Monte Carlo simulation of the water in a channel with charges

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ABSTRACT A Monte Carlo simulation of water in a channel with charges suggests the existence of water in immobile, high density, essentially glasslike form near the charges. The channel model has a conical section with an opening through which water molecules can pass, at the narrow end of the cone, and a cylindrical section at the other end. When the charges are placed near the narrow section of the model, the "glass" effectively blocks the channel; with the charges removed, the channel opens. The effect can be determined from the rate of passage of the water molecules through the pore, from the average orientation of the water molecule, and from distortion of the distribution of molecules. In the simulations carried out to date, no external ions have been considered. In addition to the energy, the Helmholtz free energy has been calculated.

INTRODUCTION

Several mechanisms have been suggested for channel gating (e.g., Edmonds, 1987; Bass and Moore, 1968; Leuchtag, 1987*a*, *b*). Green (1989) proposed that water effectively "froze" under the influence of an electric field (an electrorheological effect). However, the field produced by the external cross-membrane voltage turns out to be about an order of magnitude too small to account for gating; also it leaves rotation of the water molecules as the only mechanism to account for the gating current. However, proteins contain charged amino acids which can produce much larger fields. The original hypothesis must therefore be modified to allow these charges to become the source of the fields required for the electrorheological effect.

Whereas the structure of very few channels is well enough known to allow us to assert definitely that such charges are known to exist, there is some evidence at least. The presence of gating currents in nerve channels equivalent to the motion of five or six charges moving across the membrane (Stuhmer et al., 1989; Keynes and Rojas, 1974) suggests either motion of actual charges or very large dipoles swinging through a very large distance, which is difficult to imagine geometrically if the dipole is to provide so large a charge movement. For example, a dipole with one full charge at each end, of length 10 Å, rotating 180°, would give a current equivalent to less than one-half a gating charge; over 10 would be required to account for the full gating charge. Stuhmer et al. (1989) provide evidence for charge movement. The rotation of water molecules might account for a fraction of the total gating charge also (Green, 1989). Whereas it seems to us easiest to imagine actual charges, either titratable or otherwise, mobile under the external field, this paper does not consider in detail the origin of the charges, but rather the consequences which would ensue, assuming they are actually present.

The model, therefore, can be described as follows: charges in the narrow region of the channel cause water to "freeze," blocking the channel. During gating, these charges are removed (possibly as a consequence of events remote from the location of the charges), freeing the water and allowing ions to move through the channel.

There is evidence for conical sections in what is presumably the critical section of several channels (Dani, 1986; Eisenmann and Dani, 1987). We therefore consider channel models with an opening large enough to allow water molecules to go through, and differing in the angle of the cone and some details of geometry, as well as in the charges placed in the channel. The procedure is a standard Monte Carlo simulation, using the TIP4P potential of Jorgensen et al. (1983) for the water molecules.

DESCRIPTION OF SYSTEM

The model for the interior of the channel is, geometrically, the frustum of a cone at the bottom, with a cylinder attached above (see Fig. 1, which includes the initial placement of the molecules in Fig. 1 *a*). The dimensions depend on the angle at the bottom of the cone, which, for the cases we have simulated, ranges from 0.40 to 0.55 rad for the half angle (0.8–1.10 rad for the entire cone).

