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Quantum calculations on water in the KcsA channel cavity with permeant and non-permeant ions

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ABSTRACT

Different ions in the pore of the KcsA channel behave differently, and we relate this to their solvation. We show that the selectivity is dependent, in part, on the solvation in the cavity (sometimes referred to as the vestibule, it is the region containing water molecules between the intracellular gate and the selectivity filter at the extracellular end of the pore). We have shown earlier that potassium is more dependent at the upper end of the cavity region on solvation by the threonines there, while sodium ion has more water molecules as ligands. In addition, sodium ion is placed asymmetrically, while potassium is nearly exactly symmetric with respect to the four-fold symmetry of the channel. We have now extended these calculations to rubidium and cesium ions, and find that rubidium solvation resembles that of potassium (and both are permeant ions), while cesium resembles sodium (and both are non-permeant), in terms of the geometry of up to eight hydrating, and four non-hydrating, water molecules. In each case, a maximum of 12 water molecules are relevant to the calculation. The placement of the water molecules in the two cases is essentially the same as found from the electron density in the X-ray structure of Zhou and MacKinnon. For Na^+ and K^+, we show that energy decreases from bulk to the cavity to the lowest position in the selectivity filter (accurate energy could not be calculated for the heavier ions). A separate calculation shows that fixing the Na^+ ion at the position of the K^+ minimum, followed by re-optimization produced a significantly modified system, not something that could be produced by thermal fluctuations. Moving the K^+ into the Na^+ position in the upper cavity led to a small increase in energy, $\approx 3\ k_BT$, but was accompanied by large shifts in the positions of hydrating waters, which would create a major kinetic barrier. Therefore, thermal fluctuations could not invalidate the conclusions of the main calculations.

1. Introduction

We discussed the selectivity of the KcsA channel in terms of the solvation of the K^+ and Na^+ ions in an earlier paper [1]; positions in the cavity and in the lowest (S4) position of the selectivity filter at the extracellular end of the pore were considered and this work has been extended recently. However, the ions may also find a position slightly further down in the cavity, where all ions can be hydrated. We have now further expanded the calculations to see the water in this central cavity position, and we have found that sodium and potassium ions are hydrated differently. Zhou and MacKinnon [2] obtained very similar results from X-ray structures, in which they found different electron density from water molecules when different ions were present. In addition to Na^+ and K^+, they also studied Rb^+, Cs^+, and Tl^+. We have now carried out *ab initio* calculations for the ions Na^+, K^+, Rb^+, and Cs^+, (there are technical difficulties with Tl^+) finding the same positions for water molecules as would be inferred from the electron density determined by Zhou and MacKinnon, within the limits of resolution of the two techniques. Cs^+ behaves like Na^+, and Rb^+ like K^+. The latter two have rings of water above and below them, while Na^+ and Cs^+ have part of the lower ring missing. Also, Zhou and MacKinnon found two positions for Cs^+ in the cavity, and we found two local energy minima in approximately the same locations (although in our calculation only Na^+ was proven unequivocally not to have two minima; K^+, and other permeant ions, can move along the center of the pore).

There is relatively little literature on ions in the cavity position, the work of Zhou and MacKinnon being the most important for testing our results. However, there have been several molecular dynamics (MD) simulations that have covered this subject [3–5] (see Domene for a review [6]); the related question of the selectivity filter has been more widely studied. Guidoni et al., in an MD study, emphasized the water–helical dipole interactions [7]. They followed this by a density functional study of the water in several positions in the selectivity filter chosen as snapshots of the MD study [8]. Noskov and Roux compared the NaK channel to KcsA, and concluded that differences in hydration were responsible for the differences in selectivity [9] (the NaK channel does not select between Na and K). In an earlier study, they examined the role of carbonyl groups and their electrostatic interactions [10]. Boiteux et al. examined the role of water in both closed and open states in a channel partitioned into sections, using

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both MD and *ab initio* calculations [11]. Going beyond channels, there have been far more studies of water–protein interactions than we can review here, but there is no doubt that water is an important component of proteins generally, so that we should not be surprised by its importance for ion selectivity in channels.

