Monte Carlo Study of the Effect of Ion and Channel Size on the Selectivity of a Model Calcium Channel†

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Our earlier simulation (*J. Phys. Chem. B* **2000**, *104*, 8903) of the selectivity of a model calcium channel, where all ions were assumed to have the same diameter and the channel dimensions were fixed, is extended to allow for different ionic size and variable channel size. We find that for equal valence, the channel selects cations of the smallest size. If higher valence cations are present, the channel selects cations with the highest valence, and as a result, Ca^{++} will replace monovalent cations. This replacement is more efficient for larger monovalent cations than for smaller ones. This is what would be expected on the basis of the charge/space competition mechanism that has been postulated earlier. Of course, if the size ratio is very large, size might be selected over valence. In addition, we consider the effect of the channel diameter and find that narrow channels are less Ca^{++} selective. This suggests that the recent theory of Nonner et al. (*Biophys. J.* **2000**, *79*, 1976) is most useful for wide, but still microscopic, channels.

Introduction

It is a pleasure to dedicate this article to Howard Reiss. Howard has been a good and faithful friend and supporter. More importantly, he has made many important contributions to physical chemistry/chemical physics. He participated in the development of the Scaled Particle Theory that was one of the very first reliable theories of a dense gas and that influenced our work on perturbation theory. He shares our admiration for Henry Eyring and his work.

Nonner et al.[1] (NCE) have proposed a simple model for a calcium channel and have made calculations of the ion selectivity of this model channel using the mean spherical approximation (MSA) for a homogeneous (or bulk) primitive model (PM) electrolyte. As well as being of scientific importance, the NCE/MSA calculation is interesting because the results of a theory of a homogeneous fluid are applied successfully to what is clearly an inhomogeneous fluid in a narrow channel. It is far from obvious that such a procedure is reasonable.

Recently, we have made a Monte Carlo (MC) simulation for an idealized representation of their model and found the NCE/MSA theory to be quite accurate.[2] In our earlier study, we assumed that all the ions had the same ionic diameter, $d = 2.5$ Å. In this study, we refine our calculations and allow the ions of different species to differ in size. This way we can distinguish between various cations. This new work could be regarded as similar to, but more fruitful than, the electoral recounts that took place recently in Florida. The ionic diameters used in this study are $d(Li^+) = 1.2$ Å, $d(Na^+) = 1.9$ Å, $d(K^+) = 2.66$ Å, $d(Ba^{++}) = 2.7$ Å, $d(Ca^{++}) = 1.98$ Å, $d(Cl^-) = 3.62$ Å, and $d(O^{-1/2}) = 2.8$ Å. These values are taken from the crystal ionic radii in the Sargent−Welch Company periodic table. Different sources may give slightly different values; at most, such differences would have a minor effect on our results.

The NCE approximation yields the result that the selectivity of the channel depends solely on the concentration of the glutamate side chains of channel and does not depend explicitly on the diameter of the channel. To test this approximation, we examine the selectivity of our model calcium channel as a function of channel width when the glutamate concentration is fixed. We would find little or no change if the NCE approximation was fully satisfactory.

Model and Method

The geometry used in our simulation is shown in Figure 1. The channel is represented by an infinitely long cylinder of radius $R_1 = 5$ Å and contains $O^{-1/2}$ ions that represent the glutamate groups that are part of the channel structure. The Ca^{++} ions in calcium binding structures are coordinated with the oxygen ions that belong to the carboxylate, carbonyl, and hydroxyl groups of the amino acid residues of proteins, of which glutamate is one example. Thus, representing the glutamates by oxygen ions seems reasonable. One electron charge is distributed over the two oxygens in a glutamate. For this reason, we assign half an electron charge to each oxygen ion. The $O^{-1/2}$ ions are confined to the interior of the channel. In the first part of our study, where ion size effects are considered, we assume