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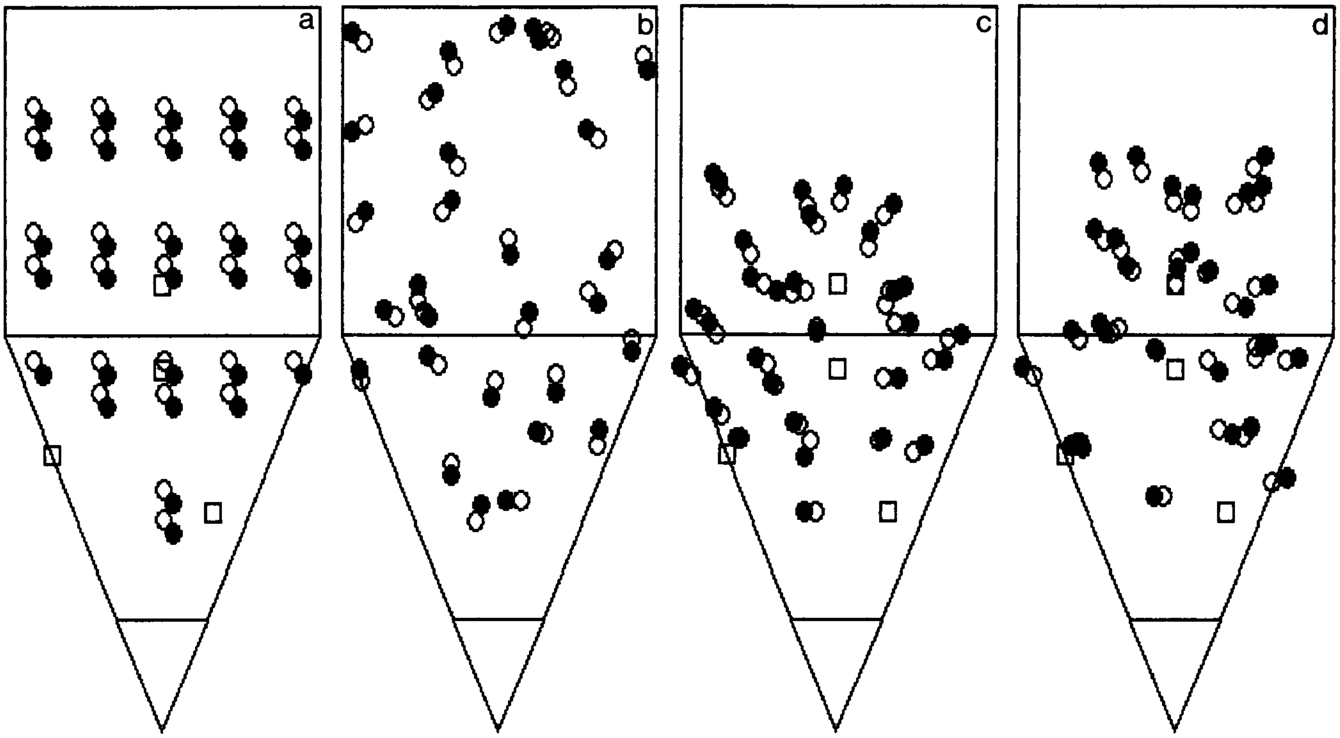

FIGURE 1 Two dimensional projection of positions of water dipoles in the channel after 1,500 iterations per molecule, with 5 Å opening and four charges as shown by squares. The dipoles are represented as paired circles, the solid circles the positive, the open circles the negative end of the dipole. Each charge equals the electronic charge times a factor *g*. (*a*) initial position, all cases, (*b*) 0 charge (*c*) $g = 0.5$, (*d*) $g = 1.0$.

Most of the data we will discuss refer to a half angle of 0.45 rad. The boundary between the cone and the cylinder is at 11 Å above what would be the apex of the cone, multiplied by a factor, equal to 0.97 in all the runs reported here, used in making the density equal that of liquid water at the start of the run. The water molecules are first arranged in an ice lattice, at ice density, then contracted to water density, and finally rotated to random orientations at the beginning of each run. The upper boundary is at 20.1 × 0.97 Å above the apex of the cone, and the lower boundary chosen to allow either a 3 or 5 Å orifice at the cutoff, so that water molecules could pass through. For the channel selectivity filter, at least, a 3 Å opening was first found for the K^+ channel of squid axon by Bezanilla and Armstrong (1972), and is still considered correct (Zimmerberg et al., 1990). (Some zeolites with main channel apertures <3 Å can be dehydrated (Breck, 1974), so that 3 Å is not implausibly small for the passage of water molecules.) Some gated channels are larger, so we have also considered a 5 Å opening. Charges, if present, are placed on the wall of the cone; the first charge is ~6 Å above the apex, the second ~3 Å above this and rotated 90° around the cone, the third ~3 Å higher still, and rotated another 90°. If a fourth is present, it is another 3 Å up, rotated by 90°. For the 5 Å orifice, the lowest charge is just above the opening. A few runs, not discussed here, have charges placed higher in the cone.

If, in the course of the simulation, the oxygen atom of any molecule passes through the aperture in the lower boundary, the molecule reenters through the top of the volume; the new configuration is accepted with probability determined by the relative energy of the new configuration, just as in any other step. Molecules are not permitted to pass in the reverse direction (from top to tip); for geometric reasons, such transitions would be improbable, especially with the 3 Å aperture, so very few moves are excluded.

