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Rapid report

Potassium and sodium ions in a potassium channel studied by molecular dynamics simulations

P.C. Biggin [a,b], G.R. Smith [a], I. Shrivastava [a], S. Choe [b], M.S.P. Sansom [a,*]

[a] *Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, The Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK*

[b] *The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92036, USA*

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Abstract

We have performed simulations of both a single potassium ion and a single sodium ion within the pore of the bacterial potassium channel KcsA. For both ions there is a dehydration energy barrier at the cytoplasmic mouth suggesting that the crystal structure is a closed conformation of the channel. There is a potential energy barrier for a sodium ion in the selectivity filter that is not seen for potassium. Radial distribution functions for both ions with the carbonyl oxygens of the selectivity filter indicate that sodium may interact more tightly with the filter than does potassium. This suggests that the key to the ion selectivity of KcsA is the greater dehydration energy of Na^+ ions, and helps to explain the block of KcsA by internal Na^+ ions.

Keywords: Molecular dynamics; KcsA; Selectivity; Potassium; Ion channel

Ion channels are found in organisms ranging from viruses and bacteria to mammals, and play a key role in the electrical properties of neurones and muscle [1]. An increasing number of diseases ('channelopathies') are associated with malfunctions of channels [2]. A key property of channels is their selectivity as to which ions may permeate. For example, potassium channels are highly selective for potassium over sodium. K channel selectivity resides in a region of the transmembrane pore known as the selectivity filter that is associated with a signature sequence, TVGYG, which is strongly conserved across all potassium channel families. Permeation studies using ions of different crystal radii lead to the suggestion [3] that the diameter of the filter region was between 3 and 3.3 Å. How then does the channel select for K^+ (crystal radius = 1.33 Å) over Na^+ (crystal radius = 0.95 Å)? A widely accepted view is that the filter is better able to make good contacts with a desolvated potassium ion than with a desolvated sodium ion. Such filter/ion interactions replace the ion/water interactions of the ion in bulk aqueous solution. Note that the solvation of Na^+ by water is tighter than that of K^+ [4].

The X-ray structure of the bacterial potassium channel KcsA [5] has provided a valuable insight into how oxygen–ion interactions within the filter may determine the selectivity of the channel. This structure reveals that the filter is lined by backbone carbonyl oxygens oriented inwards towards the centre of the pore in such a fashion as to coordinate

* Corresponding author. Fax: +44-1865-275172;
E-mail: mark@biop.ox.ac.uk

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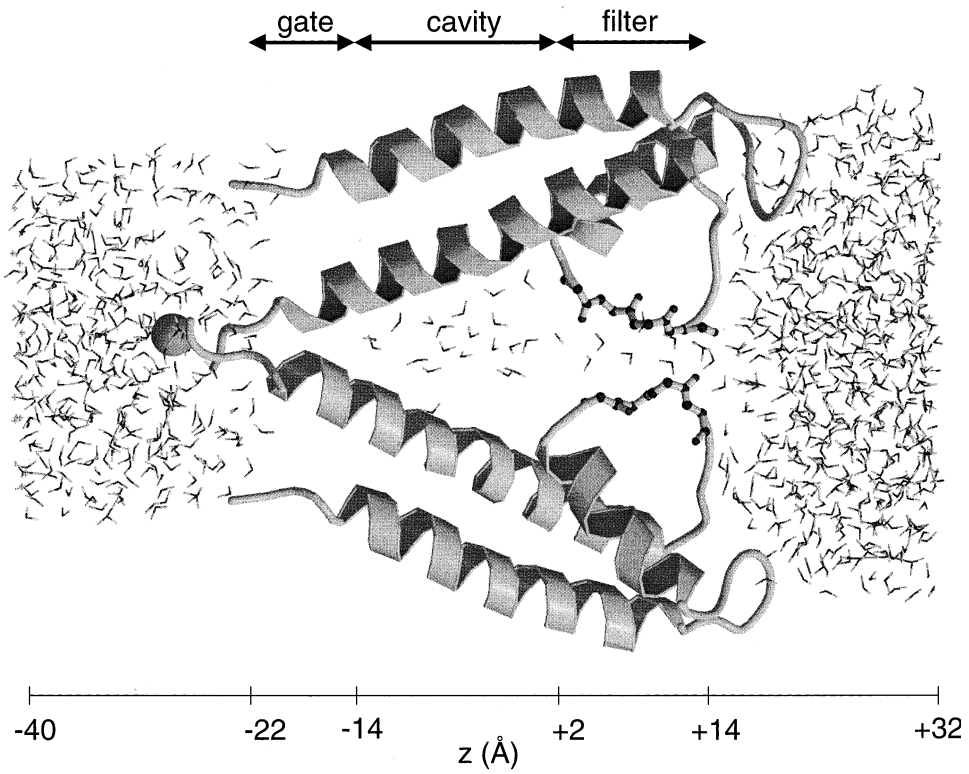