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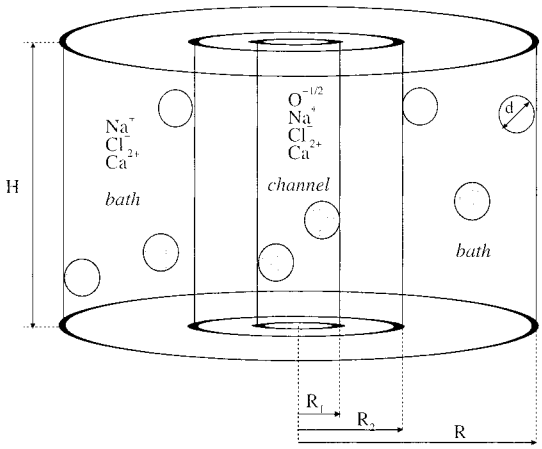

Figure 1. Geometry used in our simulations.

that there are two $O^{-1/2}$ ions in each 2.5 Å section of the channel. Using the volume of the cylinder, we find the concentration of ions to be 16.9 M, and using the volume accessible to the center of an oxygen ion, we find the concentration of oxygen ions to be 32.7 M. This is one of the concentrations used in our earlier work. The other two concentrations were twice this (i.e, 32.8 or 65.4 M, depending on the choice of volume used to compute the concentration) and zero. The highest concentration of $O^{-1/2}$ ions considered before[2] is not used here because of the greater difficulty in obtaining accurate simulation results for this high concentration of oxygens. The value for the concentration of oxygens used by NCE is 35.4 M. The issue of whether the volume accessible to the centers should be used does not arise in their work since they use the results for a homogeneous fluid. Their value differs from the value of 32.8 M that we used because we used the dimensions given to us by NCE; it turned out that the dimensions that were given to us were only approximate and, amusingly, correspond to $\pi = 3$. This small difference in concentration (35.4 and 32.8 M) is not important. The electrolyte consists of two species of cations and one species of anions (Cl^-— in the bulk (or bath) that can permeate into the channel. The bath is represented by the region between the outermost cylinders of radii R_2 and R. Periodic boundary conditions are applied in the z direction.

We use the PM for the electrolyte, where the solvent (water) is represented by dielectric continuum of dielectric constant $\epsilon = 78.5$. For simplicity, we assume the dielectric constant to be everywhere the same. Quite obviously, a molecular model of the solvent is preferable and is feasible in the NCE approach if the chemical potential can be determined without undue labor. However, a molecular model of the solvent does not lend itself to a simulation of selectivity because a very large system is necessary in order to obtain reasonable statistics for the ions, some of which are present at extreme dilution, as is often the case in biological systems.

In the first part of this study, our procedure is to start with a pure 0.1 M NaCl, KCl, or LiCl or a pure 0.05 M $BaCl_2$ system in the bath and add small amounts of $CaCl_2$, observing to what extent the cations are (or are not) replaced by Ca^{++} ions in the channel. In the second part of our study, the oxygen concentrations is held constant at 32.7 M, and the channel diameter is varied. We add $CaCl_2$ to a 0.1 M NaCl bath and study the selectivity.

By appropriately choosing the simulation parameters (number of particles and size of the simulation cell), we kept the concentration of the original cations in the bath fixed, to a high degree of accuracy. The simulations were performed in the canonical (NVT) ensemble, and particle exchanges between the channel and the bath ensured that the two regions were in equilibrium. The use of a μVT ensemble could, in principle, allow the concentration of the original cations in the bath to be held exactly constant. However, the use of this ensemble gives rise to difficulties with particle insertions and requires additional simulations to establish the chemical potential of the bath. The details of our simulation are the same as those given in ref 2. Although this study is a modest conceptual extension of our earlier work, it represents considerable effort extending over several months and involves hundreds of hours of computer time with a Silicon Graphics Power Challenge computer.

