Coordination Numbers of K$^+$ and Na$^+$ Ions Inside the Selectivity Filter of the KcsA Potassium Channel: Insights from First Principles Molecular Dynamics

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ABSTRACT   Quantum mechanics/molecular mechanics (QM/MM) Car-Parrinello simulations were performed to estimate the coordination numbers of K$^+$ and Na$^+$ ions in the selectivity filter of the KcsA channel, and in water. At the DFT/BLYP level, K$^+$ ions were found to display an average coordination number of 6.6 in the filter, and 6.2 in water. Na$^+$ ions displayed an average coordination number of 5.2 in the filter, and 5.0 in water. A comparison was made with the average coordination numbers obtained from using classical molecular dynamics (6.7 for K$^+$ in the filter, 6.6 for K$^+$ in water, 6.0 for Na$^+$ in the filter, and 5.2 for Na$^+$ in water). The observation that different coordination numbers were displayed by the ions in QM/MM simulations and in classical molecular dynamics is relevant to the discussion of selectivity in K-channels.

Potassium channels are membrane proteins that can catalyze K$^+$ ions permeation across cellular membranes while simultaneously discriminating the permeation of Na$^+$ ions by several orders of magnitude. To uncover the mechanism of K-channel selectivity in theoretical studies, the KcsA channel has proved to be a particularly useful system, because it is relatively small, and it contains the essential structural elements that are shared by all potassium channels (1). The selectivity of the KcsA channel is believed to originate from the conserved TVGYG signature sequence, which forms a narrow constriction in the tetrameric pore called the selectivity filter (Fig. 1). The K$^+$/Na$^+$ selectivity in the KcsA channel was initially proposed to arise from the geometrical arrangement of ligands in the filter (1), the so-called snug-fit hypothesis. However, molecular dynamics (MD) simulations have shown that the selectivity filter is relatively flexible and that Na$^+$ ions are able to interact with the carbonyl ligands that pave the interior of the selectivity filter (2). This leads to the idea that other interactions, such as the repulsion between carbonyl groups, play an important role in the selectivity (3). In addition, the selectivity has been discussed as arising from the favorable arrangement of the carbonyl in the filter, which may promote high coordination numbers, compared to the ion coordination numbers in water (4,22).

Therefore, the ion coordination numbers (CNs) are key facets for the different theories of selectivity. The ion CNs in the selectivity filter can be computed in MD simulations (2). However, calculations performed with different force fields do not necessarily agree (5). Besides, the use of nonpolarizable force fields in classical MD studies remain problematic, as demonstrated by QM/MM (6,7) studies that

indicate significant ion-induced dipole shifts on the carbonyl groups of the filter. On top of that, the CNs of K$^+$ and Na$^+$ ions in aqueous solution are slightly overestimated by classical nonpolarizable force fields with respect to both scattering experiments (8,9) and ab initio simulations (10,11).

Here, to circumvent these problems, we have studied the CNs of K$^+$ and Na$^+$ ions in the selectivity filter of the KcsA channel using quantum mechanics/molecular mechanics (QM/MM) simulations (12,13). Adequate sampling was obtained by concentrating the computational effort on the (S$_2$) binding site of the filter and on the (0101) occupancy state.

