Minimalist Molecular Model for Nanopore Selectivity

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Using a simple model it is shown that the cost of constraining a hydrated potassium ion inside a narrow nanopore is smaller than the cost of constraining the smaller hydrated sodium ion. The former allows for a greater distortion of its hydration shell and can therefore maintain a better coordination. We propose that in this way the larger ion can go through narrow pores more easily. This is relevant to the molecular basis of ion selective nanopores and since this mechanism does not depend on the molecular details of the pore, it could also operate in all sorts of nanotubes, from biological to synthetic.

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There has been a great deal of interest in understanding the molecular basis for currents of different ionic species through nanopores, in particular, channels in biological membranes since Hodgkin and Huxley [1] proposed the cell excitability model based on an electric circuit with different ionic paths. This has led to the discovery of membrane proteins that work as selective ion channels and have multiple functions in the biological cell. The high selectivity these proteins have for particular ions is one of their most important properties. Several x-ray structures of these molecular arrangements have been published recently [2–6], leading to considerable progress in our understanding of this phenomenon. Subsequent to the determination of these structures multiple molecular dynamics simulations have been performed [7–17], yielding some information on how the selectivity could be operating. It is now clear that there is a narrow pore that acts as the selectivity filter. Subtle differences in the interaction of the walls and the semihydrated ion within this passage must make the difference that, for example, leads to a greater potassium (K^+) over sodium (Na^+) current when a similar electrochemical gradient is applied to a K^+ selective channel. On the other hand simple synthetic nanopores that present ion selectivity have been constructed [18,19]. This and the simplicity of the selectivity pore of the K^+ channel suggest that some general physical-chemical process is behind ion selectivity.

It seems clear, from the simulations and the experimental structures showing the ions inside the narrow biological pore, that pore motion and the coupled passage of several (up to three) ions is behind the discrimination. It is also clear that the differences in the interactions between the channel walls and Na^+ or K^+ are small; both ions have a similar behavior, which indicates that there must be subtle distinctions behind the molecular basis of selectivity. This poses a paradox for the use of numerical simulations, since interaction potentials that are not refined must be used to treat the complex macromolecular structure. These interaction potentials consequently lack polarizability in a surrounding environment with multiple charges at close distances. They also lack nonadditive effects in structures that are closely packed, and, as a result, with small 3-body distances. Finally, they lack intramolecular relaxation of water in a medium with strong inhomogeneities that could easily distort the molecule.

With this in mind, we decided to look into the ionic selectivity of Na^+ vs K^+ using the simplest possible model. In this way, it was feasible to use very refined force fields, which adequately reproduce the subtle differences between the ions. The model also focused where the differences matter; that is, the coordinating properties of the ions. Hence, the mobile charge densities in harmonic oscillators model potential for water [20], Na^+ and K^+ ions [21] was used.

The simplest system that could be found to differentiate between the ions inside a pore is as follows. The ion surrounded by 72 waters (including up to the third hydration shell) was placed inside a cylindrical pore, of radius R_c, built by a repulsive wall with a hard core rejection (Fig. 1). This "drop" of water having either one Na^+ or one K^+ ion at its center was used in Monte Carlo simulations in order to find the structural and thermodynamic behavior of each system. The same system without an ion was also simulated in order to obtain the average energy values of ion hydration.

A series of Monte Carlo simulations was done on an *NVT* ensemble at 298 K without periodic conditions. The initial pore radius was 9.75 Å, where no effect from the

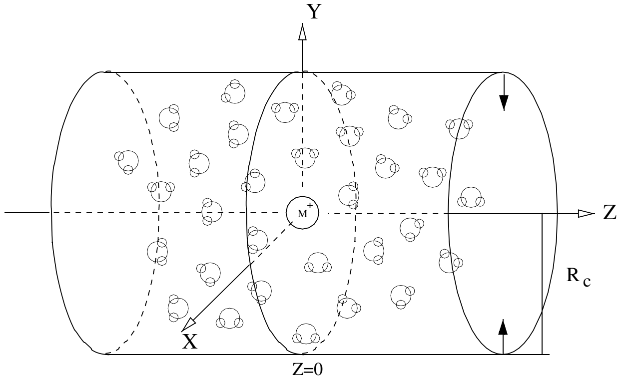

FIG. 1. Minimalist model of channel passage of ions. The system consists of an ion (center), either sodium or potassium, surrounded by water molecules, inside a cylindrical pore with no molecular detail, hard walls and radius R_c. The ion is placed at $Z = 0$, with freedom to move on the XY plane. Reducing R_c simulates narrower pores.