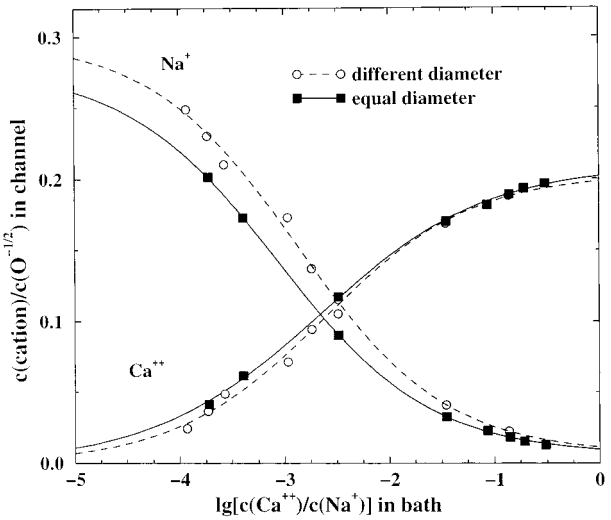

Figure 2. Average monovalent Na^+ and Ca^{++} concentrations in the channel (relative to the oxygen concentration) as a function of the bulk Ca^{++} concentration (relative to the bulk Na^+ concentration) for the case where there are 2 $O^{-1/2}$ ions (representing the glutamate groups) for each 2.5 Å of channel length for the cases where we use the same diameter (2.5 Å) for all ions and differing ion diameters. The points give the simulation results and the curves are fits obtained using eq 7 of ref 2.

Results

Our MC data are given in Table 1. The statistical uncertainty of these data is 1%. There is an additional 1% uncertainty due to the fact that the concentration of the original cations in the bath cannot be held exactly constant in our NVT ensemble.

First we consider the effect of differences in ionic size. The case where $CaCl_2$ is added to a 0.1 M NaCl solution is considered in Figure 2, where our new results are compared with those of ref 2, where all ions were assumed to be of equal size. The reader should be aware that the abscissa in Figures 4 and 6 of ref 2 should have been a base 10 logarithm (denoted by here by lg) and not a natural logarithm. For this case, the effect of ion size is small. This is because Na^+ and Ca^{++} have nearly the same size and the selectivity of the channel is determined almost exclusively by the valence of the ions. The adsorption of the Cl^- ions into the channel is small, and the precise diameter of the Cl^- ion is unimportant.

The results of our simulations for other cations are given in Figures 3 and 4 and are consistent with the mechanism proposed in the NCE/MSA study that we have called the charge/space competion (CSC) mechanism. As is seen in Figure 3, the channel prefers divalent ions over monovalent ions because they deliver twice the charge for a similar excluded volume. Thus, the Ca^{++} ions are more successful in replacing larger monovalent cations than smaller monovalent cations. From Figure 4, we see that