Fig. 1. Simulation system. For clarity, only two of the four KcsA subunits are shown. The water molecules are represented as wire-frame drawings and an initial coordinate of the ion is represented as a sphere. The approximate extents along the pore (z) axis of the filter (f), cavity (c) and intracellular gate (g) regions are indicated. Diagram created using Molscript [33] and Raster3D [34].

passing ions, thus replacing their waters of solvation. The pore region was indicated to be held more or less rigidly in place by a network of hydrogen bonding, thus suggesting that it would be much harder for the filter region to interact strongly with a dehydrated sodium ion than with the larger potassium ion. However, a crystallographic structure provides a time- and space-averaged picture of a protein. In particular, it is difficult to address directly the question of to what extent a channel protein might change its structure and dynamics as an ion passes, and how this might effect the energetics of permeation. To address the question of what actually happens in a dynamic atomistic sense as an ion passes through the pore a number of groups have performed simulations based on the KcsA structure [6–11] and models of related K channels [12]. In this paper we compare the results of molecular dynamics (MD) simulations with a single potassium ion vs. a single sodium ion moved along the pore axis. In particular, we analyse the dynamics and energetics of the ions within the pore, and the strength of ion/pore oxygen interactions in order to provide insights into the physical basis of selectivity.

The model of the KcsA protein was taken from a simulation of KcsA embedded in a fully solvated phospholipid bilayer of 1 ns total time with no ions present [13]. The protein and waters within the pore and in the cap regions (see Fig. 1) were extracted from this full bilayer simulation. The lipid and remaining waters were discarded to simplify the system and thus save CPU time. To prevent water from escaping from the caps and from the channel through the protein, an hourglass restraint was included, implemented in the mmfp module of Charmm [14]. For the simulations discussed below no protein restraints were included in the simulations. However, further controls were done whereby harmonic restraints were imposed on the Cα atoms of the M1 helix, and helical NOE type restraints were imposed on the M2 and P helix region Cα atoms. The diffusion properties and ion interaction energetics were not found to differ beyond the error bars.

Simulations were performed using Charmm23 [15]

on Silicon Graphics Indigo2, Origin 2000 and Pentium II processors (the latter running Red Hat Linux 5.2). The param 19 force field was used and only polar hydrogens were represented explicitly. The TIP3P water model was used. Non-bonded interactions were cut off at 14 Å using a switched function, but were extended in the energetic analysis to 20 Å [16].

Each simulation comprised a series of runs whereby the ion was placed at successive points along the channel (z) axis. The ion was placed at each point and the nearest water molecule was removed. Thus for each sample point along the channel axis, a separate 100 ps simulation run was performed. The protocol has already been described [17] and so only a brief outline is provided here. The system was subjected to minimisation, heating and equilibration runs before the production run. The system was minimised for 3000 steps, during which the ion was held at its initial z coordinate by a harmonic restraint (10 kcal mol^{-1} $Å^{-2}$). This was followed by 6 ps of heating to 300 K, followed by 9 ps of equilibration (both with the ion restrained) followed by another 9 ps of equilibration with the harmonic restraint on the ion removed. Finally each production run was 100 ps long and performed using Nose–Hoover dynamics [18] and no restraints on the ion. The time step was 1 fs and SHAKE was used on the X–H bonds.

