induced at dielectric discontinuities amplifies the net charge and thus electrostatic energies of the selectivity filter, increasing charge selectivity between Na^+ and Ca^{2+} while maintaining size selectivity between Na^+ and K^+.

The balance of steric repulsion (from the excluded volume of mobile ions and protein side chains) and electrostatic attraction (between mobile ions and protein side chains)—amplified by the surrounding dielectric protein—can account for the main properties of the Na channel in this model. In our model, any small pore with a -1 permanent charge and side chains that occupy a significant volume is an Na-selective channel. In our results, the balance between steric repulsion and electrostatic attraction forms a design principle for selectivity likely to be used in many channels (95–98), transporters (99–101), proteins (102–106), and enzymes (107). The lysine K does not play a special role in this balance in our model beyond its volume and charge. Thus, our vision of the design principle needs to be refined to understand the particular role of lysine in the DEKA Na channel as well as other atomic detail when that detail is determined from structures of these channels.

METHODS

Channel model

The channel protein is represented as a dielectric continuum that surrounds the selectivity filter with a hard wall. Similar dielectric descriptions of solvation are widely used in physical chemistry. Tomasi (108) reviews this enormous literature and describes the strengths and weaknesses of such descriptions. The selectivity filter contains mobile ions Na^+, K^+, Ca^{2+}, and Cl^- and structural ions representing charged side chains of some of the amino acids of the protein (Fig. 1). The structural ions of the selectivity filter mix with the mobile ions and the dielectric that represents water implicitly (109). Mobile ions are charged hard spheres with radii $Na^+ = 1$, $K^+ = 1.33$, $Ca^{2+} = 0.99$, and $Cl^- = 1.81$ Å. The structural ions are charged hard spheres used to (crudely) represent side chains of the protein with permanent negative (acidic) charge or permanent (basic) positive charge. The permanent charge of the carboxyl COO^- groups of the acidic aspartate D and glutamate E side chains are assumed to be spread uniformly on the two oxygens of the carboxyl group because the oxygens are indistinguishable and an ordinary single bond joins the carbon of the carboxyl to the rest of the amino acid. These structural ions are represented as two independent negative half-charged structural ions, each an oxygen ion $O^{-1/2}$ of radius 1.4 Å, confined within the pore. The amino group of the basic lysine K side chain is a positively charged structural ion, represented here as an NH_4^+ ion with radius 1.5 Å. Alanine A is not represented because it is small. A selectivity filter of radius 3 Å and length 10 Å has a volume of 283 $Å^3$. A DEKA Na channel will have four oxygen ions $O^{-1/2}$ and one NH_4^+ giving an average concentration of structural ions of 30 M. This article deals mostly with the natural Na^+ selective channel wild-type DEKA (Asp-Glu-Lys-Ala, permanent charge $-1e$), and the Ca^{2+} selective DEEA mutant (Asp-Glu-Glu-Ala, permanent charge $-3e$). A neighboring EEDD locus is known to influence permeation in Na channels but has not been included because it modifies conductance, not selectivity (110).

The dielectric coefficient ε_w of all solutions containing mobile ions is $\varepsilon_w = 80$, while the dielectric coefficient ε_p of the protein has various values between $\varepsilon_p = 2$ and 80. Bulk solutions are thus represented as a primitive model electrolyte, namely as spherical ions in a dielectric continuum (16,22,111). The qualitative effect of dielectric discontinuities depends on the sign of $\varepsilon_w - \varepsilon_p$ (in this article, $\varepsilon_w - \varepsilon_p \geq 0$). Polarization charge induced at dielectric boundaries (see Eq. 20 of Nadler et al. (79)) varies as $(\varepsilon_w - \varepsilon_p)/(\varepsilon_w + \varepsilon_p)$, and thus one ion induces a charge of the same sign as the ion itself in our simulations. The ion is repelled by the polarization charge the ion itself induces at the dielectric boundary (although the net charge at the dielectric boundary, produced by all ions, might be of either sign so the net dielectric boundary force might be of either sign). Computation time is reduced by assigning a dielectric coefficient of 80 to the membrane, but this value does not change our results (47).