TABLE 1

		bath		channel	
cation	R_1/Å	$\frac{c(Ca^{++})}{c(\text{cation})}$	$\lg\frac{c(Ca^{++})}{c(\text{cation})}$	$\frac{c(\text{cation})}{c(O^{-1/2})}$	$\frac{c(Ca^{++})}{c(O^{-1/2})}$
		0	$-\infty$	0.258	0
		7.41×10^{-5}	−4.130	0.174	0.0498
		2.87×10^{-4}	−3.542	0.136	0.0743
K^+	5	3.93×10^{-4}	−3.405	0.101	0.0990
		1.01×10^{-3}	−2.995	0.0703	0.123
		3.01×10^{-2}	−1.521	0.0185	0.181
		1.35×10^{-1}	−0.869	0.0096	0.198
		0	$-\infty$	0.0367	0
		1.69×10^{-2}	−1.773	0.0347	0.0066
		2.54×10^{-2}	−1.594	0.0332	0.0100
		3.41×10^{-2}	−1.467	0.0341	0.0117
Na^+	3	4.27×10^{-2}	−1.370	0.0330	0.0148
		6.43×10^{-2}	−1.192	0.0311	0.0194
		1.30×10^{-1}	−0.886	0.0284	0.0348
		2.64×10^{-1}	−0.579	0.0241	0.0508
		4.43×10^{-1}	−0.353	0.0210	0.0641
		6.69×10^{-1}	−0.175	0.0184	0.0753
		0	$-\infty$	0.190	0
		1.70×10^{-3}	−2.770	0.134	0.0424
		2.72×10^{-3}	−2.565	0.113	0.0628
		5.49×10^{-3}	−2.260	0.0987	0.0758
Na^+	4	9.07×10^{-3}	−2.042	0.0890	0.0849
		1.17×10^{-2}	−1.931	0.0762	0.0977
		1.94×10^{-2}	−1.713	0.0611	0.115
		2.96×10^{-2}	−1.529	0.0563	0.121
		8.20×10^{-2}	−1.086	0.0378	0.146
		1.39×10^{-1}	−0.858	0.0306	0.158
		0	$-\infty$	0.299	0
		1.13×10^{-4}	−3.947	0.249	0.0247
		1.82×10^{-4}	−3.741	0.230	0.0371
		2.64×10^{-4}	−3.579	0.210	0.0494
Na^+	5	1.06×10^{-3}	−2.973	0.173	0.0725
		1.76×10^{-3}	−2.756	0.137	0.0958
		3.16×10^{-3}	−2.500	0.105	0.117
		3.42×10^{-2}	−1.465	0.0407	0.172
		1.38×10^{-1}	−0.859	0.0225	0.192
		0	-∞	0.351	0
		9.55×10^{-5}	−4.020	0.291	0.0332
		2.44×10^{-4}	−3.612	0.228	0.0663
Na^+	6	1.70×10^{-3}	−2.769	0.120	0.131
		1.49×10^{-2}	−1.827	0.0510	0.179
		5.13×10^{-2}	−1.290	0.0326	0.195
		1.18×10^{-1}	−0.928	0.0235	0.203
		0	-∞	0.328	0
		4.67×10^{-4}	−3.331	0.280	0.0239
		6.90×10^{-4}	−3.161	0.261	0.0359
		1.15×10^{-3}	−2.939	0.242	0.0472
Li^+	5	2.32×10^{-3}	−2.635	0.204	0.0695
		5.32×10^{-3}	−2.274	0.175	0.0876
		7.92×10^{-3}	−2.101	0.145	0.107
		4.09×10^{-2}	−1.388	0.0737	0.158
		1.43×10^{-1}	−0.844	0.0456	0.182
		0	$-\infty$	0.202	0
		3.39×10^{-2}	−1.470	0.180	0.0242
		7.41×10^{-2}	−1.130	0.163	0.0415
		1.57×10^{-1}	−0.804	0.132	0.0739
		2.51×10^{-1}	−0.600	0.114	0.0944
Ba^{++}	5	3.42×10^{-1}	−0.467	0.0967	0.112
		4.51×10^{-1}	−0.345	0.0877	0.123
		5.52×10^{-1}	−0.258	0.0747	0.137
		6.58×10^{-1}	−0.181	0.0669	0.145
		7.66×10^{-1}	−0.116	0.0603	0.152
		8.75×10^{-1}	−0.058	0.0568	0.157
		9.83×10^{-1}	−0.007	0.0507	0.163
		1.09	0.039	0.0470	0.168

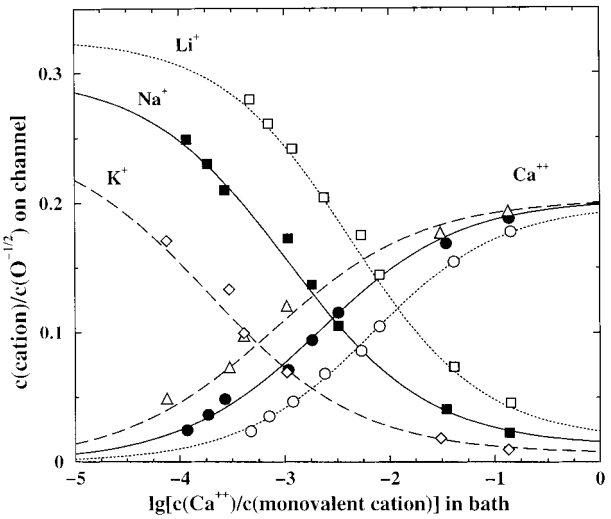

Figure 3. Average monovalent cation and Ca^{++} concentrations in the channel (relative to the oxygen concentration) as a function of the bulk Ca^{++} concentration (relative to the bulk cation concentration) for the case where there are 2 $O^{-1/2}$ ions (representing the glutamate groups) for each 2.5 Å of channel length and using differing ion diameters. The points give the simulation results whereas the curves are fits obtained using eq 7 of ref 2.

