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Selective Complexation of K^+ and Na^+ in Simple Polarizable Ion-Ligating Systems

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Abstract: An influx of experimental and theoretical studies of ion transport protein structure has inspired efforts to understand underlying determinants of ionic selectivity. Design principles for selective ion binding can be effectively isolated and interrogated using simplified models composed of a single ion surrounded by a set of ion-ligating molecular species. While quantum mechanical treatments of such systems naturally incorporate electronic degrees of freedom, their computational overhead typically prohibits thorough dynamic sampling of configurational space and, thus, requires approximations when determining ion-selective free energy. As an alternative, we employ dynamical simulations with a polarizable force field to probe the structure and K^+/Na^+ selectivity in simple models composed of one central K^+/Na^+ ion surrounded by 0−8 identical model compounds: *N*-methylacetamide, formamide, or water. In the absence of external restraints, these models represent gas-phase clusters displaying relaxed coordination structures with low coordination number. Such systems display Na^+ selectivity when composed of more than ∼3 organic carbonyl-containing compounds and always display K^+ selectivity when composed of water molecules. Upon imposing restraints that solely enforce specific coordination numbers, we find all models are K^+-selective when ∼7−8-fold ion coordination is achieved. However, when models composed of the organic compounds provide ∼4−6-fold coordination, they retain their Na^+ selectivity. From these trends, design principles emerge that are of basic importance in the behavior of K^+ channel selectivity filters and suggest a basis not only for K^+ selectivity but also for modulation of block and closure by smaller ions.

Recent growth in knowledge of biological ion transport protein structure has motivated investigations to identify principles underlying ion-selective binding site design. A potentially incisive means of isolating and directly probing specific design principles lies in the construction of simplified ion-ligating model systems whose ionic selectivity (taking bulk water as a reference) can be determined computationally.[1−10] Such systems are composed of a single ion, such as K^+ or Na^+, interacting with a set of surrounding molecular species. The functional groups of these surrounding species are typically chosen to interrogate the effect of ligand chemistry on specific local interactions. For example, to model ion interactions with the peptide backbone, simple uncharged compounds like formamide, formaldehyde, or *N*-methylacetamide (NMA) are chosen to surround the ion.

Despite the simplicity of such models, subtleties in their design and analysis can lead to quantitatively or even qualitatively different ion-selective behavior. This notion manifests itself most apparently in simplified model studies aiming to probe mechanistic aspects of K^+ selectivity over Na^+ in carbonyl-lined K^+ channel binding sites.

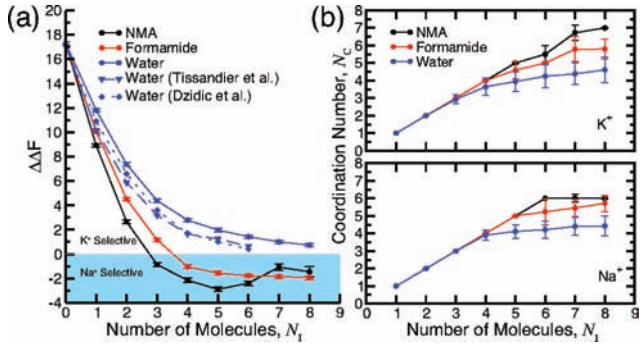

Figure 1. Analysis of unrestrained (gas-phase) simplified ion-ligating models. (a) Helmholtz free energy of selectivity versus the number of (NMA, formamide, or water) molecules, N_I, in the cluster, calculated as $\Delta\Delta F = \Delta F_{\text{model}}^{K\rightarrow Na} - \Delta F_{\text{bulk}}^{K\rightarrow Na}$, where $\Delta F_{\text{model}}^{K\rightarrow Na}$ and $\Delta F_{\text{bulk}}^{K\rightarrow Na}$ are free energies to alchemically transform $K^+\rightarrow Na^+$ in the cluster and in bulk water, respectively. $\Delta\Delta F > 0$ and $\Delta\Delta F < 0$ indicate K^+ and Na^+ selectivity, respectively. For comparison we show experimental values for water molecules[19,20] (blue dashed/dotted lines). Error bars were obtained by block averaging (see Supporting Information). (b) Average coordination number, N_C, versus the number of molecules, N_I, included in the cluster. Vertical bars represent standard deviation of the sample.