METHODS

The electronic structure problem was solved with DFT and the BLYP density functional (14,15). Although BLYP tends to overestimate the average K-O distances in water by almost 0.2 Å (16) with respect to scattering experiments (8,9), the average ion CNs (17) in water can be accurately reproduced. The QM calculations were performed with a plane-wave cutoff of 80 Ry and a supercell of dimensions ($18 \times 18 \times 16$ Å^3). The valence-core interaction was described by norm-conserving Troullier-Martins pseudopotentials (18) that were generated by including semicore states explicitly in the valence (2s2p for Na$^+$, and the 3s3p states, for K$^+$). MD simulations were run for 2 ns with the AMBER/parm99 force field (19) to generate equilibrated coordinates and compute the classical CNs. For each ion, three QM/MM simulations were then run, starting from different initial coordinates. The first 0.5 ps were used for equilibration, and the remaining 10 ps were used to collect statistics. The adequacy of the sampling time was checked by performing 8×5 ps simulations that were started from different
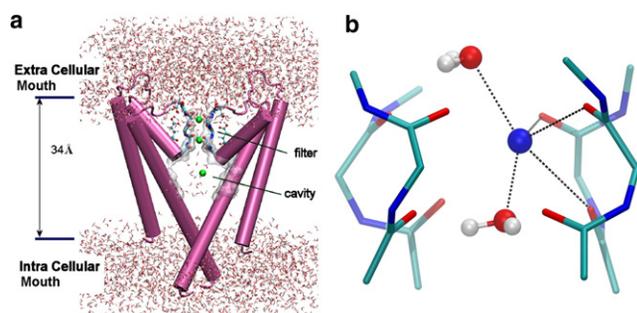
FIGURE 1 Simulation system. (*a*) Cartoon representation of two of the four KcsA units that form the channel. (*b*) S_2 binding site of the selectivity filter. A typical coordination geometry for Na$^+$ in the selectivity filter is shown.

initial CNs. These simulations are described in the Supporting Material. The ion CNs were also determined in water by performing 3×10 ps simulations. In the QM simulations, a water box with 63 H_2O was used. The CN for K$^+$ was calculated by using a distance cutoff d_0 of 3.5 Å, which corresponds approximately to the first minimum of the radial distribution function in water. A distance cutoff d_0 of 3.1 Å was used for Na$^+$.

RESULTS

Overall, the radial distribution function and the CNs are very similar for both the AMBER and BLYP/MM simulations. However, several differences could be observed. In the filter, the first peak in the radial K-O distribution function (Fig. 2 *a*) is centered at 2.85 Å, in AMBER MD, and shifted toward slightly longer distances, 2.93 Å, for BLYP/MM. K$^+$ displays a preferred CN of 6 in water, and of 6 in the filter (Fig. S6). The average CN is 6.2 in water, and 6.6 in the filter. This result suggests that K$^+$ is only very slightly over-coordinated in the filter. A similar result is obtained with AMBER. The preferred CN is 7, both in water, and in the selectivity filter, and the average CN is 6.6 in water, and 6.7 in the filter.
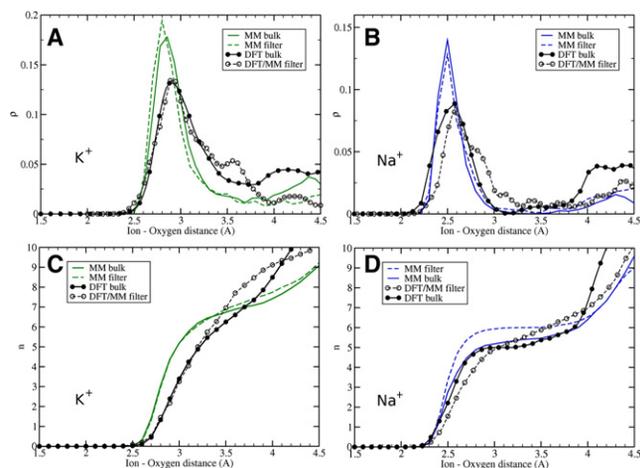

FIGURE 2 Radial distribution functions and coordination numbers in AMBER, and BLYP/MM simulations. (*A*) Radial distribution function for K$^+$, and for Na$^+$ (*B*), and running coordination number $n(r)$ for K$^+$ (*C*), and for Na$^+$ (*D*).

In the case of Na$^+$, the first peak in the radial Na-O distribution function is at 2.45 Å in classical MD, and at 2.55 Å, for BLYP/MM. Na$^+$ displays a preferred CN of 5, both in water and in the filter (Fig. S6). The average CN is 5.0 in water, and 5.2 in the filter. In AMBER MD, the preferred CN is 5 in water, and 6 in the filter. In Table 1, our results concerning the ion CNs are compared with estimates from previous studies. We find that all classical studies indicate a larger value of the CNs compared to DFT/MM.