pore walls occurred. It was decreased to a final radius of 1.25 Å in 0.25 Å size step diminutions, thus squeezing the droplet. The starting point for each simulation was the final configuration of the system at the previous pore radius. A very large number of configurations (500×10^6) was done for each point (radius). This number of configurations is greater than the one required to ensure a statistical sampling longer than the correlation time (70×10^6) as estimated with the method of Flyvbjerg and Petersen [22]. All points in the series of simulations had the same number of configurations for both ions, and since the interest lay in the relative behavior between them, the partial evaporation that might occur did not pose a problem. In addition, a recent work [23] has shown that ions tend to keep the structural and dynamical properties of their first hydration shell intact as they are moved into the liquid-vapor interface.

The last 300×10^6 configurations were used for computing statistical properties such as the internal energy (H) and the Gibbs free energy (G). The latter was obtained using the Jayaram and Beveridge algorithm [24], which, without being very precise, is adequate for relative comparisons between ions [25]. The coordination number for water and the hydration numbers for the ions as a function of the pore radius were also computed.

The average enthalpies and free energies for water, Na^+ and K^+ are presented in Figure 2(a). As expected, it can be seen that all energies increase as the pore radius is decreased. There is a particular behavior for the pure water system that consists of a sharp decrease in the enthalpy at a pore radius of 2.75 Å. This decrease is due to a very ordered arrangement that water acquires in a pore this size (see Fig. 3), presenting an interesting behavior—crystallization by simple constriction. This singularity is reflected in the free energy, albeit reduced because of the entropic contribution of such a structured

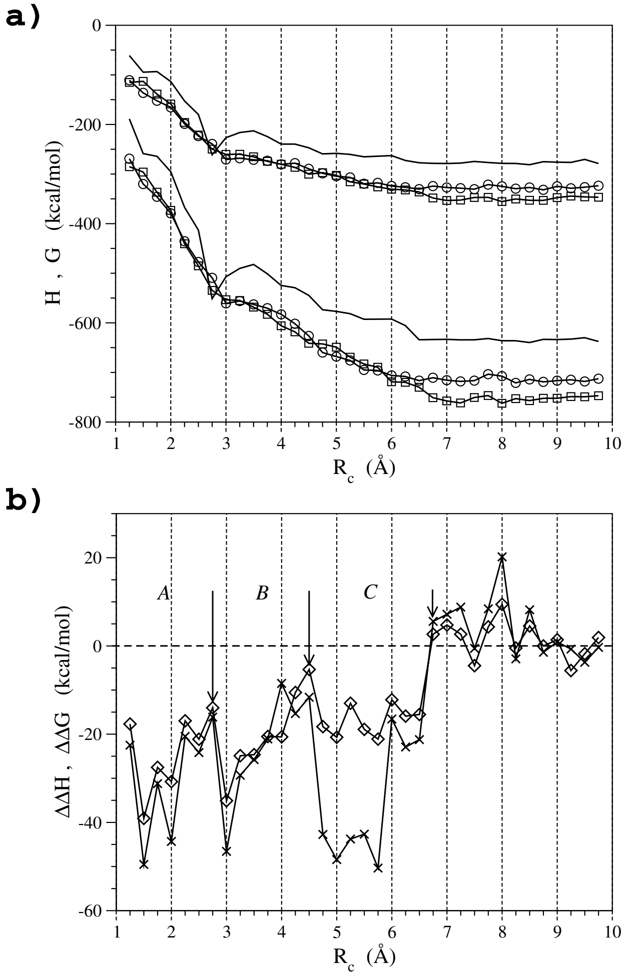

FIG. 2. Energetic cost of constraining the hydrated ions. (a) Enthalpy (lower curves) and free energy (upper curves). Variation of the water droplet (solid line), the droplet with a sodium ion (□) and the droplet with the potassium ion (○) as a function of pore radius. (b) Difference between the variation in enthalpy (×) and free energy (◇) of potassium minus sodium as a function of pore radius. Negative values represent an energy advantage of potassium over sodium. Black arrows indicate the three regions corresponding to the different ion hydration shells.