Molecules with initial placement within 0.5 Å of the wall were allowed to remain immobile, as if they were hydrogen bonding groups on the protein. The remaining walls of the protein are almost hard walls. They include a van der Waals interaction with the water; the strength of the attraction is approximately that estimated by Ben-Naim et al. (1989). However, this attraction turns out to be relatively a small effect. Finally, a hard core is added to the water molecules, so that oxygen atoms are not allowed to come within 2.3 Å of each other, or of the charges.

SIMULATION OF SYSTEM

(*a*) To produce the initial random orientation of the molecules, each molecule is given 100 free rotations, without translation and without checking energy. Each orientation step is between −20 and 20°, with equal probability for all 21 possible step sizes, in 2° increments; the rotation is about all three axes. These were the same step sizes used throughout the simulation.

(*b*) For the remainder of the run, each molecule is rotated as described above, and translated along each axis (one step therefore requires six random numbers, three for the rotations and three for the translations). The translations range from −0.9 to +0.9 Å in equal increments of 0.09 Å. A lookup table for the cosines of the rotation angles is used to speed computation. Because the average displacement in any direction is 0.47 Å, the mean displacement per step becomes 0.87 Å.

(*c*) After the molecule has moved, its energy is computed twice: once with the new coordinates, and once with its old coordinates (the coordinates of the other molecules having changed since the previous iteration). If the new coordinates give the lower energy they are accepted; if they give higher energy, they are accepted with probability exp $(-\Delta\epsilon/kT)$, where $\Delta\epsilon$ is the energy difference (Metropolis et al., 1953), which is the most common procedure in Monte Carlo simulations. If the new coordinates are not accepted the old coordinates are accepted as the new configuration. If the translation carried the oxygen atom of the molecule within 2.3 Å of another molecule or a charge, or within 0.5 Å of the wall, the old configuration was accepted without any further test. Overall acceptance rates were $>30\%$ for the systems without charge, but dropped to near 1% at the highest charges (after the initial rearrangement, see below). At no charge was there a noticeable difference between 750 and 1,500 iterations/mobile molecule. (An external field, parallel to the *z* axis, was sometimes left in, in the early runs; however, it turned out to be too small to matter.) To obtain statistics, multiple runs were carried out. In all cases, at least two runs were done; in about half, four to six were carried out, and these sets are the basis for the error bars shown in Figs. 2–4.

Computations were carried out on a 16 MHz model 386 computer with 387 math coprocessor (Fountain Technologies, Inc., Somerset, NJ), using FTN77/386 Fortran (essentially, Fortran 77, from the University of Salford, Salford, England). The random number generator which came with the compiler was used with a random (date-time based) seed. The Fortran output, in ASCII form, was used as input for a graphics program written in FORTH (Harvard Softworks, Springboro, OH), which produces the figures of the water dipoles. The output of this program is shown in Figs. 1 and 5; all other graphs were produced with Easyplot (Spiral Software, Inc., Brookline, MA).

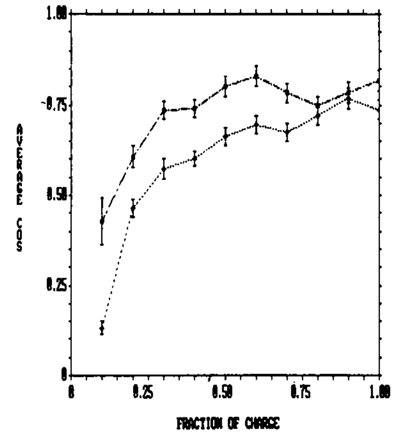

FIGURE 2 Rates of passage of water molecules, in units of passages per total iterations, vs. fractional charge. (total iterations = iterations/molecule *x* mobile molecules) Upper curve, 5 Å opening, four charges, lower, 3 Å, three charges. Error bars are 1.5*x* SD for cases where four or more runs have been carried out, and are estimated by interpolation for the remainder.

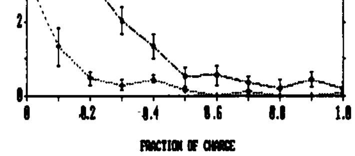

FIGURE 3 Average cosine of water dipoles with respect to the direction to the center of charge, as a function of charge. Error bars estimated as in Fig. 2. Upper curve, 5 Å opening, lower, 3 Å, with charges as before.

