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ARTICLE

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Test of molecular dynamics force fields in gramicidin A

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Abstract The force fields commonly used in molecular dynamics simulations of proteins are optimized under bulk conditions. Whether the same force fields can be used in simulations of membrane proteins is not well established, although they are increasingly being used for such purposes. Here we consider ion permeation in the gramicidin A channel as a test of the AMBER force field in a membrane environment. The potentials of mean force for potassium ions are calculated along the channel axis and compared with the one deduced from the experimental conductance data. The calculated result indicates a rather large central barrier similar to those obtained from other force fields, which are incompatible with the conductance data. We suggest that lack of polarizability is the most likely cause of this problem, and, therefore, urge development of polarizable force fields for simulations of membrane proteins.

Keywords Molecular dynamics · Force fields · Ion channels · Gramicidin A

Introduction

Understanding the function of biomolecules using the available structural data is one of the grand challenges of the twenty-first century. The problem is too complex for analytical approaches; thus, progress in the field has been made mainly via computer simulations. Thanks to rapid developments in computer technology, the growth in the field, especially in molecular dynamics (MD) simulations, has been phenomenal (Wang et al. 2001, Karplus and McCammon 2002). From the early 1980s, three MD simulation packages with their associated force fields have distinguished themselves, namely, AMBER (Weiner et al. 1984), CHARMM (Brooks et al. 1983) and GROMACS (Hermans et al. 1984). Currently most of the simulation work on biomolecules is carried out using one of these packages. Although they were developed for a specific system initially—AMBER for nucleic acids, CHARMM for proteins and GROMACS for lipids—they have eventually developed into all-purpose packages and currently they are used as such.

A great deal of work has gone into optimization of the parameters employed in these force fields over the last 2 decades. Despite all this effort, one aspect of the force fields just mentioned has remained virtually untouched, namely, neglect of the polarization interaction. Unlike the Coulomb and Lennard-Jones interactions, which are two-body and can be simply implemented, the polarization interaction has a many-body character and requires a computationally expensive iteration process for implementation. The computer power in the early 1980s was simply not enough for explicit inclusion of the polarization interaction. Since they cannot be neglected entirely, effects of polarization were included in a mean-field sense by increasing the permanent dipole moments of molecules. For example, in the common TIP3P water model (Jorgensen et al. 1983), the dipole moment of water is increased from the experimental value of 1.86–2.35 D. This approximation has apparently worked very well for globular proteins (and also for lipids and nucleic acids) so that the simple nonpolarizable form of the force fields has become standard in MD simulations. While the computational overhead associated with the inclusion of polarization is not a problem anymore, development of an entirely new force field from scratch is rather costly. Thus, in order to motivate such an effort, one needs to provide a much better justification for inclusion of polarization than is currently available. For a recent review of the current efforts on the development of polarizable force fields, we refer to Rick and Stuart (2002).

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The membrane proteins are much harder to crystallize compared with the globular ones. Until recently, structures of very few membrane proteins have been available for MD simulations, and therefore they have had virtually no impact in the optimization of force fields. This situation is likely to change in the near future. After the initial breakthrough of the MacKinnon group, who determined the crystal structure of the KcsA protein that forms a potassium ion channel in membranes (Doyle et al. 1998), the structures of many other membrane proteins have followed suit (Chang et al. 1998, Sui et al. 2001, Dutzler et al. 2002, Jiang and coworkers 2002, 2003, Kuo et al. 2003, Yernool et al. 2004). These events have attracted the interest of the MD community in membrane proteins (and more specifically in ion channels), so much so that hundreds of simulations have been carried in the last few years (for reviews see, Kuyucak et al. 2001, Tieleman et al. 2001, Roux et al. 2004, Ash et al. 2004). A common thread in these MD simulations involving membrane proteins is that they all use the standard nonpolarizable force fields. While the mean-field treatment of polarization effects may be justified for globular proteins in a homogeneous water environment, this is a difficult proposition to make for the membrane proteins, which are embedded in a heterogenous lipid–water environment that has vastly different polarization characteristics.