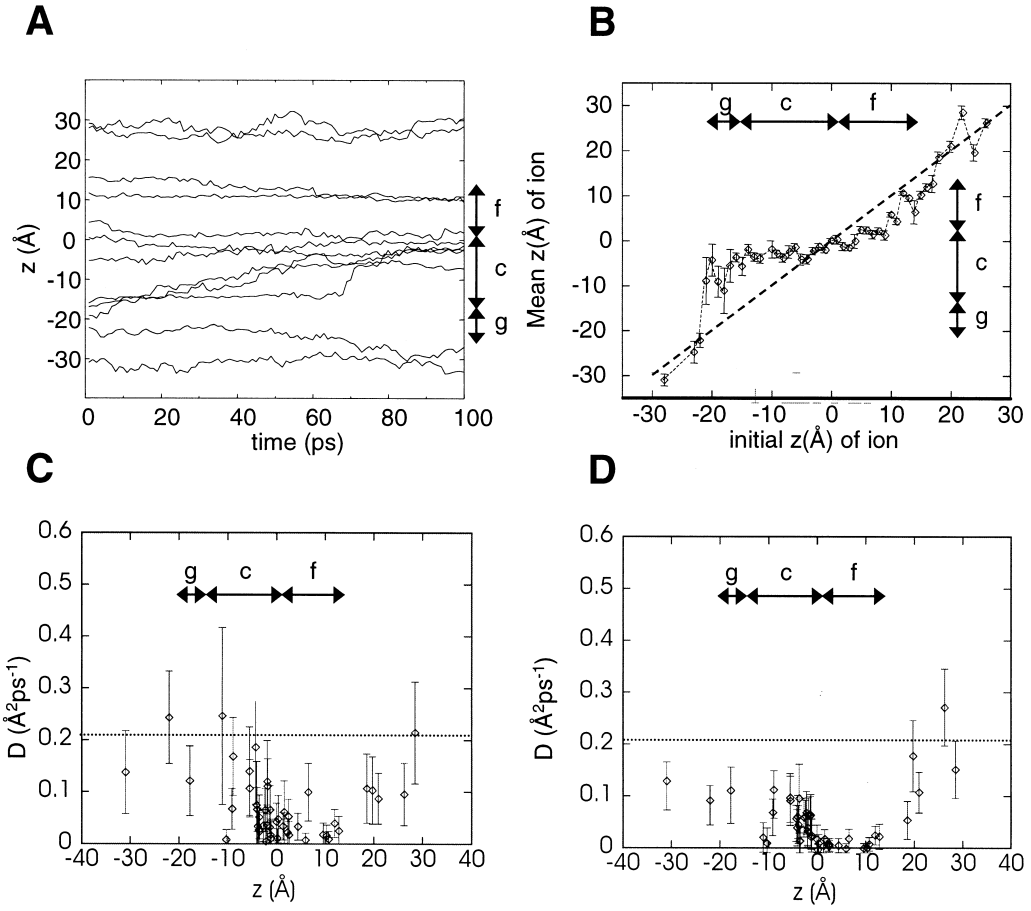

Fig. 2. Motion of a K^+ ion in different regions of the pore. (A) Sample z coordinate trajectories for a K^+ ion in the channel. Note that if the ion starts in the cavity (c) or filter (f) region it tends to remain within that region throughout the 100 ps, whereas if it is initially positioned in the gate (g) region it tends to leave this region during the course of the simulation. (B) Mean z coordinate of an ion (averaged over 100) vs. initial z coordinate. It can be seen that ions initially positioned in the gate region at the bottom of the cavity move towards the area at the bottom of the filter, at z ca. 0 Å. The reduced diffusional mobility of a K^+ ion in the cavity and filter regions is shown in plots of (C) D_Z and (D) D_{XY} vs. z. The value for a K^+ ion within bulk from previous simulations [35] is shown by the dotted line and is 0.21 $Å^2$ ps^{-1}.

Coordinate sets were saved every 0.1 ps for analysis. Sampling was performed every 2 Å along the channel axis and in the case of potassium in the channel sampling was extended to every 1 Å. The extra sampling did not produce significantly different results. As the flexibility of the protein may be effected by the lack of explicit lipid, a further reduced set (with initial ion positions within the selectivity filter) of simulations were performed with harmonic restraints on the secondary structure elements of the protein.