Thomas et al. [12] also found a critical role for the coordination number of the ion in potassium channel selectivity. They used both MD and quantum calculations, although the latter were on a system so small that the reliability of the results is uncertain. They did not allow the adjustment of the liganding oxygens to the ions, so did not find the asymmetric minima for Na^+ and Cs^+. They attributed the selectivity in the end to the restraints on the carbonyl oxygens; we find that there are other oxygens, especially on water, and on the threonine side chain hydroxyls, that are important. Nevertheless, their paper is important as the first attempt to combine MD with *ab initio* calculations on this system.

2. Methods

2.1. Optimizations

The positions of the ions and water molecules were optimized by Hartree–Fock (HF) *ab initio* calculations, with basis sets 3-21G* (Na^+ and K^+) and 3-21G for all four ions; the results for the two basis sets for Na^+ and K^+ were nearly identical, so the additional polarization functions in 3-21G* do not significantly affect the geometry of the

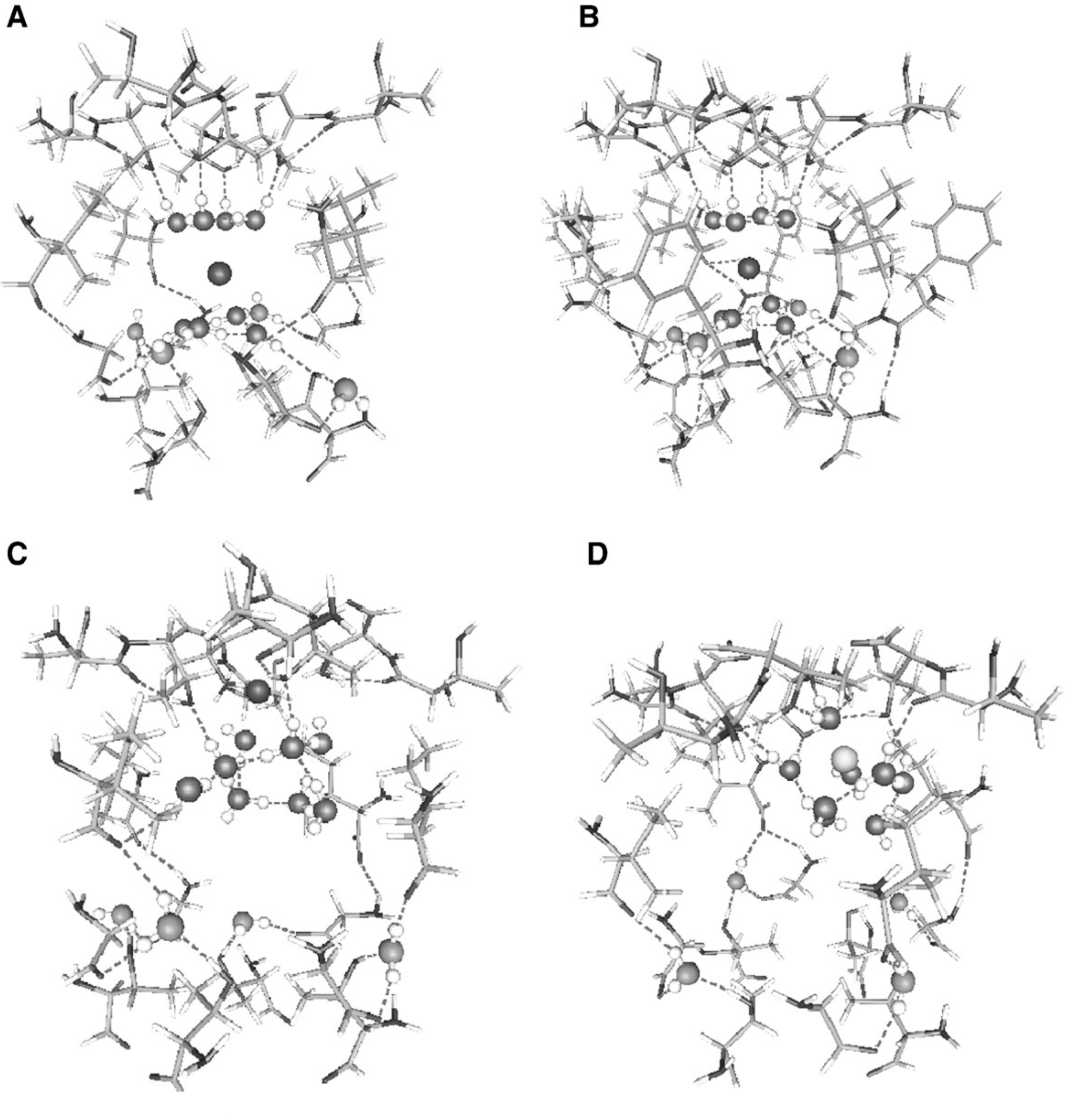

Fig. 1. A) Cavity with 12 water molecules plus K^+ in the *middle* position minimum in the cavity. Those water molecules that hydrate the ion are shown with oxygen atoms as large gray spheres; outer waters (below) also have water oxygens as large gray spheres; both have hydrogen as small white spheres. For the protein (stick diagram), optimized backbone atoms are gray; K^+ ion is the largest sphere; this convention is followed in