The importance of the polarization effects in ion permeation across membrane channels was recognized earlier (Lee and Jordan 1984, Jordan 1990, Duca and Jordan 1998). However, owing to lack of compelling evidence, these studies did not have the desired impact in the field. To achieve that end, one needs to show that use of the nonpolarizable force fields leads to a clear disagreement with an observable quantity and agreement can only be achieved by including polarization. Until recently, such a computation could only be carried out with a fast supercomputer over long times, which was beyond the reach of most laboratories. Developments in cluster technology (e.g., Beowulf systems), however, revolutionized high-performance computing, bringing cheap supercomputer power to the masses. Thus, rigorous testing of force fields can now be performed in a relatively modest computational environment.

The gramicidin A (GA) peptide, whose head-to-head dimer forms an ion channel in membranes, offers one of the best systems for that purpose (Roux and Karplus 1994). Its molecular structure has been determined from NMR studies (Arseniev et al. 1986, Ketchem and coworkers 1993, 1997, Koeppe et al. 1994), and its functional properties are well characterized from numerous physiological experiments (Andersen and Koeppe 1992, Busath 1993). The peptide's structure—a right handed β-helix formed from only 15 residues (Urry 1971)—is one of the simplest known among the membrane-soluble peptides. Further advantages of GA compared with other ion channels are (1) the channel structure is known in the open state, so no modifications are required to create an open structure, e.g., the KcsA channel, and (2) the conduction mechanism involves a single ion under normal concentrations, whereas multiple ions are involved in most other channels (Hille 2001), which complicates the calculations and their interpretation. Another property of GA making it an exceptionally stringent test system is the single-file nature of the ion–water complex in the channel. This requires a rearrangement of the hydration shell of the ion upon entry to the pore, which has the potential to expose any shortcomings arising from the mean-field treatment of the polarization interaction.

The most straightforward test of the force fields would be the calculation of the conductance of ions across the GA channel from MD simulations. Unfortunately this is still beyond the reach of MD and an indirect method has to be employed, namely, calculate the potential of mean force (PMF) of ions across the GA channel first and then feed this result in Brownian dynamics simulations to estimate the conductance. In fact, the second part has already been performed for a variety PMF profiles and plausible parameters for the PMF of potassium ions that reproduce the conductance data have been determined (Edwards et al. 2002). Thus, calculation of the PMF of potassium ions from MD is all that is required. This was carried out for the CHARMM and GROMACS force fields recently, resulting in central barriers in excess of 20 kT (Allen et al. 2003a). Compared with the 5-kT barrier extracted from the data (Edwards et al. 2002), this is unacceptably large and, if confirmed, could provide the sought after evidence for the breakdown of the nonpolarizable force fields. Subsequently, Allen et al. (2004) repeated the previously mentioned PMF calculation for the CHARMM force field using an improved MD protocol and obtained a similarly high barrier (20 kT), although they argued that inclusion of membrane polarizability and other simulation artefacts (e.g., periodic boundary conditions and high electrolyte concentration) could lower the barrier down to 14 kT. Clearly, further tests of the force fields are desirable to settle the outstanding questions. Here we present the PMF results for a potassium ion obtained using the AMBER force field and compare them with the previous PMF results and experimental data.

Methods

The methods have been described in an earlier paper (Allen et al. 2003a), so we summarize the basic ingredients of the computations here, stressing the changes made compared with that work. The model system consists of the GA dimer embedded in a bilayer consisting of 96 dimyristoylphosphatidylcholine (DMPC) molecules and approximately 3,200 water molecules. Nine pairs of potassium and chloride ions are included in the solution, corresponding to a concentration of 150 mM. In the work of Allen et al. (2003a), the GA monomers were taken from the work of Ketchem et al.

(1997) and Koeppe et al. (1994), for the lower and upper parts, respectively. Here we have used the 1MAG structure of Ketchem et al. (1997) for both monomers. There are some differences in various NMR structures of GA, especially in the rotameric states of Trp9. But as shown by Allen et al. (2003b), after sufficient equilibration all the structures converge, so it does not really matter much which one is chosen initially. A snapshot of the equilibrated simulation system is shown in Fig. 1.