For example, one such set of studies concluded that the eight carbonyl (C═O) ligands of a canonical K^+ channel binding site, by virtue of their electrostatic properties alone (especially their large dipole moment), are intrinsically suited to select K^+ over Na^+.[4,5,11] Moreover, it was suggested that K^+ selectivity is entirely lost or even reversed when replacing a model's C═O ligands with water molecules.[5,11] Thus, it was argued to be unlikely that a Na^+-selective site could be formed exclusively by backbone C═O ligands.[11] However, other simplified model studies found this trend (i.e., loss or reversal of K^+ selectivity) upon C═O/water replacement could not be reproduced,[1,2,8−10] and closer investigation suggested external restraints (i.e., architectural, topological, or structural forces) from the protein *are crucial* for K^+ selectivity by the C═O ligands.[1−3,7−10,12−15]

More recent works,[16,17] though oppositional, do not present evidence or theory that conflicts the conclusions of the latter studies[1−3,7−10,12−15] but vigorously question the methods used in their simplified model calculations. While such questioning is mostly abated by the literature,[1,3] the outstanding issue pertains to those works[7−10,15] that employ quantum mechanical (QM) methods to investigate selectivity in simplified models.[17] Although such approaches are attractive because they explicitly account for electronic degrees of freedom, questions are raised[16,17] because their free energy estimates are based on optimized molecular configurations around K^+/Na^+ and only implicitly consider the effects of dynamic sampling.

In this study, we consider simple models, comprised of one ion and 0−8 surrounding model compounds—NMA, water, or forma-

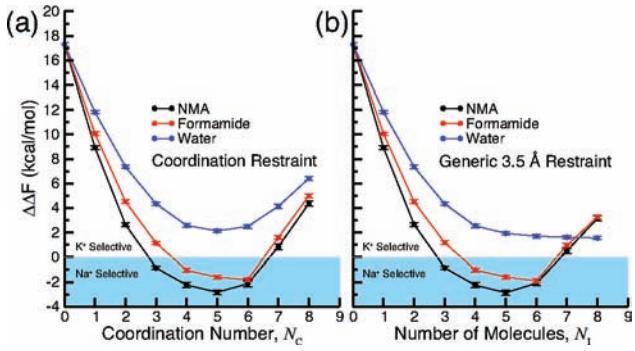

Figure 2. Simplified models with external restraints. Helmholtz free energy of selectivity as a function of the number of molecules (a) coordinating K^+/Na^+ and (b) included in a model imposing 3.5 Å volume-confinement. Error bars were obtained by block averaging.

mide—using the AMOEBA polarizable force field[18] (see Supporting Information). These models are more computationally accessible and simultaneously model polarizability, as available in QM studies. As such, we are able to sample configurational space using molecular dynamics simulation to obtain precise free energy measures of K^+/Na^+ selectivity. Furthermore, we can probe the models' emergent coordination structure to see how it affects K^+/Na^+ selectivity.

Figure 1a displays the Helmholtz free energy of selectivity with respect to bulk water, $\Delta\Delta F$, for models where surrounding compounds are unrestrained (i.e., gas phase). For 0−2 molecules, all such models produce K^+ selectivity (positive $\Delta\Delta F$). However, as the number of included molecules, N_I, is increased toward eight, all models produce $\Delta\Delta F$ approaching the expected bulk liquid values for their respective compositions. As N_I becomes very large, $\Delta\Delta F$ is expected to reach exact bulk liquid values. For example, bulk water is definitively nonselective ($\Delta\Delta F \equiv 0$), and prior work (as pointed out by many[3,7,8]) suggests that $\Delta\Delta F$ in the organic solvents is either Na^+-selective ($\Delta\Delta F < 0$) or nonselective.[18,21] In contrast, widely used pairwise additive models of liquid NMA and formamide are known to provide K^+ selectivity in the range of ∼1.3−3.8 kcal/mol, depending on the model.[4,18] However, it is of particular interest that all models composed of water molecules in Figure 1a (blue data) are invariably more K^+-selective than the C═O-containing models.