arrangement. At the largest pore radius the results for Na^+ are $\Delta H = -110.0$ and $\Delta G = -68.0$ kcal/mol and for K^+ $\Delta H = -75.0$ and $\Delta G = -44.5$ kcal/mol. The enthalpy results compare well with the corresponding experimental values [26], Na^+ $\Delta H = -96.9$ and $\Delta G = -98.2$ kcal/mol and K^+ $\Delta H = -76.7$ and $\Delta G = -80.6$ kcal/mol, mainly when considering that these systems are far from the bulk hydration conditions. The ΔG values are far from the experimental ones, which is not surprising taking into account the size of the system and the method used to estimate them. However, the relative differences are reasonable. At a pore radius around 6.5 Å the energies for both ions become similar. One can get a better idea of their relative behavior in Fig. 2(b), where

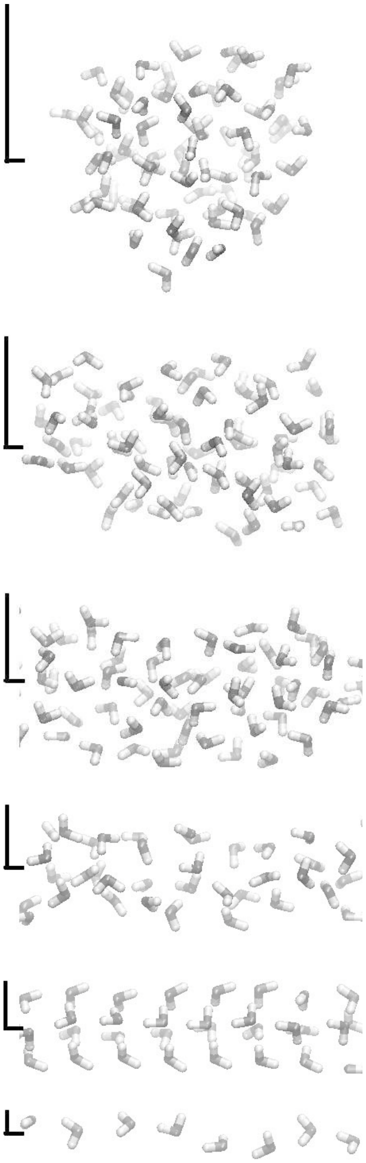

FIG. 3. Snapshot of the water droplet inside the pore at a radius of (from top to bottom) 9.75, 6.75, 5.25, 3.75, 2.75, and 1.50 Å. Note the semicrystalline structure at a pore radius of 2.75 Å and the hydrogen bonded filament at a pore radius of 1.50 Å. Horizontal black bars indicate the location of the pore axis. Vertical black bars represent the length of the pore radius.

the subtraction of the K^+ minus Na^+ energies, $\Delta\Delta H$ and $\Delta\Delta G$, are presented. The reference points for the $\Delta\Delta$ energy values are those for a pore radius of 9.75 Å. It can clearly be seen that constriction of the K^+ ion with its hydration shells into pores narrower than 6.75 Å leads to increments in the enthalpy and free energy that are smaller than those corresponding to Na^+. That is, there is a selectivity of K^+ over Na^+ at pores with radii in the range of the pores of biological potassium channels.

However, there is not a monotonic behavior in $\Delta\Delta H$ and $\Delta\Delta G$ as the pore radius decreases. Rather, a certain structure appears. Three regions can be identified: (*A*) from 1.50 to 2.75 Å, (*B*) from 2.75 to 4.50 Å, and (*C*) from 4.50 to 6.75 Å. In the region *C* the enthalpy for K^+ is quite favored, but the addition of the entropy term brings the free energy back into the general trend. The limit between regions *A* and *B* coincides with the limit of the ions' first hydration shell, while the limit between regions *B* and *C* coincides with the limit of the ions' second hydration shell; 2.79 Å for the first shell and 4.7 Å for the second shell of K^+, and 2.37 and 4.35 Å for the corresponding values for Na^+ [21]. The correspondence between the structure in energy variations and the occurrence of the ions' hydration shells highlights the role the coordination properties have.