In this study, the AMBER7 MD code with the force field parameter set of AMBER94 was employed. The original set does not have the parameters for the formyl and ethanolamide groups attached to the either ends of the GA monomer. We created them from the existing data set with similar residues. There are no parameters for the DMPC bilayer in AMBER, and these were adapted from the CHARMM27 data set. The CHARMM and AMBER parameters are sufficiently close, and also the lipid atoms play a secondary role in ion permeation compared with the water and GA atoms. Thus, such a treatment of lipid atoms should not have much influence on the results. Default AMBER parameters were employed for water (TIP3P) and ions. Electrostatic interactions were computed using the particle-mesh Ewald algorithm. The simulations were performed in the *NPT* ensemble with Berendsen pressure coupling. Berendsen temperature coupling was used during all equilibration to maintain a temperature of 298 K. A time-step of 2 fs was employed for all simulations and the trajectory data were written at 2-ps intervals during equilibration and also during analysis runs.

We replaced the GA structure in our previous simulation (Allen et al. 2003a) with the 1MAG structure (Ketchem et al. 1997), ensuring a maximal overlap between the two structures during the process. This was followed by an energy minimization of 5,000 steps while keeping all the GA atoms fixed using harmonic constraints with force constants of 2,500 $kT/\text{Å}^2$. During a 200-ps MD simulation, the constraints on the GA atoms were reduced to 500 $kT/\text{Å}^2$. In order to ensure proper equilibration of the GA dimer in the lipid–water environment, we simulated the system for a further 1 ns while keeping the 500-$kT/\text{Å}^2$ constraints on the GA atoms. In the next 1-ns MD simulation, the constraints were removed from the GA atoms gradually. After totally relaxing the GA dimer, we equilibrated the system for a further 2 ns to remove any artefacts associated with the initial NMR structure. One problem with long MD simulations is that the whole system rotates. To prevent such undesired rotations (and thus keep the channel axis parallel to the z-axis), we applied weak planar harmonic constraints at $z = 17$ Å with $k = 0.2\ kT/\text{Å}^2$ on the phosphate atoms of lipids.

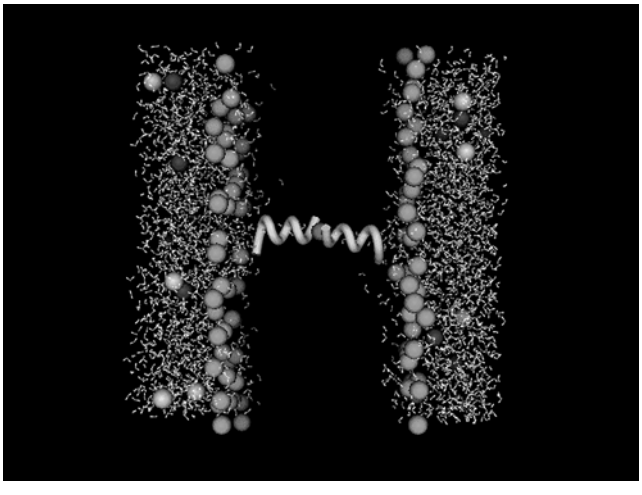

Fig. 1 The model system: gramicidin A (*GA*) dimer embedded in a dimyristoylphosphatidylcholine bilayer and solvated with approximately 3,200 water molecules and nine pairs of KCl ions. The backbone atoms of GA are indicated by the two helices in the *middle* (with a K^+ ion at the centre), K^+ ions by *light-grey balls* and Cl^- ions by *dark-grey balls*. For the lipid, only the phosphate head groups (*grey balls* forming the lipid–water interface) are shown

In the previous work (Allen et al. 2003a), we dragged a potassium ion along the channel axis to create the ion positions for the umbrella sampling. To avoid possible equilibration problems associated with this process, here we used a substitution scheme as in the work of Allen et al. (2003b). After the equilibration of the water-filled channel, each of the eight water molecules inside the pore were replaced with a potassium ion, creating eight different starting positions for the umbrella potentials. During this process a potassium ion outside the pore is converted to a water molecule to keep the whole system charge neutral. By moving the ion to the left and right of these positions by up to 1 Å, we created umbrella-sampling positions at 0.5-Å intervals along the channel axis. After each substitution or after each movement of the ion, the system was equilibrated for a further 300 ps. Finally at each ion position, production runs with umbrella potentials of $k = 20\ kT/\text{Å}^2$ were carried out for 400 ps, and the positions of the potassium ion were recorded at every time step of 2 fs.