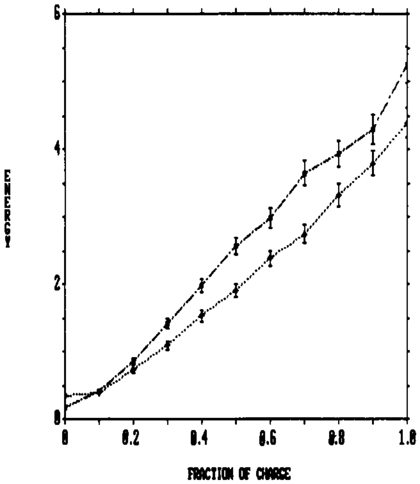

FIGURE 4 Energy as a function of charge in units of 10^{-18} *J* total energy for the system. Errors estimated as in Fig. 2. Upper curve, 5 Å opening, lower 3 Å, charges as before.

RESULTS AND DISCUSSION

The qualitative results can be briefly summarized: with no charge in the channel, there are a relatively large number of molecules moving through the bottom aperture while the spatial distribution maintains roughly constant density. We first found that for the smaller channels, the presence of two or three charges results, at 300 K, in a drop of a least a factor of 30 in the number of water molecules per time (i.e., steps) allowed to pass through. In some runs, no molecules were allowed through. With charges, the density is greatly distorted, with many molecules piled up near the center of charge if the charges have the same sign. There is a near vacuum at the top of the volume if the charges are placed near the bottom, implying something resembling a transition to a glassy "microphase" of high density. If the channel is large enough (half angle 0.50 or 0.55 rad) some molecules are sufficiently distant to move with some freedom, and for the 0.55 rad channel, there is still some traffic through the bottom; even with three charges, about 10% of the zero charge water current persists. Narrower channels are essentially completely shut. Mezei and Beveridge (1981) found, in a simulation with longer range interactions present (isotropic solution), that only the first layer of water is tightly held, whereas molecules beyond this are in a "structure breaking" region. With the "protein," structureless, or, in effect, hydrophobic, walls of our simulation, more distant competing hydrogen bonds are absent, which may be the reason for the absence of these structure breaking effects. In the conical region, especially toward the bottom, there is little more than one layer distance to the nearest wall. The results therefore would not be surprising in any case.

If the molecules are held by the charges, we expect the dipole moments of the molecules to orient with respect to the charges; they do. Values of $\langle \cos \theta \rangle$, the mean cosine of the angle between the dipoles of the water molecules and the vector to the center of charge, were computed for each run. The average is taken over all molecules, including those distant from the charges. Not surprisingly, these are not as well oriented as those close in. (TIP4P water generally produces dielectric coefficients of ~50 at 300 K in a molecular dynamics simulation (Neumann, 1986), slightly below that of the experimentally known 80). Molecules very close to the charges will orient toward the individual charges, rather than towards the center of charge. A few runs were also done with one charge of each sign. These also make the water "glassy," but the predicted mean cosine is zero, and the values obtained have been <0.3 in absolute value. The $\langle \cos \theta \rangle$ values were also small in a few runs when two charges of one sign and one charge of the other was tried. The averages were taken twice during the simulation, once after half the iterations, the other at the end. In simulations of 3,000 steps/molecule, there was a difference in $\langle \cos \theta \rangle$ of $<10\%$ (generally $<5\%$) between the halfway value and the final value when the charges were of the same sign. This strong alignment suggested that the configuration was equivalent to the final average in $<1{,}500$ iterations per molecule. The energy had also reached its final value by this number of steps, with fluctuations at most of the order of 10%. We therefore standardized on 1,500 iterations/mobile molecule. The distributions around the center of charge, as averaged for the charges on the channel wall, are shown after 750 iterations/mobile molecules, and after 1,500 iterations/mobile molecule, in Figs. 6 and 7. Three charge states are shown: 0, 0.5, and 1.0 times three charges for the 3 Å aperture, and times four charges for the 5 Å aperture. The distribution is further described in the figure caption. There were 30 mobile, 12 fixed molecules in the 5 Å aperture runs, 36 mobile, 2 fixed in the 3 Å runs.