Bulk values for diffusion coefficients were obtained from simulations in two cubic boxes, one of 15.6 Å×15.6 Å×15.6 Å containing 124 TIP3P waters and the other of 31.1 Å×31.1 Å×31.1 Å containing 999 TIP3P waters. One potassium and separately one sodium were placed in the centre of the boxes and four sets of 100 ps dynamics were performed for each. The pair distribution function, $g(r)$, was calculated for some ion interactions and gives the probability of finding an atom or molecule at distance r from another atom or molecule. All values are relative to the ion in the bulk solution described above.

Inspection of the z coordinate of the ion vs. time for various trajectories of a potassium ion within the pore (Fig. 2A) revealed a general trend that if the ion is placed anywhere between the cytoplasmic entrance to the pore (at $z=-24$ Å) and the bottom of the selectivity filter (at $z=-2$ Å), the ion tended to move towards the base of the selectivity filter before the production run started. Furthermore, even if a K^+ ion is present within the gate/cavity region at the end of equilibration, it spends little time in this region during the production run (Fig. 2B). The gate/cavity region is predominantly a hydrophobic part of the channel. The ion seems to prefer to be located at the cavity/filter interface. This is close to the 'focus' of the P helix dipoles suggested on the basis of continuum electrostatic calculations to form a favourable site for a K^+ ion within the cavity [19].

The mobility of the K^+ ion was examined further by calculating the ion diffusion coefficients, D (Fig. 2C,D). A plot of D vs. z shows that there is a significant reduction of diffusion when the ion is located in the filter. Interestingly, the values in the gate/cavity region are higher than might be expected. Although the error bars are rather large (as the ions do not spend much time in this region) it appears that the diffusion rate here may be as high as (and quite possibly higher than) that in bulk water.

It is possible to analyse the energetics of ion/protein/water interactions as a function of the position of an ion within the channel, and compare the results for K^+ vs. Na^+. It is important to note here that because the minimisation and heating were performed independently for each ion position, the total self-interaction energy of the whole system shows variations with the position of the ion. These variations are small compared to total energies of the system but are however $\gg RT$. Nevertheless, specific interactions can be calculated if considered separately. In these calculations the non-bonded cut off was set to 20 Å. Note that in constructing the profiles, the instantaneous (rather than average) position of the ion was used for each step of each simulation. Thus several samples contributed to each point on the profile and the sampling was adequate for all regions.

It is instructive to first compare the total interaction energy profiles of the two species of ion (Fig. 3). These profiles show the interaction potential energy of an ion with the remainder of the system (i.e. water plus protein) as a function of the position of the ion along the channel axis. The overall shape of the profiles for both ions reveals a significant barrier (ca. +50 kcal/mol) at the intracellular mouth of the channel. This is the region of the putative channel 'gate' as suggested by spin label experiments [20] and by simulations [9]. The potential energy profiles show that even in an unrestrained simulation this region provides a large (ca. 80 RT) barrier to ion permeation, and thus a conformational change in the protein is required to open the gate. Of course, this conformational change could be relatively small.

The potential energy falls below zero in the centre of the cavity (z ca. -10 Å) and reaches a minimum close to the boundary between the cavity and the filter (z ca. +2 Å). Thus the minimum in the potential energy profile clearly explains the positional drift that was evident in the diffusion analysis. The interaction potential profiles for both ions are almost entirely dominated by electrostatic energies. The K^+ ion appears to have a lower interaction potential energy in the filter region than does the Na^+ ion (Fig. 3C). In particular it seems that Na^+ ions are rather less stable in the extracellular half of the filter than