The PMF $W(z)$ of potassium ions along the GA channel axis is related to their average distribution function $\langle \rho(z) \rangle$ as

$$W(z) = W(z_0) - kT \ln \left(\frac{\langle \rho(z) \rangle}{\langle \rho(z_0) \rangle} \right), \quad (1)$$

where z_0 is a reference point in the bulk solution. The biased distributions obtained from the MD production runs with umbrella sampling were unbiased and combined to give a PMF using the weighted histogram analysis method (WHAM) (Kumar et al. 1992).

Results and discussion

The results of the PMF calculations for a potassium ion obtained using the AMBER force field are shown in Fig. 2. For comparison, the previous PMF results ob-

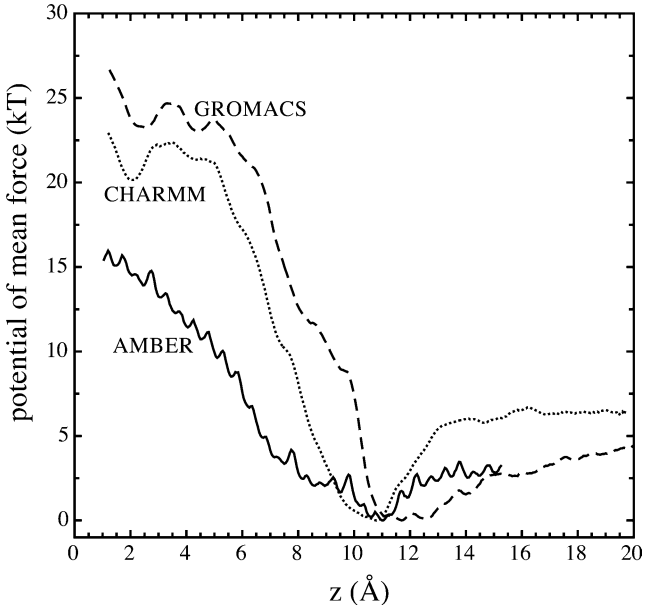

Fig. 2 Potential of mean force of a K^+ ion traversing the GA channel. The *solid line* depicts the AMBER result. The potentials of mean force (*PMFs*) obtained from the CHARMM (*dotted line*) and GROMACS (*dashed line*) force fields are included for comparison. The PMFs are set to zero at the binding site at $z = 11$ Å to make the comparisons easier

tained using the CHARMM and GROMACS force fields are also included in the figure (Allen et al. 2003a). To make the comparison easier, the minimum energy at the binding site is set to zero in all 3 PMFs. The AMBER PMF has a more jugged appearance because the intervals used in umbrella potentials are larger (0.5 Å instead of the 0.2 Å used earlier). Larger intervals with longer simulation times are preferred here because our target is the overall barrier rather than the local structures in the PMF. The central energy barrier found using AMBER is about 16 kT, roughly 5 kT lower than that of CHARMM. This is still some 10 kT higher than the barrier determined from the experimental data by inverse methods (Edwards et al. 2002). Assuming that the current depends on the barrier height exponentially and using the 10 kT figure in the Boltzmann factor, the corresponding discrepancy in the conductance would be $e^{-10} = 4.5\times10^{-5}$, which is more than a factor of 10,000. Thus, there is clear evidence that the traditional force fields have difficulties when they are applied to membrane proteins and something needs to be done about it.