all subsequent parts. B) The same system, with the side chains of F103 included; everything remains almost exactly the same, including the positions of the waters, showing that whatever the role of the F103 sidechains, they do not affect the hydration of the K^+ ion. C*****) K^+ in a different minimum, further up in the cavity. D*****) Na^+ in the only minimum position it has in the cavity, essentially at the upper position (there is no middle position minimum for Na^+). The ion shading changes, with the non-permeant ions Na^+ and Cs^+ shown light gray rather than dark gray. E) Rb^+ middle position in cavity: like K^+ this is a permeant ion, and, like K^+, it is hydrated above and below with the ion dark gray. F) Cs^+, upper position; this is a non-permeant ion, and is hydrated asymmetrically, very much like Na^+; again, the ion is light gray. G) Cs^+ middle position (Cs^+ has two local minima). The hydration is drastically asymmetric. H) No ion; in the absence of an ion, the water molecules are spaced wide apart; in this calculation the gate is not included, and the channel adopts an open configuration. We have earlier published a discussion of the gate [20], which lies below the region included in this calculation. Our present result (no gate) resembles the open state found by Miloshevsky and Jordan from a normal mode following Monte Carlo simulation [21]. The space is large enough for an ion to enter. *Note: Panels C and D are a replotting of a calculation previously published in reference [1]. All other parts are presented here for the first time.