Analyzing ion−oxygen coordination in these models (see Supporting Information), we find that the number of molecules included in each model, N_I, is *not equal to* the number of molecules actually coordinating the ion, N_C (Figure 1b). Thus, one may not necessarily use such gas-phase models to draw conclusions about the effect of N_C on $\Delta\Delta F$ as suggested by recent work.[16] Figure 1b shows that, upon including more than ∼4 (water, NMA, or formamide) molecules around K^+ or Na^+, second and/or third solvation shells form (see also Figure S1, Supporting Information). When $N_I = 8$, water provides the lowest N_C (∼4−5 for K^+ and Na^+) of all compounds. Under the same conditions, formamide provides ∼5−6-fold coordination, and NMA provides ∼7-fold and ∼6-fold coordination for K^+ and Na^+, respectively.

If an external potential (see Supporting Information) is imposed to ensure ion−oxygen coordination by all included molecules (i.e., $N_I \equiv N_C$), the selectivity trend in unrestrained models (Figure 1a) is qualitatively reproduced for ∼0−6 molecules (see Figure 2a). However, $\Delta\Delta F$ is increased drastically with ∼7−8 coordinating molecules (Figure 2a) in comparison with the unrestrained models. Coordinating models composed of the organic C═O-containing compounds become K^+-selective when more than ∼6 oxygen atoms coordinate both K^+ and Na^+. Following prior studies,[4,5,11,16] we tested selectivity in models using a "generic" harmonic volume-confinement that restrains all ion−oxygen distances to be less than ∼3.5 Å (Figure 2b). Figure 2 shows that the qualitative trend in such models is the same. However, the generic confinement models (Figure 2b) provide lower (but still positive) $\Delta\Delta F$ for ∼7−8 included molecules. The quantitative difference in K^+ selectivity between the models is explained by observing the dependence of N_C on N_I. Beyond 6−7 molecules, full coordination (i.e., $N_I = N_C$) is not necessarily enforced by the generic confinement (Figure S4, Supporting Information). The difference between N_I and N_C is largest in the 3.5 Å generic confinement model when $N_I = 8$ water molecules. This model provides ∼6−7 and ∼5−6 coordinating water molecules for K^+ and Na^+, respectively (Figure S5, Supporting Information), and as a result, $\Delta\Delta F$ is lower than that provided by the C═O-containing compounds of the corresponding model (Figure 2b, $N_I = 8$).

These unrestrained and restrained models support, despite the caveats[17] suggested for QM-based studies, the qualitative conclusion of prior works:[1−3,7−10,12−15] that sole enforcement of >6-fold coordination by the organic C═O-containing compounds or water is a sufficient, though not a necessary, condition for K^+/Na^+ selectivity. This result is in stark contrast with inferences from pairwise-additive models of C═O-containing ligands,[4,5,11] which are shown to provide a residual K^+ selectivity both in bulk liquids and in simplified models "across the board" (i.e., for all N_I).[1,2,4,5,11,18] Taken together, Figures 1 and 2 suggest that the imposed external restraints applied in this study "cause" K^+ selectivity in models including ∼7−8 C═O-containing compounds. More to the point, the K^+ selectivity in these models (Figure 2) is *not* "caused" or "controlled" by the C═O ligands themselves, nor is it caused by specified (e.g., ion−ligand, ligand−ligand, etc.) interaction energy or entropic contributions (see Discussion in the Supporting Information). Instead, the external potential applied to the ligands generally determines *all* such contributions (Table S1 and Discussion, Supporting Information) to yield a positive net $\Delta\Delta F$. In fact, without the external potential (i.e., "topological" control[1−3,7−10,12−15]), these contributions would yield net Na^+ selectivity (Figure 1a).

Our findings have implications for permeant ion effects on K^+ channel behavior.[22−25] Both Na^+ and K^+ are known to bind the K^+ channel selectivity filter, but at different sites, and with different specific interactions with C═O and water.[13,14,22] If C═O ligands were always K^+-selective, then interactions with smaller ions like Na^+ or Li^+ would not modulate filter block[22,23] or closure.[25] Some modes of Na^+ binding in the filter can involve ∼4−6 C═O ligands[13,14,22] and are favorable. Others will impose larger coordination numbers[4,10,12,13] on both K^+ and Na^+, will be K^+-selective, and provide barriers to Na^+ permeation. These concepts appear rudimentary.

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Supporting Information Available: Discussion, methods, and additional figures from simplified model analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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