The width of the filter was monitored by computing the oxygen-oxygen distances between diagonally facing carbonyl groups. In several previous classical MD studies, the filter structure was seen to pinch, in order to cradle Na$^+$ ions (3,20,21). In the DFT/MM simulations, on the other hand, such distortion of the filter structure was not observed. The O-O distance of the Gly-77 residues remained stable between 4.5 and 5.5 Å, also in the presence of Na$^+$ (Fig. S5). Interestingly, QM simulations on cluster models (Table S1 and Fig. S7) indicate that the average Na-O distance is ~2.18 Å for a cluster with a single acetamide ligand, and it increases to ~2.55 Å when five ligands are present. 2.55 Å is also the Na-O distances observed in the filter, which suggests that the carbonyl repulsion is the main source of the long Na-O distances. The neglect of explicit polarization in the classical studies, which leads to an underestimate of the carbonyl-carbonyl repulsions (3,6,7,17), can provide an explanation for the different behaviors of the filter in the presence of Na$^+$, in classical, and DFT/MM simulations.

Inspection of the trajectories reveals that Na$^+$ is often positioned at the transition limit between two adjacent K-binding sites (20), where it is coordinated by one-to-two water molecules, and three carbonyl ligands, in agreement with a recent x-ray structure with Na$^+$, which also indicates that Na$^+$ does not bind at the center of the K-binding sites (23). Alternatively, Na$^+$ can be coordinated by the channel wall, and interact only with two of the four KcsA subunits (Fig. 1 *b*). Both coordination geometries lead to a distortion of the local fourfold symmetry of the filter, which is not observed in classical MD, but which has been reported in a previous QM study (24).

TABLE 1   Average ion coordination numbers

	K$^+$	Na$^+$	Ref.
Bulk water			
OPLS	6.6	6.5	(25)
CHARMM27	6.9	5.9	(25)
AMOEBA	7.0	6.0	(25)
AMBER99	6.6	5.3	This work
DFT	6.2	5.0	This work
Exp.	5.9–6.4	4.4–5.4	(8,9)
Selectivity filter			
GROMOS96	8	6.3	(5)
CHARMM27	8.4	7.1	(26)
AMBER99	6.7	6.0	This work
DFT/MM	6.6	5.2	This work

In conclusion, three main observations can be made from the QM/MM simulations:

Observation 1. The CNs of both K^+ and Na^+ ions are very similar in the filter and in water.

Observation 2. The pore size is not reduced significantly when one K^+ is substituted by Na^+.

Observation 3. The long ion-oxygen distances observed in the filter are similar to those observed in ion-acetamide clusters containing a large (>4) number of ligands.

Taken together, these results suggest that the carbonyl repulsion alone can cause the large Na-oxygen distances observed in the filter, independently of the topological-constraint (e.g., forces) applied by the protein on the carbonyl ligands (4). In gas phase models, the ion-oxygen distance between a K^+ or Na^+ ion and acetamide ligands is elongated by ~0.4 Å when the number of ligands is increased from 1 to 5–6. (K^+ shifts by 0.41 from 2.53 Å to 2.94 Å, and Na^+ shifts by 0.37 Å from 2.18 Å to 2.55 Å). With water molecules, the shifts are considerably smaller, ~0.2 Å (K^+ shifts by 0.21 from 2.68 to 2.89, and Na^+ shifts by 0.24 Å from 2.26 Å to 2.50 Å). This sheds new light on the underlying cause for the ion-ligand distances observed in the selectivity filter, and on the important chemical differences between waters and carbonyls. Furthermore, it suggests that using a polarizable force field that can describe accurately ion-induced polarization effects (16) may be a necessary step to take before we can fully discriminate between different mechanisms of selectivity in theoretical studies of K-channels.

SUPPORTING MATERIAL

Seven figures and one table are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00260-2.

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