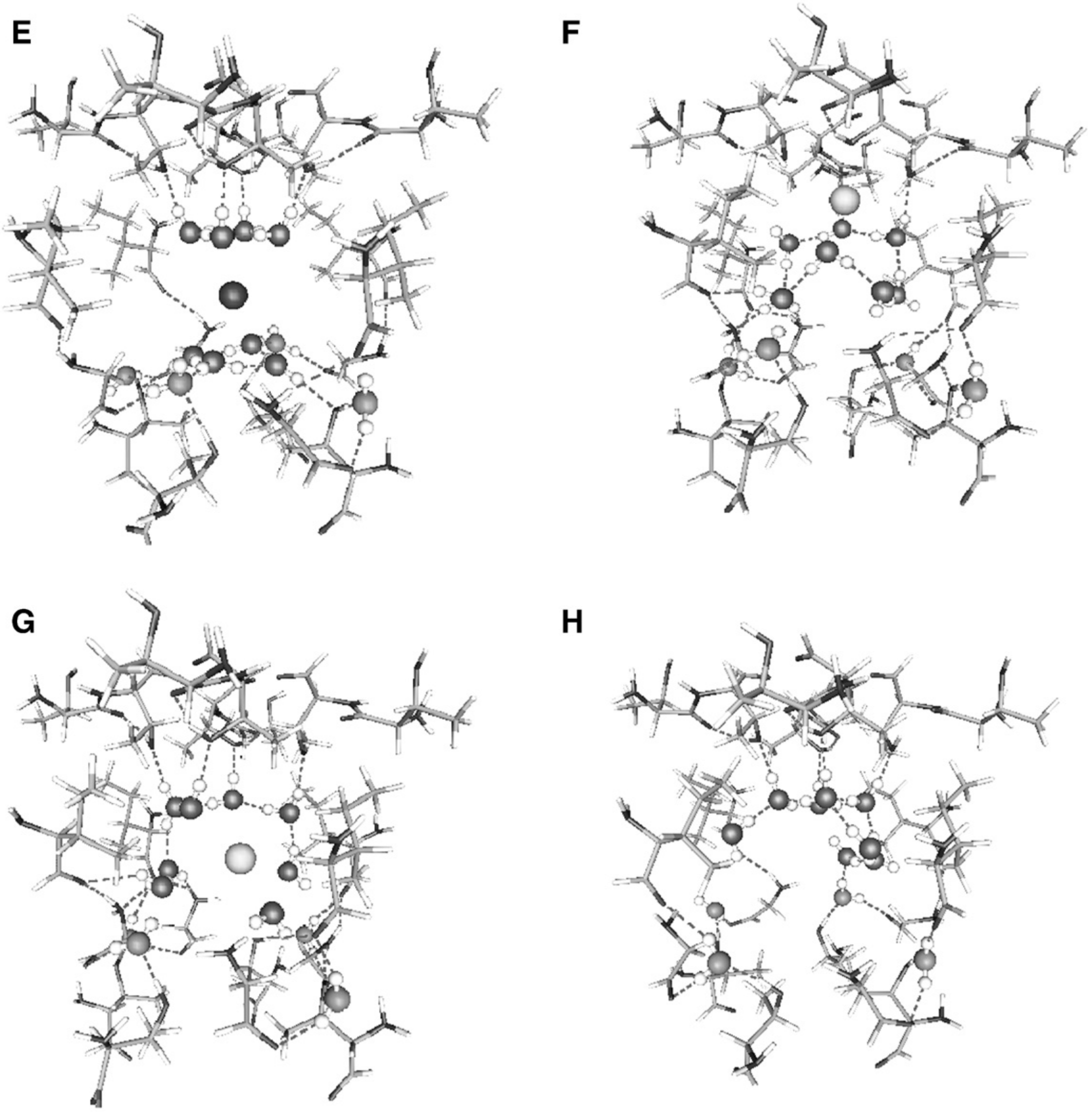

Fig. 1 (*continued*).

minimum, including the positions of the water molecules; all conclusions that could be drawn from 3-21G* calculations are reproduced by 3-21G calculations. Starting positions for the atoms were taken from the X-ray structure for all but H atoms. The system was truncated (see Fig. 1) leaving 341 atoms counting the ion. The side chains of the following amino acids were included: T74, T75, I100, G104, and T107. The outermost methyl groups of the side chain were frozen, with the remainder of the atoms allowed to optimize. The optimizations, except those described in [5] below, were started with the ion in the "middle" position of the cavity, at a position shown in the X-ray structure for K^+. All optimizations were done using NWChem [13] at the EMSL computer facility at Pacific Northwest National Laboratory. For Na^+, there is no minimum in the center position of the cavity, but for K^+, Rb^+, and Cs^+, there is. The energy in the cavity minimum is higher than it is further "up" in the selectivity filter ("up", meaning in the extracellular direction) The cavity position is therefore a local minimum (the permeant ions move up, the non-permeant ions appear to be trapped). For energy results, see Table 1. The results showed that the protein heavy atoms (non-hydrogen) took positions quite close to those they had in the X-ray coordinates relative to each other and to the frozen positions. Truncated groups were replaced with H atoms to maintain correct valence.

2.2. No ion case

A separate optimization was carried out on the cavity without any ion present. This showed how the water places itself with respect to the protein alone (Fig. 1H). The positions of the protein side chains move somewhat outward in the absence of an ion.

2.3. The F103 side chain

For K^+, there is very little difference in the water positions if F103, a highly conserved residue, has its side chain truncated (Fig. 1A, B).