The ion-water radial distribution function (RDF) was calculated, and by integrating it up to the first minimum one gets the number of water molecules in the first hydration shell (n_{H_2O}). The RDF shows clear maxima and minima for all pore radii even if excluded volume emerges as the pore narrows in this model. Figure 4 presents the behavior of n_{H_2O} for both ions and pure water as a function of the pore radius. It can be seen that n_{H_2O} remains almost constant for water and Na^+ as R_c decreases up to the point where, because of steric conditions, it falls rapidly to a value of two; one neighbor on each side. On the other hand, n_{H_2O} presents a very different behavior for K^+: contrary to what could be expected the number of neighboring waters increases as the pore narrows. For pore radii from 7 to 4.5 Å the hydration number increases from 8 to 9, explaining the advantage in enthalpy in Fig. 2(b). This extra water also leads to additional struc-

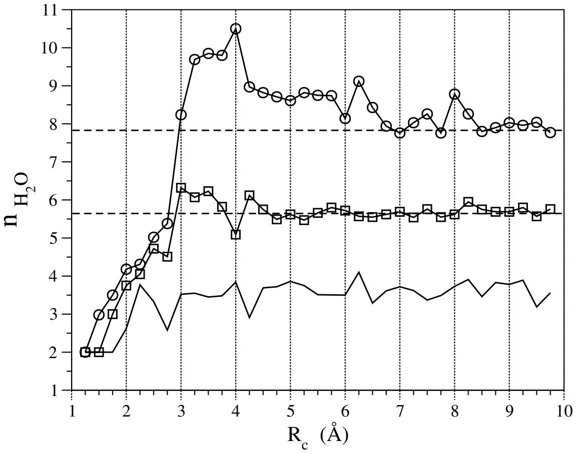

FIG. 4. Hydration numbers. Number of water molecules in the first hydration shell of pure water (solid line), sodium (□) and potassium (○) as a function of pore radius. Dashed horizontal lines indicate the values for the hydration number in bulk conditions for the ions. Note the almost constant behavior for sodium and water until the steep descent as compared to an increasing hydration number for potassium up to the steep descent as the pore narrows.

ture that contributes to a $\Delta\Delta G$ that is still advantageous, yet not as much as the $\Delta\Delta H$. The K^+ hydration number increases by another one to two water molecules at the 3.5–4 Å pore radius range. The large increase in the hydration number for K^+ seems surprising. In order to make certain that this was not due to some statistical trapping resulting from the initial configuration, other simulations for pore radii in the aforementioned range were done. These systems had a reduced hydration number for the ion as the initial configuration. The final result of the simulations was, again, a hydration number around ten to eleven. This sudden increase can be understood by the fact that the first hydration shell gets wider, going from a radius of 3.5 Å to a radius of 4 Å. It then becomes quite clear that constraining a K^+ ion with its hydration shells into a narrow pore within the range of biological channels presents a different behavior than that of Na^+ in the same conditions.

These results are relevant for all nanopores, since the pore wall had no molecular detail, i.e., the results are due only to the relative responses of the ion-water systems to constriction. Of course the particular lining of the biological pore walls, or the charged surface of synthetic nanopores [18], can play an important role in providing coordination sites for the ions and could add to the selectivity. But the results suggest that simple pores with radii in the range of 1.5 to 6.5 Å should select potassium over sodium ions as a result of K^+ being able to increase its coordination under constriction. They also explain why the selectivity filter of biological channels can be formed with the backbone of the protein instead of with the more differentiated chemical groups of the side chains, as was previously thought [27]. Furthermore, these results suggest that sodium selective channels should have wider pores so the potassium advantage would be diminished. This agrees with the experimental observation [28] that Na^+ selective channels are less selective and allow for the passage of molecules more than twice the size of those that go through K^+ channels. This had comprised a rather strange observation since Na^+ is smaller than K^+ considering either the bare or the hydrated ion [21]. There is also interest in producing narrower synthetic pores [29] where the present results can be tested accurately.

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