The orientations were sometimes as large as 0.85 in channels with half angle < 0.50 (especially 0.40) rad and only one charge, so that the charge and the center of charge were coincident. Charges were first taken equal to integral multiples of the electronic charge. If we had taken account of the dielectric constant of the protein, it would have had the effect of renormalizing the charge (i.e., changing its effective value). However, our major qualitative conclusion holds as long as the total charge is at least one to two charges, and essentially holds for charges of opposite sign (one of each sign). Multiplying

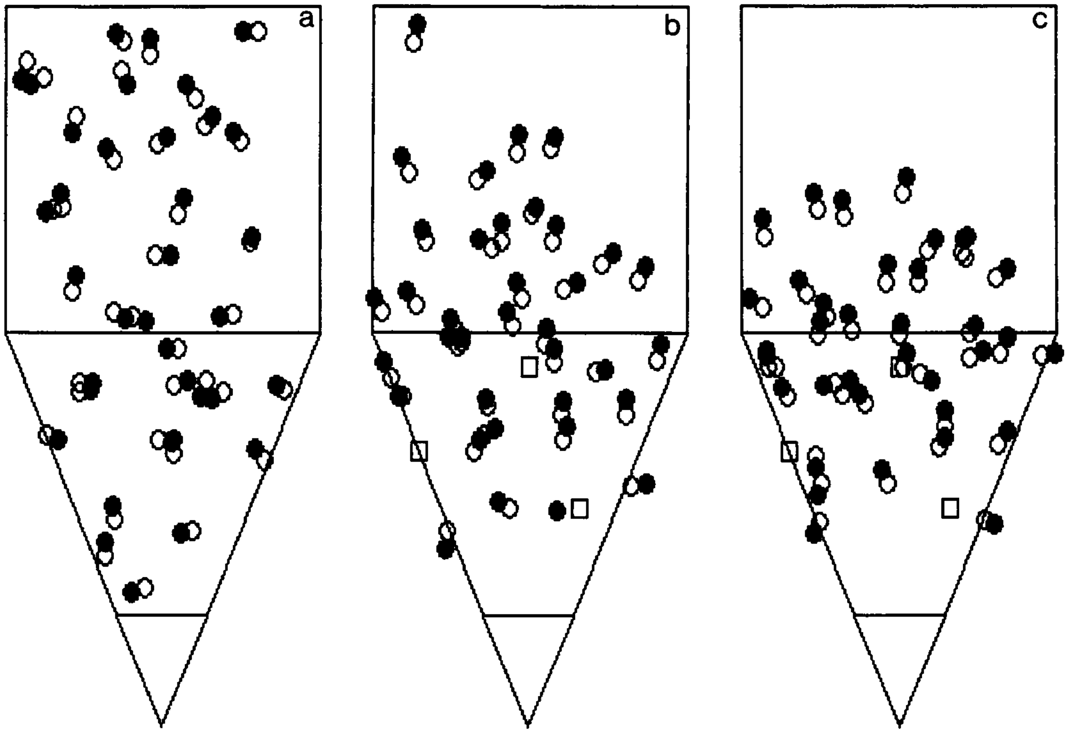

FIGURE 5 Distribution of positions of the oxygen atoms in the 5 Å case with respect to the center of charge, defined as the average position of the four charges present on the boundary of the channel. "Number of molecules" is the number with oxygen atoms between the distance shown on the abscissa, and 1 Å less. (e.g., the value for 4 Å is the number with oxygen atom between 3 and 4 Å from the center of charge). Dotted curve, positions after 750 iterations/molecule, dashed 1,500 iterations/molecule. (*a*) 0 charge, (*b*) $g = 0.5$, (*c*) $g = 1.0$.

charges by any reasonable factor would not be expected to make a qualitative difference. Because real channels probably have four or more charges, it is likely that only quantitative results would be affected by taking into account the actual field. To test this, an extensive series of runs were made with fixed conditions: 1,500 iterations/molecule, 280 K, 0.45 rad half angle, 3 Å opening with three charges or 5 Å opening with four charges. In these runs, the charges were multiplied by 0.0, 0.1, 0.2, 0.3, . . . , 1.0, making possible some estimate of the effect of a diminished electric field. Qualitatively, it appears that the channel largely shuts down (see Figs. 2 and 3, as well as the distribution of molecules in the channel, Figs. 1 and 5–7) with the equivalent of approximately one complete charge, when the opening is 3 Å, or nearly two charges when the opening is 5 Å. It should be emphasized that we are concerned with relative rates, not with absolute rates, which would depend on knowledge of the time scale of the calculation. While we can make an order of magnitude estimate of time scales, by comparing displacements in the upper part of the channel to known diffusion constants, this work is too preliminary to report on here. The relative rates do not depend on this extension of the work.

The fact that only one charge (equivalent) suffices to close the channel in the 3 Å case is significant also in evaluating the probable effect of the protein as dielectric, with dielectric coefficient of perhaps five. If four to six actual charges are present, the polarization effect will be inadequate to lower the field sufficiently for the channel to be open. The fact that about this number of charges appears to be actually present is not surprising according to this result, as that is the number needed to control the channel.