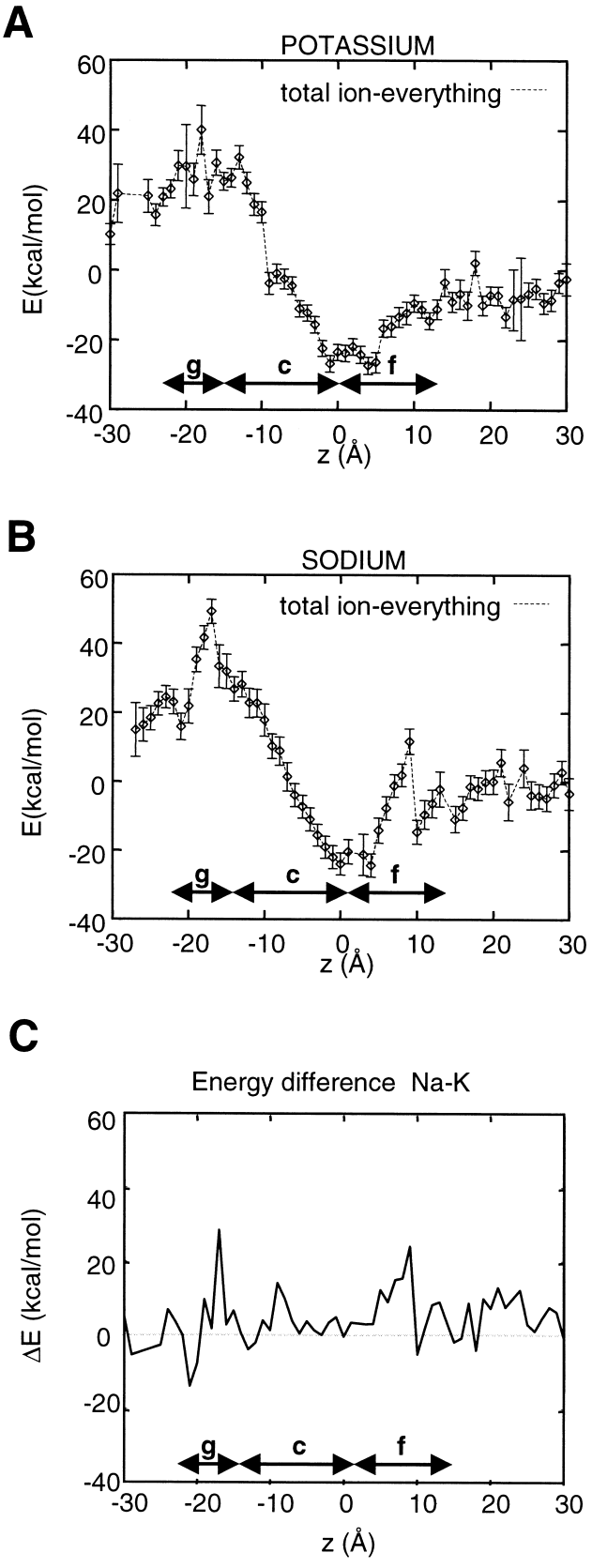

Fig. 3. Potential energy profiles for K^+ and Na^+ compared. (A) and (B) Total interaction energy profiles for the interaction of the ion (A: K^+; B: Na^+) with all other components of the system (i.e. protein plus waters). The interaction potential energies are calculated relative to that of a hydrated cation at infinity (estimated from a simulation of each ion in a 46 $Å^3$ box of water). (C) Difference potential energy profile, calculated as $\Delta E = E(Na^+) - E(K^+)$. Note that a positive value of ΔE indicates a less favourable interaction for Na^+ than for K^+.

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are K^+ ions. The difficulties in estimating errors in differences between potential profiles means that one should not overinterpret these results. However, it is tempting to speculate that this provides a basis for ion selectivity, and thus further analysis of the compounds of the interaction energy profiles is worthwhile.

The overall interaction energy profiles may be usefully decomposed into ion/water and ion/protein interaction energies. The ion/water profiles (Fig. 4A,B) for both ions show that the potential barrier at the gate results from dehydration of the ion (giving an ion/water interaction energy of ca. +50 kcal/mol) that is not compensated for by a favourable ion/protein interaction. Turning to the filter region, the ion/water profiles for both ions reveal a maximum in the region of the selectivity filter (z ca. +10 Å). This corresponds to (partial) dehydration of the ion in the filter. Note that the maximum ion/water energy is higher for Na^+/water (ca. +180 kcal/mol) than for K^+ (ca. +160 kcal/mol) reflecting the stronger hydration of the smaller ion. This must play an important role in channel selectivity.