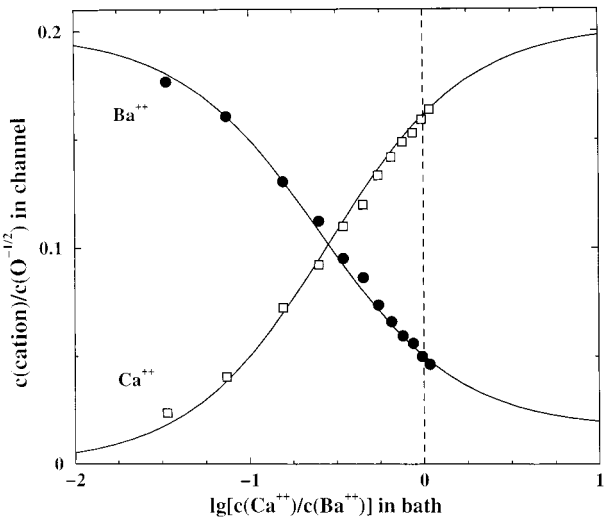

Figure 4. Average Ba^{++} and Ca^{++} concentrations in the channel (relative to the oxygen concentration) as a function of the bulk Ca^{++} concentration (relative to the bulk Ba^{++} concentration) for the case where there are 2 $O^{-1/2}$ ions (representing the glutamate groups) for each 2.5 Å of channel length and using differing ion diameters. The meaning of the points and the curves is the same as in Figure 3.

because of their smaller size, Ca^{++} ions will replace Ba^{++} ion. The purpose of the fitted curves is to aid in visualizing the connection of the simulation points and in extrapolation of the data to ∞. They are both arbitrary and scientifically unimportant. The values at $-\infty$ are simulation results but cannot be plotted because of the logarithmic scale. The values at ∞ are not simulation values but are reasonable extrapolations. The approach to $-\infty$ is not obtained easily by simulation; the fits supply this information in a plausible manner. The curves are, to a good approximation, shifted and scaled versions of each other. The results are consistent with those of NCE, who also considered different ion sizes in their study.[1]

Let us now examine the effect of changes in channel diameter at fixed $O^{-1/2}$ concentration. According to NCE, the selectivity should not change. In Figure 5, $CaCl_2$ is added to a 0.1 M NaCl solution. In these simulations, the number of $O^{-1/2}$ ions in the

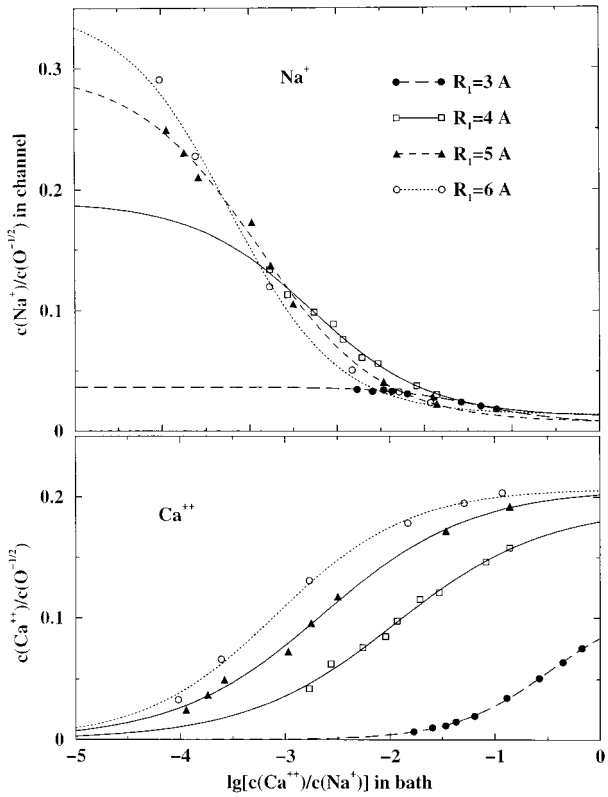

Figure 5. Average monovalent Na^+ and Ca^{++} concentrations in the channel (relative to the oxygen concentration) as a function of the bulk Ca^{++} concentration (relative to the bulk Na^+ concentration), using differing ion diameters, for the case where the $O^{-1/2}$ concentration in the channel is constant. The meaning of the points and curves is the same as that in Figure 3.