The added free energy on charging is the one thermodynamic quantity which can be found from the simulation at the present level of accuracy. Thermodynamic integration (Mezei, 1988; Beveridge and DiCapua, 1989) allows determination of ΔA by integration of energy as a parameter is varied from zero to one. Here, the parameter is charge. In general, with λ as parameter,

$$\Delta A = \int_0^1 \epsilon_\lambda \, d\lambda, \tag{1}$$

where ϵ_λ is the energy for a given value of λ, which in our case is the fraction of total charge. By obtaining values of energy at charge intervals of 0.1 of total charge, from 0.0 to 1.0 (i.e., at 0.3 charge intervals for three total charges

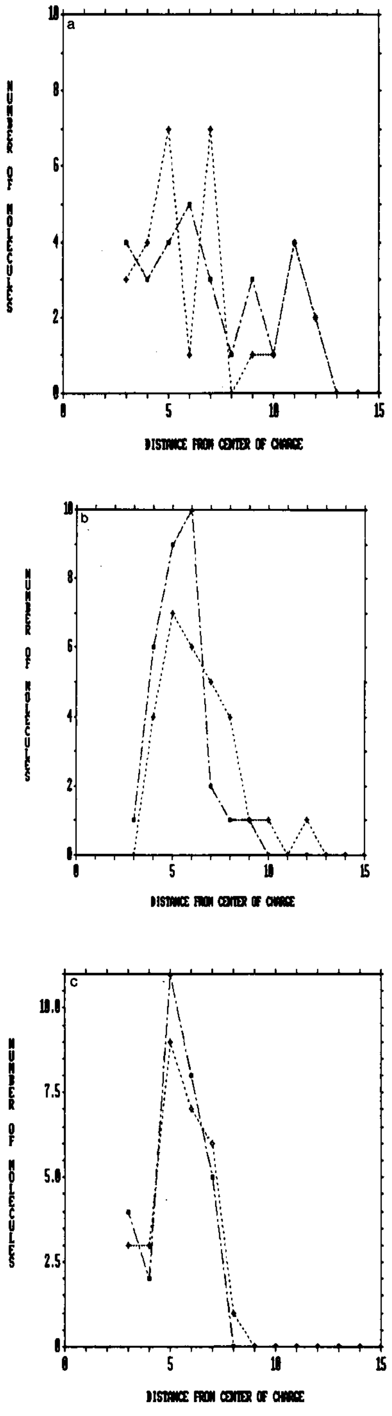

FIGURE 6 Same as Fig. 1 for channel with 3 Å opening. Initial positions are very similar to those in Fig. 1 *a*, and are not shown. Values of *g* for Fig. 5, *a–c*, correspond to those in Fig. 1, *b–d*.

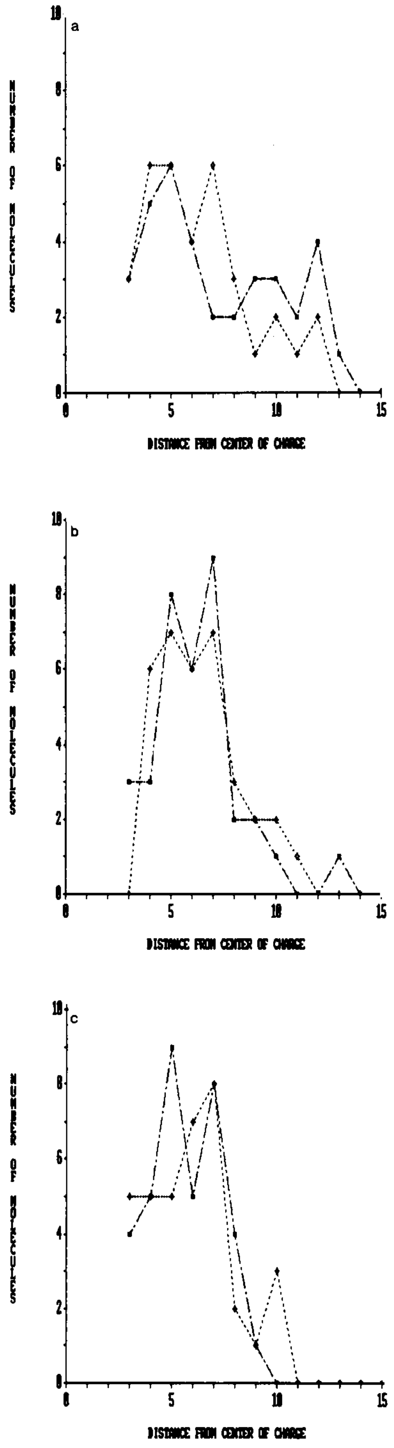

FIGURE 7 Same as Fig. 6 for channel with 3 Å opening with three charges. Values of *g* correspond to those of Fig. 6.