In our model, the structural ions of the selectivity filter of the protein mix with the mobile ions in a dielectric continuum that represents water implicitly. The mixture of water, mobile ions (here Na^+, Ca^{2+}, K^+, and Cl^-), and structural ions (here D, E, and K) form a liquid self-adjusting environment resembling an ionic liquid (28,29), which allows the mobile ions (from the surrounding bulk solutions) to enter the selectivity filter. All ions, both mobile and structural, are represented as charged hard spheres and cannot overlap with the walls of the channel pore or the membrane; these are hard walls the ions cannot cross. The spherical structural ions are also entirely confined longitudinally to the selectivity filter (±5 Å from the center of the pore, Fig. 1 *A*). The selectivity filter has spatially nonuniform selectivity (see Fig. 7) and so we chose to plot occupancy in the central, most-selective

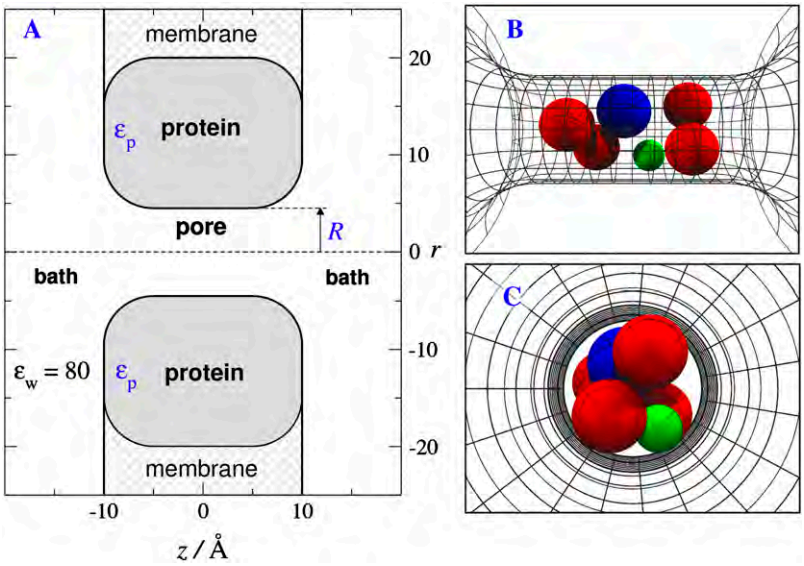

FIGURE 1 The channel model. Computations are done in a much larger region than shown (see text). (*A*) Baths containing bulk solution on either side of a membrane containing a channel protein. (*B*,*C*) Snapshots of ions in the pore (-10 Å $< z <$ 10 Å). The cross-sectional view Fig. 1 *C* vividly shows the crowding of ions and the competition for space in the narrow pore. The dielectric coefficient of the bulk solution is $\varepsilon_w = 80$. The dielectric coefficient of the protein is ε_p, ranging from 2 to 80. Side chains are restricted to the central region of the channel (-5 Å $< z <$ 5 Å) which is called the selectivity filter for that reason. The selectivity filter has spatially nonuniform selectivity (see Fig. 7) and so later figures plot occupancy in the central most selective region of the filter ±2.5 Å from the center of the pore.

region of the filter ±2.5 Å from the center of the pore after considering several possible choices, and many conditions, beyond those illustrated in this article. Confinement is with a hard-wall potential and enforced by rejecting MC moves; springlike restraining forces are not used. Future computations should compare different types of restraining forces.

It is important to remember that the effective radius of the pore is reduced dramatically by the side chains of the channel protein, the structural ions. The side chains exclude volume that would otherwise be available to the mobile ions. The channel protein provides a pore with an effective diameter smaller than the distance between the walls of the pore because the side chains extend into the pore from the walls. So little space is available in the pore that ions pile up outside the pore proper, as we shall soon see. When side chains pile up at the ends of the region in which they are constrained, ±5 Å from the center of the pore, the effective length available to ions is reduced as well.

All ions, including structural ions, assume configurations of minimal free energy, which vary depending on experimental boundary conditions imposed on the bulk solution (bulk electrolyte composition, temperature, pressure). Configurations depend also on the charge, composition, and assumed structure of the channel protein itself (e.g., DEKA versus DEEA). Different configurations of structural (and mobile) ions produce different electric fields, and different steric interactions (produced by excluded volume) between mobile and structural ions. Thus, the spatial distribution (i.e., profile) of both electrical and chemical free energy in the selectivity filter varies with experimental conditions imposed on the bulk solution and also with the composition of the channel protein itself. In this way, the mixture of water, mobile ions (here Na^+, Ca^{2+}, K^+, and Cl^-) and structural ions (here D, E, and K) form a liquid self-adjusting environment that allows the mobile ions (from surrounding bulk solutions) to enter the selectivity filter and carry electric current.

Simulations

Calculations are performed in a cylindrical compartment forming a simulation box much larger than shown in Fig. 1. The simulation box and procedure has been shown (see Supplementary Material of Boda et al. (47)) to allow the formation of bulklike solutions in both baths. The compartment has a 75 Å radius representing two baths (each 170 Å long) separated by a membrane 20 Å thick containing a protein with a pore (radius R) through it. MC moves that put an ion outside the simulation box are rejected. Electrostatic boundary conditions are not imposed on the simulation box. Rather the dielectric material ε_w extends to infinity. Electric potentials are found at the edge of the simulation box, if, for example, ions are of different diameter, as arise in any double-layer calculation (112,113). Care is taken to be sure these potentials do not reach the channel. (See Supplementary Material of Boda et al. (47) for computation and discussion of these effects.)