Turning to the ion/protein interaction energy profiles (Fig. 4C,D) the picture around the filter is a little more complex. Both profiles show a minimum in this region, reflecting the strongly favourable ion/carbonyl oxygen interactions in the filter. One has to be careful to dissect out the direct ion/backbone interactions within the filter and the through space ion/sidechain interactions. Note that the latter are likely to vary according to the assumed ionisation state of the protein sidechains, as discussed in detail in [21]). Focussing on the ion/backbone profiles (which are dominated by the ion/carbonyl oxygen interactions) we see that the potential well is slightly deeper for Na^+ (ca. −145 kcal/mol than for K^+ (ca. −130 kcal/mol). The value of this difference should be treated

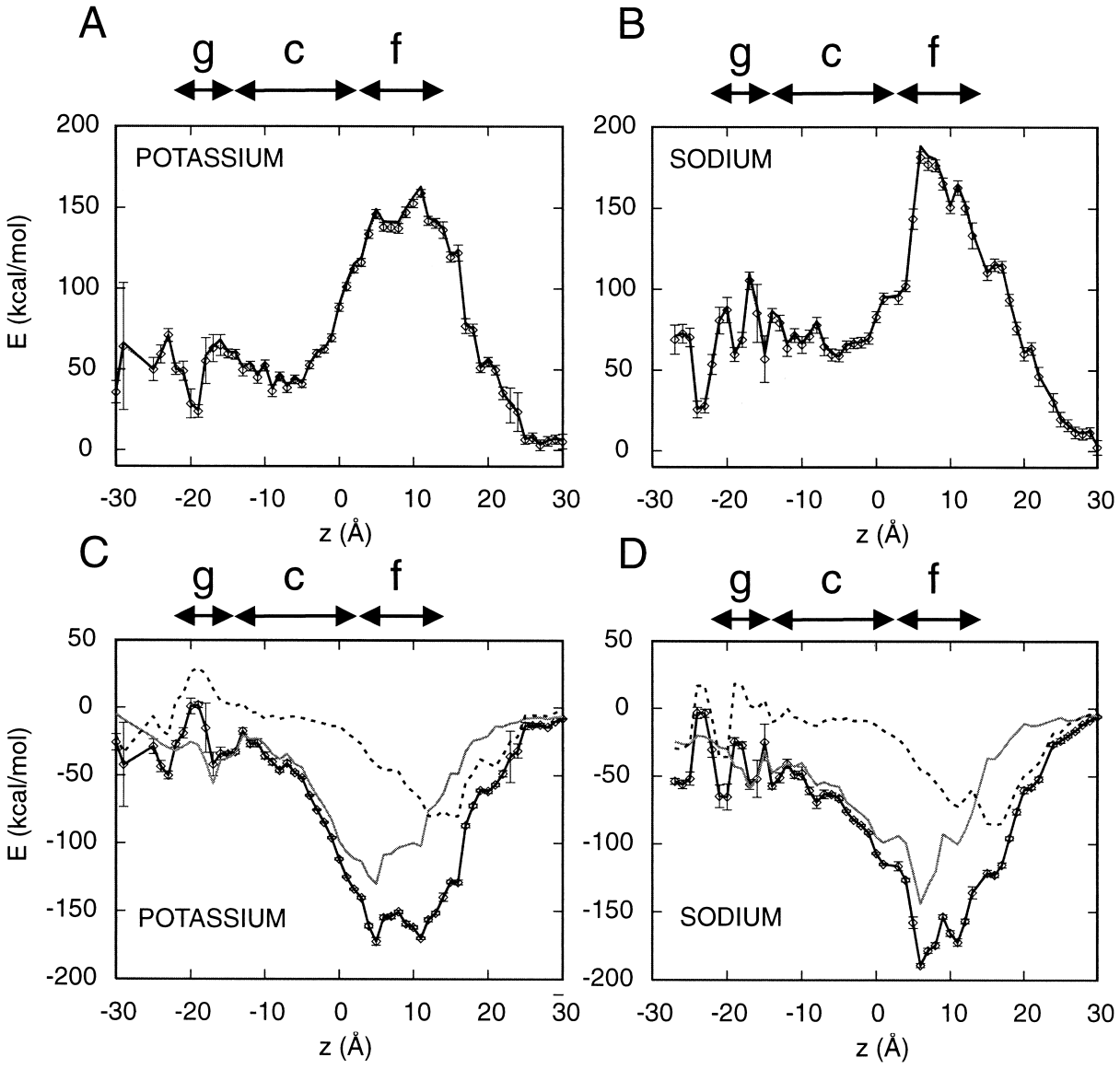

Fig. 4. Analysis of contributions to ion potential profiles. (A) and (B) show the ion/water interaction energies as a function of z for potassium and sodium respectively. Both energies are relative to that of the same ion in a box of waters. (C) and (D) show ion/protein interaction energies for potassium and sodium respectively. The solid black lines plus points show the total ion/protein interaction energy profiles; the solid grey line shows the ion/backbone profiles; and the broken black line shows the ion/sidechain profiles.