as in the 3 Å opening, 0.4 charge intervals for four total charges, as in the 5 Å opening) we were able to carry out the integration numerically. Fig. 4 shows the results in terms of total energy for the system in units of 10^{-18} *J*. It is also useful to consider energy per mobile molecule. For the 3 Å opening, 3 charge case, $\Delta A = 5.5 \times 10^{-20}$ *J*/mobile molecule, or nearly 14 *kT* per mobile molecule. The corresponding numbers for the 5 Å, 4 charge case are 8.3×10^{-20} J/mobile molecule, or $> 20\,kT$ per mobile molecule. Even if diminished by polarization effects, a lowering of energy by several times kT for each mobile molecule is found, which should still slow molecular motions. The fact that the lowering of energy is $> kT$ means that the "frozen" configuration is fairly stable, and will not be easily disturbed by thermal fluctuations.

The errors in the energies (as shown by the error bars in Fig. 2, representing scatter in replicate runs) is sufficient that it was not practical to attempt to obtain thermodynamic quantities which depend on a temperature derivative (ΔU, ΔS).

The question of the mechanism by which depolarization changes the charge state of the channel has not been dealt with in this calculation, and is not the point of this paper. However, it is of interest to know that at least one plausible method can be suggested, involving hydrogen and hydroxide ions. For one, we observe that the charges are associated with carboxylic acids (if negative) or amines. These are weak electrolytes, and their ionization depends on the motion of hydrogen ions. Hydrogen ions, unlike any other species (except the complementary hydroxide ions), can move through water or ice by the Grotthuss mechanism (Moore, 1972), which requires only molecular rotation for ion transport. However, the water structure which facilitates this mechanism is altered here. Conceivably, a less efficient form of the mechanism is adequate for the small number of charges involved.

It is also known that weak electrolytes in solution in a dielectric liquid will have their pK values altered in a sufficiently high electric field (Second Wien effect). The theory for this phenomenon, first given by Onsager (1934) and little changed since, was the basis for a gating mechanism proposed by Bass and Moore (1968). However, it is unlikely that this theory can be applied directly in the channel, which is a confined space linked to a surface, whereas Onsager's theory pertains to electrolyte in an isotropic solution. The effects of high fields at surfaces on dissociation of electrolytes are difficult to compute. An interesting outline of a theory was proposed by Jenard and Hurwitz (1968), and may give some idea of the magnitude of relevant effects. Their work appears not to have been extended. However, even in its original form, it suggests that an increment of $\sim 0.3 \times 10^8\ \mathrm{V \cdot m^{-1}}$ at an electrode surface could shift pK by over one unit, if the underlying field is large enough. There are two sets of weak electrolyte here: the amino acids (acid or base), and water (water dissociation in a high field is a possible alternate source of charge). Obviously, additional work is needed to understand the effect in the peculiar geometry of a channel; however, this does suggest that it is at least plausible that ionization of weak acids or bases, and therefore charge, may change in response to an external field of about the right order of magnitude.

CONCLUSIONS

The principal outcome of this simulation is that channels apparently can be shut by charges in the wall of the channel; when the charges are absent, the channel allows water to flow through. No studies of the effects of mobile ions have yet been included; if these are not held by the walls of the channels, they should flow through as water does; there does not appear to be any reason for them to be held, as the groups responsible for the charges (presumably amine and carboxyl groups) should not, when neutral, hold ordinary alkali metal ions. Gating currents would then correspond to the neutralization of the charges on the channel walls. Until the actual distribution of charges in a channel is known, and the changes in these charges upon gating determined, the model cannot be regarded as complete.

The "glassy" state reached may be metastable, albeit reproducible in all features of interest from our point of view. It is worth noting that the energy of the system does become stable; however, we cannot claim to be sure that alternate final configurations, even if also "glassy," and with similar characteristics to the states found, may not produce a slightly lower energy. However, the limited scatter in replicate runs (error bars in Figs. 2–4) suggests a fairly reproducible set of configurations. Studies as a function of charge suggest that one to two charges suffice to close a channel, if polarization of the protein is ignored; with polarization, about three to five charges probably would be required.