Occupancy of species i is defined as the number of (centers of) ions of that species in the central region, namely the 5 Å of the selectivity filter -2.5 Å $< z <$ 2.5 Å. The occupancy determined in MC simulations is an average. If a channel were occupied half of the time by one ion, and the other half of the time by zero ions, the occupancy we determine would be 0.5.

Snapshots from an MC simulation illustrate our reduced model of the selectivity region (Fig. 1, *B* and *C*). Fig. 1 *C* particularly shows the crowding of ions and the competition for space. The central, cylindrical part of the pore contains charged side chains extending from polypeptide backbone of the channel protein into the pathway for ionic movement: the side chains are free to move inside the selectivity filter of the channel, and in this sense are dissolved, but they cannot leave the selectivity filter; they are kept within it.

We perform calculations for cylindrical selectivity filters of fixed length 10 Å with hard walls at radii between $R = 3$ Å and $R = 5$ Å. Roth and Gillespie (114) have shown that a cylinder of protein surrounding a pore of radius ρ (representing the wall of a channel) has properties similar to those of a cylinder with hard, smooth walls surrounding a pore of slightly larger radius $\rho + \Delta\rho$ when the cylinder of protein is represented as a fluid of wall particles.

We simulate an equilibrium system in the canonical ensemble with temperature $T = 298$ K. The volume of the computational compartment and the number of atoms of the various ionic species are fixed. The length and radius of the simulation box are chosen so that the number of Na^+ determines a previously chosen bath concentration. In a few cases, where small bath Ca^{2+} concentrations were computed, we simulated the grand canonical ensemble. We simultaneously inserted (or deleted) one Ca^{2+} and two Cl^- ions while maintaining a fixed chemical potential for $CaCl_2$ (47). All bath concentrations, including Ca^{2+} concentrations in the bath, are outputs of the calculations in every simulation of this article.

An essential part of our MC procedure is a biased particle exchange between the channel and the bath to accelerate the convergence of the average number of various ions in the channel (27,39), but the acceleration of convergence does not change our results. The electrostatic energy of the system is determined using the induced-charge computation method (45), which numerically solves an integral equation for the surface charge induced on dielectric boundaries. Previous work (see Supplementary Material of Boda et al. (47)) has shown the accuracy of the method and the need to check that accuracy when boundaries are curved (44,45).

RESULTS

We simulate selectivity in a reduced model of a channel protein over a wide range of conditions and show that a treatment involving only a few forces can do quite well. The protein in our model is represented by a dielectric boundary surrounding structural ions described in Methods and Fig. 1. The highly concentrated and charged selectivity filter resembles an ionic liquid (28,29) more than an ideal dilute electrolyte solution.

Charge selectivity Ca^{2+} versus Na^+

Fig. 2 shows the dramatic effect of the side chains of the channel protein on the contents (occupancy) of the selectivity filter. Simulations were done in which a variable amount of Ca^{2+} was added to a constant, approximately physiological, concentration of Na^+ (100 mM). Simulations compare a Ca^{2+}-selective DEEA mutant (Asp-Glu-Glu-Ala, permanent charge $-3e$, Fig. 2 *A* with logarithmic abscissa) with the natural Na^+ selective channel wild-type DEKA (Asp-Glu-Lys-Ala, permanent charge $-1e$, Fig. 2 *B* with linear abscissa). DEEA has been shown to conduct substantial Ca^{2+} currents: Ca^{2+} can easily enter this channel (54,115,116). In our simulations of DEEA, Ca^{2+} easily enters the channel to give the titration curve (Fig. 2 *A*, logarithmic abscissa) typical of a Ca channel (58–60,117–133).

As Ca^{2+} is added to the bulk solutions, more and more Ca^{2+} enters the channel, displacing Na^+ from the selectivity filter. In the case shown, half of the Na^+ in the selectivity filter is replaced with Ca^{2+} when $[Ca^{2+}]_{bulk}$ is just 10^{-4} M, compared to $[Na^+]_{bulk} = 10^{-1}$ M. This DEEA Ca channel has an apparent binding constant of 10^{-4} M under these conditions. In calcium channels, Ca^{2+} at just 10^{-4} M successfully competes for space with the Na^+ counterions at 10^{-1} M and displaces them from the crowded selectivity filter, as we have described previously (47). The filter of the DEEA Ca channel is crowded because structural ions are at high concentration ($[O^{1/2-}]_{selectivity\ filter} \simeq 35$ M) comparable