with some caution given our earlier statement about difficulties in estimating errors, but it does suggest that the filter is almost equally capable of interacting with an Na^+ and a K^+ ion. One may also be concerned that in the original description of the KcsA structure [5], the conformation of the filter region was based on modelling the polypeptide chain backbone into electron density derived from diffraction data at 3.2 Å resolution. However, recently presented data [36] suggest that a higher resolution electron density map supports the same conformation of the filter region as originally presented.

A geometric measure of the 'strength' of ion/oxygen interactions is provided by the ion/oxygen radial distribution functions $g(r)$. For both potassium and sodium the ion–water oxygen $g(r)$ values in the cap regions were indistinguishable from the bulk simulations, as expected. When the ion is within the filter

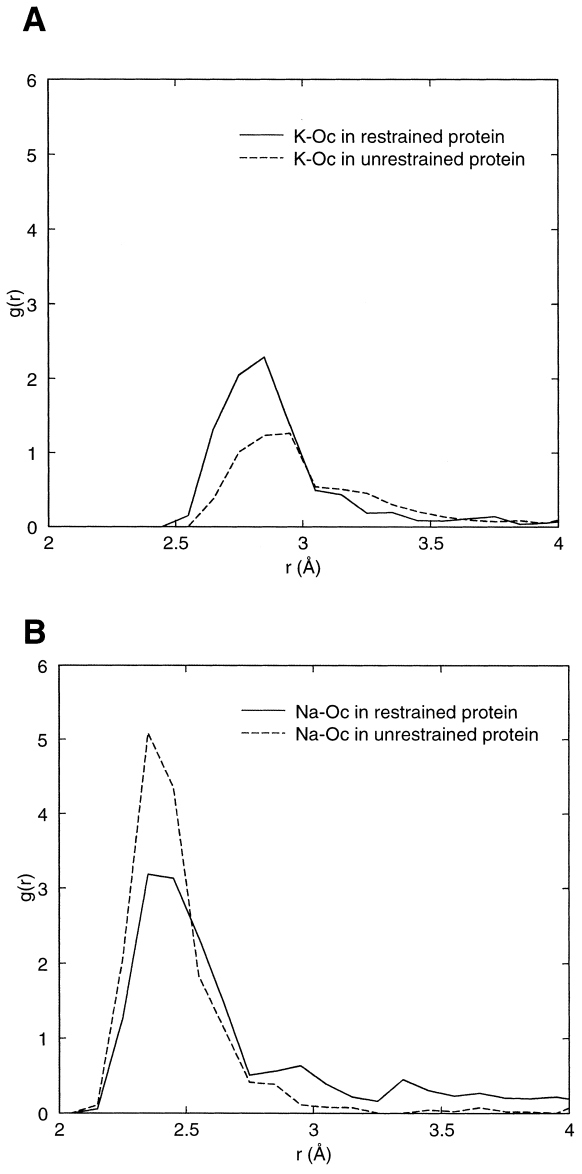

Fig. 5. Radial distribution functions for ions in the filter. (A) shows K–OC radial distribution functions for (i) K^+ ion/filter carbonyl oxygen with the protein restrained (solid black line); (ii) K^+ ion/filter carbonyl oxygen with the protein unrestrained (dashed line). (B) shows the corresponding radial distribution functions for Na^+.

region a significant reduction in the value of peak ion/water oxygen $g(r)$ value was observed, as one would expect given the partial dehydration of the ion in this region. The peak $g(r)$ values for both ions within the filter are about the same. In bulk solution the peak $g(r)$ is much greater for Na^+/water than for K^+/water. Thus, on the basis of this geometric criterion the loss of hydration of Na^+ is greater than that of K^+. One can calculate ion/carbonyl oxygen $g(r)$ functions (Fig. 5) to investigate how well the carbonyls replace the water oxygens in solvating the ion as it moves through the filter. Our results are somewhat different from those of Chung and colleagues [22] who showed better solvation of K^+ by the filter. In the restrained simulations the peak $g(r)$ is a little higher for Na–OC interactions than for K–OC interactions. In the unrestrained simulations, the difference was much more pronounced, with a significantly higher $g(r)$ for Na–OC than for K–OC. Tentatively, one might suggest that these results indicate a selectivity filter that fits K^+ ions rather loosely whereas it fits Na^+ ions rather more tightly. This is compatible with the results of (independent) unrestrained simulations of KcsA in a full bilayer environment [23].