Our result requires a modification of the electrorheological model of gating proposed by Green (1989). The simulation shows the field responsible for a transition to a highly viscous state of water must come from ions, presumably fixed to the channel wall. In a sense this makes the electrorheological model more specific.

Given this much information, it is possible to go much farther in modeling channels, their interactions with ligands or membrane potentials, and channel energetics. However, this modeling should take account of individual channel properties, and will need additional information on the protein structure, as well as more thorough simulations. Therefore, such further description will be reserved for later work.

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REFERENCES

Bass, L., and W. L. Moore. 1968. A model of nervous excitation based on the Wien dissociation effect. *In* Structural Chemistry and Molecular Biology. A. Rich and N. Davidson, editors. Freeman Publications, San Francisco, CA. 356–369.

Ben-Naim, A., K. L. Ting, and R. L. Jernigan. 1989. Solvation thermodynamics of polymers: I. Separation of the volume and surface interactions with estimates for proteins. *Biopolymers*. 28:1309–1325.

Beveridge, D. L., and F. M. DiCapua. 1989. Free energy via molecular simulation: applications to chemical and biomolecular systems. *Annu. Rev. Biophys. Biophys. Chem.* 18:431–92.

Bezanilla, F., and C. M. Armstrong. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axon. *J. Gen. Physiol.* 60:588–608.

Breck, D. W. 1974. Zeolite Molecular Sieves. Wiley-Interscience, New York. Tables 2.4 and 6.1. 48–51, 449–451.

Dani, J. A. 1986. Ion channel entrances influence permeation. *Biophys. J.* 49:607.

Edmonds, D. T. 1987. A physical model of sodium channel gating. *Eur. Biophys. J.* 14:195–201.

Eisenmann, G., and J. A. Dani. 1987. An introduction to molecular architecture and permeability of ion channels. *Annu. Rev. Biophys. Biophys. Chem.* 16:205.

Green, M. E. 1989. Electrorheological effects and the gating of ion channels. *J. Theor. Biol.* 138:413–428.

Jenard, A., and H. D. Hurwitz. 1968. Ion pairing in strong electric fields. *Interfacial Electrochem.* 19:441–445.

Jorgensen, W. L., J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79:926.

Keynes, R. D., and E. Rojas. 1974. Kinetics and steady state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.).* 239:393.

Keynes, R. D., and E. Rojas. 1976. The temporal and steady state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. *J. Physiol. (Lond.).* 255:157.

Leuchtag, H. R. 1987*a*. Indications of the existence of ferroelectric units in excitable membrane channels. *J. Theor. Biol.* 127:321–340.

Leuchtag, H. R. 1987*b*. Phase transitions and ion currents in a model ferroelectric channel unit. *J. Theor. Biol.* 341–359.

Metropolis, N., A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller, and E. Teller. 1953. Equation of state calculations by fast computing machines. *J. Chem. Phys.* 21:1087–1092.

Mezei, M. 1988. The finite difference thermodynamic integration, tested on calculating the hydration free energy difference between acetone and dimethylamine in water. *J. Chem. Phys.* 86:7084–7088.

Mezei, M., and D. L. Beveridge. 1981. Monte Carlo studies of the structure of dilute aqueous solutions of Li^+, Na^+, F^-, and Cl^-. *J. Chem. Phys.* 74:6902–6910.

Moore, W. J. 1972. Physical Chemistry. 4th ed. Prentice Hall, New York. 435–436.

Neumann, M. 1986. Dielectric Relaxation in water: computer simulations with the TIP4P potential. *J. Chem. Phys.* 85:1567–1580.

Onsager, L. 1934. Deviations from Ohm's Law in weak electrolytes. *J. Chem. Phys.* 2:599–615.

Stuhmer, W., F. Conti, H. Suzuki, X. Wang, M. Noda, N. Yahagi, H. Kubo, and S. Numa. 1989. Structural parts involved in activation and inactivation of the sodium channel. *Nature (Lond.).* 339:597–603.

Zimmerberg, J., F. Bezanilla, and V. A. Parsegian. 1990. Solute inaccessible aqueous volume changes during opening of the potassium channel of the squid giant axon. *Biophys. J.* 57:1049–1064.