Table 1
Energy of ions in bulk and two positions in cavity

Energy (kJ mol^{-1}) for system	Bulk (expt'l) [19]	Ion plus 12 water molecules[a]	Ion in midcavity	Ion in upper cavity
K^+	−321	−345.7	−397.5	−458.1
Na^+	−405	−416.0	–[b]	−574.5

[a] This is approximately comparable to bulk water: the water molecules were optimized at B3LYP/6-31+G** around the ion (no protein), and the energy calculated as a single point calculation using B3LYP/6-31++G**; all other energy values were determined by single point calculation using B3LYP/6-31G**.

[b] No energy minimum was found for Na^+ near the mid-cavity position, suggesting the existence of a barrier to Na^+ progression in this region.

One optimization including F103 and 18 water molecules instead of 12 (total 421 atoms) was done with K^+; no significant difference in solvation was found. The closest water oxygen was 2.88 Å instead of 2.91 Å with 12 waters and no F103 side chain, and the next three waters were also similar; the closest of these in both cases was approximately 3.7 Å (3.69 Å vs. 3.73 Å), so there is little contribution of the next waters in any case. Therefore, adding waters would not greatly affect the solvation properties we found.

While the lack of effect of the side chain of F103 is not surprising, as the side chain points away from the cavity, it is useful to prove that the internal water does not interact significantly with this conserved residue.

2.4. Single point energy calculations

Single point calculations were done for Na^+ and K^+ using the density functional method B3LYP with basis set 6-31G**, with the system further truncated to 145 atoms (leaving 8 residues, 4 each of T74, and T75, plus water), using Gaussian03 [14]. The energy calculation was not done for the other two ions, as it needs to be correct within at most a few k_BT, which would not be possible for the others.

2.5. Thermal fluctuations

To determine whether thermal fluctuations could play a role, the potassium and sodium ions were placed in the position of the other ion's minimum (Na^+ frozen at the center position, where it has no minimum, K^+ frozen at the upper cavity, asymmetrically in the Na^+ position), at the same coordinates with respect to the atoms that had been frozen in the main calculation. These atoms provided an invariant frame for the remainder of the atoms in all calculations. The rest of the system was re-optimized with the exchanged ion frozen, and the energy recalculated. In each case, the optimization and the energy calculations were done using the same methods as the original. This time the positions of the protein atoms were appreciably different, and the water molecules very different, than in the main calculation, in which the ion was free.

3. Results and discussion

The primary results are the positions of the water molecules with the various ions, so the results are presented in a set of figures, Fig. 1 A–H. These should be compared to the electron densities from Zhou and MacKinnon, as described above. Several principal points are worth noting: (i) K^+ and Rb^+, both permeant ions, have eight-fold coordination by water in the middle position (Fig. 1 A, B, E), and upper position (C). This would leave electron density very much like that shown by Zhou and MacKinnon (ii) the non-permeant Na^+ and Cs^+ move up in the cavity on optimization, and leave an incomplete ring of water behind (Fig. 1 D, F, G). Cs^+ does have a minimum at the center position, unlike Na^+; it has therefore two minima, as in the Zhou and MacKinnon article. (iii) The water alone in the cavity, without ion, adopts a configuration with eight water molecules in the main and upper part of the cavity, plus four below and slightly outside, and this is the same with and without the phenyl sidechain of F103 (Fig. 1H shows the result without the side chain). The diagonal distances between oxygen of waters in the lower part of the cavity, which are not quite arranged in a square, are 9.76 Å and 11.53 Å. This is close to an open channel distance (≈12 Å) as it should be if an ion is to enter the channel. The empty channel shows the eight water molecules from the middle to upper part of the cavity to be arranged in a hydrogen bonded formation that is nearly a ring, plus four more water molecules attached through hydrogen bonds at the lower part of the channel. (iv) The energetics for Na^+ and K^+ show that both ions are at lower energy in the pore than in bulk, and the energy continues to go down as the ion moves toward the extracellular position (Table 1). Because Na^+ does not have a minimum at the middle position of the cavity, the cavity should select against it; experimentally, this is found to be true, albeit with a fraction of the selectivity of the channel as a whole [15].

