* K^+ hydration number.[18,19] Thus, Nature may exert control on the cation hydration number in the vicinity of the selectivity filter by regulating the dielectric properties of the pore.

Because the solvent exposure and thus dielectric constant inside the pore is inversely correlated with the K^+ hydration number, which in turn determines K^+/Na^+ selectivity, different degrees of solvent exposure affect the degree of K^+/Na^+ selectivity. Thus, a pore that provides a lower dielectric environment for the passing cation is expected to support a higher K^+ hydration number and consequently, a higher K^+/Na^+ selectivity. Conversely, ion-binding sites in the pore that are exposed to solvent (with a high dielectric constant) would yield a lower K^+ hydration number and reduced K^+/Na^+ selectivity. These findings are in accord with experiments showing that in low-dielectric solvents such as hexane ($\varepsilon = 2$) and dichloromethane ($\varepsilon = 9$), valinomycin prefers binding K^+ to Na^+ by -7.6 kcal/mol, whereas in a higher dielectric solvent such as methanol ($\varepsilon = 33$) where the K^+ hydration number is lower, the K^+/Na^+ selectivity is reduced to -5.4 kcal/mol[19] (see also Table 3 where $\Delta G^4 = 13.4$ kcal/mol for reaction 12 involving octahydrated K^+, but $\Delta G^{20} = 5.5$ kcal/mol for reaction 9 involving hexahydrated K^+).

Number of Coordinating Dipoles. The KcsA potassium channel provides 8 carbonyl ligands (4 from each of the two adjacent tetrameric rings) to coordinate the permeating ion. This arrangement and number of coordinating dipoles in the filter secures the highest K^+/Na^+ selectivity: the $\Delta G(K^+ \rightarrow Na^+)$ values for reactions 13−15 in Table 3 are more positive than those for filters lined with fewer carbonyl groups. Previous studies have emphasized the role of the 8-fold binding site in determining the K^+ channel selectivity.[13,17,18,24,31] On the other hand, the present calculations indicate that selectivity filters departing from the "magic" 8-fold coordination number, containing 4, 5, or 6 ligating groups can also select K^+ over Na^+, albeit to a lesser extent. For example, a filter lined with 6 instead of 8 carbonyl groups (see Figure 3a), resembling the valinomycin pore, can also select K^+ over Na^+ (positive free energies for reactions 10−12 in Table 3). In contrast to the intrinsic properties of the ligating carbonyl dipoles, previous theoretical studies focused on the higher rigidity of the valinomycin pore relative to that of the KcsA filter (see below), creating a specific cavity size matching the K^+ but not the Na^+ size, in explaining the K^+/Na^+ selectivity of valinomycin.[16,19]

Chemical Type of Coordinating Groups. The present calculations on a tetrameric filter containing four $-OH$ instead of $-CONH_2$ groups (Figure 5) show that K^+/Na^+ selectivity in ion channels is not a prerogative of the carbonyl moieties. In fact, hydroxyl groups, whose dipole moments are smaller than amide carbonyl dipoles, can even better discriminate between K^+ and Na^+ (favoring K^+) than their carbonyl counterparts. This finding is in accord with recent studies by Thomas et al.[17] who found that K^+ over Na^+ selectivity increases with decreasing dipole strength of the ligands for a given filter coordination number. Thus, it appears that, as far as the selectivity of the pore is concerned, Nature has chosen backbone carbonyl groups to line the selectivity filters of K^+ channels based not solely on their chemical properties but capitalizing on some other (physicomechanical) characteristics of these entities that enable the filter to (i) maintain proper stiffness/flexibility and (ii) provide an optimum dielectric environment ensuring maximum selectivity.