The ion parameters used in this simulation were those from the Quanta package. For K^+ the values are $r_{min} = 2.350$ Å and $e = -0.0100$ kcal/mol, and for Na^+ $r_{min} = 1.650$ Å and $e = -0.026$ kcal/mol. We have compared these potassium and sodium parameters to those from various other studies reported in the literature, for example those used by Åqvist [11,24] (for K^+ $r_{min} = 2.66$ Å and $e = -0.000328$ kcal/mol, and for Na^+ $r_{min} = 2.053$ Å and $e = -0.000845$kcal/mol). Simple calculations between ions and a single water and also between ions and *N*-methylacetamide (NMA) reveal that the parameters used here give differences in energy (compared to those used by Åqvist [11]) for the ion–water interaction by 1 kcal/mol and for ion–NMA by 0.2 kcal/mol. We note here that the differences we observe in the channel are of the order of 25 kcal/mol.

Another obvious deficiency of the model system used here is the lack of explicit lipid. This was a necessary omission given the number of simulations to be performed. However, it has been shown that the properties of water and ions inside similar model channels are not significantly affected by the presence of the lipid [25,26] and for the features we are describing here, the model is sufficiently accurate in this regard. Indeed, simulations on KcsA from other laboratories have employed either restraining functions [22] or a reduced representation of the bilayer [11]. Furthermore, a recent study of gramicidin in a lipid bilayer [27] reported that the barrier-crossing mo-

tions for water were similar for a channel in an explicit bilayer compared to simulations where the lipid was represented by artificial restraints. One must also remember that there is evidence [28–30] that the potassium channel functions as a multi-ion pore. Indeed, in the crystal structure [5] the presence of more than one ion is indicated. Further simulations will be needed to explore differences in ion/pore and ion/water energetics in the presence of multiple ions.

The energetics of the ion interaction energies presented here are first level approximations, but do seem to reveal some interesting and consistent differences between Na^+ and K^+. In particular, our results suggest that the filter is able to solvate both Na^+ and K^+ ions, thus implying that the ion selectivity of the channel lies principally in the greater dehydration energy of the smaller Na^+ ion. Recently, Åqvist and Luzhkov [11] have shown by free energy perturbation/molecular dynamics calculations how the ion permeation mechanism can be reconciled in terms of a set of stable configurations of a filter region occupied by two K^+ ions. Based on the most favourable configuration of the system for K^+ ions, their free energy perturbation calculations suggest that K^+ binds more strongly to the filter than Na^+. However, it is not clear whether the initial (K^+ favourable) configuration of the system relaxes fully when the ion is transformed to Na^+. The question of the relaxation time of the filter around an ion is difficult. Unrestrained simulations suggest that ions switch between sites in the filter on a ca. 0.5 ns timescale [9].

Turning to comparison with experimental data, it has been shown that KcsA is indeed highly selective for K^+ over Na^+ ions [31,32]. This is consistent with either model of selectivity. Interestingly, internal Na^+ ions are suggested to *block* KcsA to K^+ permeation [31]. This would seem to be consistent with a model in which, if Na^+ ions enter the filter, they bind rather tightly and are not readily displaced by competing K^+ ions. This is perhaps more consistent with a model of selectivity which emphasises differences in dehydration energies rather than differences in ion/filter interactions. However, as we have shown in this study, changes in the extent to which the protein is restrained can influence the ion/protein interactions. In simulation studies such restraints may be imposed artificially [7,21] or by embedding in a phospholipid bilayer [9,10,23] or in a bilayer-mimicking environment [6,11]. Furthermore, different simulation methods use different treatments of long-range electrostatic interactions, and it is unclear at the moment which treatment is optimal. Further detailed investigations will be necessary in order to fully determine the mechanisms of selectivity and block of K^+ channels.

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