Fig. 1A, B, E, and G, show ions in the center position: K^+ ion (permeant, A, B), Rb^+ ion (permeant, E) and Cs^+ ion (non-permeant, G). Fig. 1C, D, and F show K^+, Na^+, and Cs^+ ions, in the upper cavity position. As noted above, the X-ray electron density results of Zhou and MacKinnon [2] (see their Fig. 4) correspond very closely with the positions of the water molecules found in our calculation. The Zhou and MacKinnon electron density passes roughly symmetrically beneath the K^+ and Rb^+ ions, while the Na^+ and Cs^+ have incomplete spheres of hydrating water, with water above but not below, as in our calculation. They find two minima for Cs^+, but only one for Na^+, both the same as our result. However, we find two minima for K^+, while only one is apparent in the X-ray results; our second minimum was found by moving the ion up 3 Å from its central minimum, and reoptimizing starting from that position. The fact that K^+ can find a local minimum at lower energy in the upper cavity than the center on the path it would follow into the selectivity filter implies that the permeant ions move up (the energy of the upper minimum is lower than that of the middle position minimum). Rb^+ was not calculated for the upper position, but it should behave similarly to K^+.

A quantum calculation produces a low temperature (actually, 0K) result; it should agree with X-ray data, which are also low temperature (well above 0K, but well below the glass transition temperature of water, 220K). The fact that it does agree is evidence that the truncation of the system is not a significant problem. However, this leads to the question of relevance of the calculation, as well as of the X-ray structure, to room temperature channels. Here, we note that the agreement of X-ray structures with room temperature results is generally good, especially for the fairly rigid part of the channel with which this calculation is concerned. This is one more indication that thermal fluctuations are not a major problem for the interpretation of the results.

Table 1 shows the energy of the Na^+ and K^+ ions in the bulk, and the key positions in the cavity. Unfortunately, the methods that can be applied as far as atomic number 20 (approximately) cannot yet be applied to atoms as heavy as Rb or beyond with adequate accuracy. As we noted previously, the energy differences are large compared to thermal fluctuations. They are actually appreciably over 100 kJ for both Na^+ and K^+. This is accounted for by the greater degree of coordination for the ions in the cavity than in bulk, and the increasing tightness of the coordination. Similar effects have been noted by Varma and Rempe [16–18].

In addition to the initial calculations, we tested for the possibility that thermal fluctuations could significantly affect the system. Replacing the K^+ at its minimum position in the center of the cavity by Na^+, followed by re-optimization of all the atoms that had been optimized in the original calculation other than the ion (which is fixed in this calculation), led to a complete rearrangement of the water molecules at a much higher energy—so much so that the configuration was clearly impossible. When K^+ was placed in the position of the Na^+, however, there was a significant rearrangement of the water molecules, but not so much as to make a completely unrecognizable configuration. The energy increase was only a little more than 3 k_BT. It is less clear that this was a completely impossible position for the K^+ to reach, but it is clear that it is highly unlikely. Also, the global rearrangement of the water would be fairly slow and would have to follow an improbable path—that is equivalent to saying that there would be a large kinetic barrier preventing the rearrangement of the position of the ion. Therefore, the differences in Na^+ and K^+ positions, and configurations of hydrating molecules, are real, not easily erased by thermal fluctuations at room temperature. The fact that Na^+ cannot stay on a path to the upper cavity is consistent with the selectivity that is observed.

4. Conclusions

1) Permeant and non-permeant ions are hydrated differently in the cavity region of a potassium channel. The calculated difference appears to be consistent with what has been found experimentally from X-ray crystallography.
2) Na^+ cannot be placed in the center of the cavity (there is no minimum at that point, nor in the immediate vicinity), and the cavity should be selective against Na^+. However, Cs^+, also non-permeant, does have a minimum at the cavity center, albeit with hydration very different from that of permeant ions.
3) The energy of Na^+ and K^+ ions shows that they are at lower energy in the cavity than in the bulk, and comparison with our earlier calculations shows they continue to decrease in energy in moving up in the cavity.
4) These conclusions are unlikely to be altered by thermal fluctuations.

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