Pore Flexibility. Molecular dynamics simulations on KcsA channel have shown that its selectivity pore is quite flexible and therefore it does not select K^+ over Na^+ by providing a metal-binding site of an optimal fixed size for K^+ (see Introduction).[12,16] However, the pore should maintain some degree of stiffness to prevent conformational changes that allow the "rival" Na^+ to adopt its preferred coordination geometry and diminish/annihilate the channel selectivity for K^+ (see Figure 4). Thus, the pore should enforce Na^+ to adopt an unfavorable 8-fold coordination to enhance the K^+/Na^+ selectivity, in accord with previous findings.[18,27] Note that some simulation/energy minimization studies produce structures where Na^+ is penta or hexacoordinated to carbonyl and water ligands inside the KcsA selectivity filter.[58,59] However, reducing the Na^+ coordination number inside the filter may decrease or abolish the K^+/Na^+ selectivity of the channel, unless the Na^+ binding provokes such large conformational distortions of the pore that effectively occlude the permeation pathway and render Na^+ conductance unfavorable.[59] On the other hand, selectivity for K^+ over Na^+ was found to be maintained in a model composed of eight freely fluctuating carbonyl groups, intended to mimic the KcsA channel *without* any structural rigidity.[12,16] However, this model still retained some structural rigidity as it assumed the same coordination number for K^+ and Na^+.

Implications for Ion Selectivity in Ion Channels. The above results indicate that high selectivity of K^+ over Na^+ could be achieved from a combination of several favorable factors: (1) an octahydrated permeating K^+, (2) a filter lined with 8 carbonyl ligands, and (3) finely tuned physicomechanical properties of the channel walls providing a low dielectric medium favoring a high hydration number for the permeating K^+ and enough stiffness to force the competing Na^+ to adopt an unfavorable 8-fold coordination (Table 3, reaction 15). This implies that the intrinsic properties of the native ion, the metal-coordinating ligands, and the protein matrix could all contribute to ion selectivity in a given ion channel.

The factors affecting ion selectivity revealed herein help to rationalize why valinomycin and the KcsA ion channels are highly K^+-selective, whereas the NaK channel is nonselective, conducting both K^+ and Na^+. The KscA selectivity filter is highly selective for K^+ as it is lined with eight carbonyl groups and is found to contain an octahydrated K^+,[7] consistent with its relatively low solvent accessibility (see above). In addition, the KcsA channel may be sufficiently rigid to force Na^+ to adopt the same 8-fold coordination as K^+ in the filter. This combination of an *eight*-fold binding site and an *octahydrated* K^+ in a *low* solvent-accessible pore and a *not* too flexible KcsA selectivity filter would provide the best conditions for K^+ selectivity. On the other hand, the valinomycin selectivity filter is also selective for K^+ as it is lined with six carbonyl groups, and is found to be relatively stiff.[16,19] Unlike the K^+-selective valinomycin or KcsA ion channel, the nonselective NaK channel is wider and more solvent exposed,[8] which would favor a *lower* K^+ hydration number.[18] Furthermore, the NaK pore maybe more *flexible* than the KcsA selectivity filter,[28] which might allow Na^+ to adopt its preferred coordination geometry.[14] Thus, compared to the KcsA ion channel, the decreased Na^+ coordination number and K^+ hydration number as well as the decreased

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number of coordinating carbonyl dipoles contribute to the observed nonselectivity of the NaK pore.

The present calculations predict that other selectivity filters containing a different number/chemical type of coordinating groups could also select K^+ over Na^+. Contrary to previous findings, neither the "magic" selectivity filter coordination number of 8 nor the presence of backbone amide groups in K^+ channels is required to select K^+ over Na^+. Selectivity filters with a different coordination number and/or serine side chains instead of backbone amides could also exhibit K^+/Na^+ selectivity. However, increasing the number of coordinating groups in the pore and, thus, the K^+ coordination number enhances the K^+/Na^+ selectivity. The various factors affecting ion selectivity revealed herein could help guide the design of a binding site selective for either K^+ or Na^+.

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Supporting Information Available: Complete ref 46; calculated dipole moments of H_2O, CH_3OH and $HCONH_2$ at different levels of theory; optimized structures of Na^+ and K^+ complexes with 18-crown-6 ether; optimized structure of K^+ complex with filter3-OH; and thermodynamic parameters for the reactions in Table 3 but with the hydrated ions and water molecules in bulk water ($\varepsilon = 80$). This material is available free of charge via the Internet at http://pubs.acs.org.

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