channel divided by the volume accessible to their centers is kept fixed. As is seen in this figure, the channel becomes less Ca-selective as the diameter is decreased. The possiblity that this may be due in part to the chosen value of R_2 and the ionic interface in the bath near R_2 cannot be excluded. In any case, the value of the channel diameter must be significant if only because ions cannot be accommodated in the channel if the channel diameter is sufficiently small. Putting this aside, the crossover from a Na^+ majority to a Ca^{++} majority is shifted to higher Ca^{++} concentration in the bath. It is possible that channel diameter effects may contribute to the mechanism for the decreased affinity for calcium observed experimentally in sodium channels.

As the channel is enlarged, the change in selectivity increases. The selectivity curve seems to approach a limit. The NCE results are typical of those for a channel with a large (but still microscopic) diameter.

Discussion

We have examined the effect of ion and channel size on our model Ca channel. The channel prefers high valence and small cations. This is consistent with the CSC mechanism proposed earlier. Our simulations qualitatively explain the fact that calcium channels show, respectively, a 424-, 1170-, and 3000-fold preference for Ca^{++}, over Li^+, Na^+, and K^+, transport according to reversal potential measurements in guinea pig cardiac cells. Also, these same experiments show a size selectivity among divalent cations, with a 2.5-fold preference for Ca^{++} over Ba^{++} transport.[3−4] Interestingly, Mg^{++} does not fit into the CSC classification scheme because it is small and should compete more strongly for the selectivity filter region than the larger ions, for example, Ca^{++}; yet Ca^{++} channels are not appreciably permeable to Mg^{++}.[3] This may be an example of the reversal of selectivity on the basis of size seen in certain cases by Goulding et al.[5] Alternatively, perhaps Mg^{++} binds so tightly that it blocks the channel or it binds its inner sphere of waters so tightly that it cannot enter the channel. These latter possibilities are not addressed by our methodology.

Our simulations also shed light on the range of channel diameters for which the NCE approximation can be expected to be useful. The NCE approximation is a large channel approximation. This is not too surprising since NCE approximates an inhomogeneous electrolyte by a homogeneous electrolyte. What is pleasing is that although the channel radius should be large, it need not be exceedingly large for the NCE theory to be useful.

In this work, we assumed that the dielectric constant was the same everywhere (in the channel, in the membrane, and in the bath). The value chosen was that for water. The authors are well aware that the dielectric constant in the channel and membrane may be different from that in the bath (presumably smaller). If we used different values in these regions and did the calculation properly, each interface would be polarized. The interactions would be more complex, and the level of computational labor would be increased. The purpose of a simulation is to obtain accurate results for a well defined model, which then can be used for testing theories. The exploration of the effects of parameter changes is done more economically with an approximate theory, such as the NCE theory, that has passed this test. Further, one major goal of this work is to test the NCE approximation that does not include the effect of polarization at dielectric discontinuities. Finally, we feel that the effect of variations in the dielectric constant is better in simulations with an explicit solvent and a realistic channel structure, where such effects would arise in a natural way.

Our geometry is simplified. In reality, the channel is finite in length, the membrane is infinite in extent in the *r* direction, the bath lies above and below the membrane and channel, and the dielectric constant may vary in different regions. Nevertheless, in a statistical mechanical study, one attempts to apply as simple geometry as possible using as few parameters as possible that are able to capture the essential features of the phenomenon under study. In this case, the basic components of the model are a uniform dielectric constant and a confined region containing fixed negative charge (acting similarly to a ion-exchange resin) in equilibrium with a bath. The actual geometry of the system (the shape of the channel region and the relative position of the bath) is less important compared to the essential features of the problem that, we believe, are captured in our model. We expect that our planned simulations using more realistic geometries will yield similar results.

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