The difference between the AMBER and CHARMM results is presumably due to the larger oxygen partial charges in carbonyl groups and the opposite behaviour of the amide groups in AMBER, which creates a more favourable environment for a cation in the GA channel. To make this assertion more quantitative, we calculated the Coulomb energy between an ion fixed at the centre of the GA channel and all the charges in the GA atoms. The results obtained from the average of 100-ps MD runs are $-140\ kT$ for AMBER and $-128\ kT$ for CHARMM, which could explain the lower barrier found in AMBER. We stress that, in general, the CHARMM parameters work better for proteins, so this result should not be taken in isolation as favouring AMBER. Rather both force fields need to be revamped for a description of membrane proteins.

Clearly we need to repeat the previously described PMF calculations using a polarizable force field, and show that it resolves the discrepancies found with the nonpolarizable ones. This is a time-consuming project and will take a while to execute. In order to illustrate how inclusion of polarizability could resolve the previously mentioned problem by helping to stabilize an ion in the GA channel, we consider a simplified problem that contains the essential energetics of the system. Evidence from NMR experiments indicates that there is negligible change in GA structure upon cation binding (Tian and Cross 1999). The results of this experiment suggest that the GA atoms mostly provide a scaffold for the ion–water column but do not interact strongly with it. Thus, to a first approximation, we may consider the energy of the ion–water system, and how it changes as the ion moves from bulk to the centre of GA. The electrostatic potential energy of an ion surrounded by dipoles is given by

$$U = \frac{1}{4\pi\varepsilon_0}\left\{-q\sum_i \frac{\mathbf{p}_i\cdot\hat{\mathbf{r}}_i}{r_i^2} + \sum_{i>j}\frac{1}{r_{ij}^3}\left[\mathbf{p}_i\cdot\mathbf{p}_j - 3\left(\mathbf{p}_i\cdot\hat{\mathbf{r}}_{ij}\right)\left(\mathbf{p}_j\cdot\hat{\mathbf{r}}_{ij}\right)\right]\right\}, \tag{2}$$

where q is the charge of the ion, $\mathbf{p}_i$ denotes the dipoles, r_i is the ion–dipole distance and r_{ij} is the dipole–dipole distance. For an ion in bulk solution, the dominant contribution to this energy comes from the water molecules in their first hydration shell. Assuming an average ion–dipole distance of r and a coordination number of 6 with the dipoles placed perpendicular to the faces of a cube, we can evaluate Eq. 2 in closed form to yield

$$U_{\text{out}} = -701\frac{p}{r^2} + 173\frac{p^2}{r^3}. \tag{3}$$

Here p is in debye, r is in angstrom and U is in units of kT. Note that the dipole–dipole interaction is repulsive because the dipoles are mostly perpendicular to each other. When the ion is at the centre of the GA channel, there are three water molecules on average on either side with their dipoles aligned along the ion's electric field. The potassium–water distance is nearly equal to that of water–water. Thus, taking the distances among the neighbours as being equal, we can evaluate the corresponding potential energy for this configuration as

$$U_{\text{in}} = -318\frac{p}{r^2} - 194\frac{p^2}{r^3}. \tag{4}$$

While the ion–dipole contribution is reduced by more than half compared with the bulk case, the dipole–dipole

contribution has become attractive and thus it could compensate for the loss in the hydration energy. To see the influence of the water dipole moment on the stability of an ion inside the GA channel, we subtract the two energies in Eqs. 3 and 4:

$$\Delta U = U_{\text{in}} - U_{\text{out}} = 383\frac{p}{r^2} - 367\frac{p^2}{r^3}. \quad (5)$$

Taking $r = 2.8$ Å as the average distance between potassium and water or water and water, we see that the traditional force fields with $p = 2.3$ D yield an energy of $U = 24\ kT$, which is consistent with the barriers found in the PMF calculations. Using a typical value of $p = 2.7$ D from polarizable water models (Wallqvist and Mountain 1999), this instability in energy goes down to $U = 10\ kT$. Finally, increasing the dipole moment to $p = 3$ D as suggested by some ab initio calculations (Silvestrelli and Parrinello 1999), we obtain $U = -4\ kT$. These simple calculations show that the value of the dipole moment of water could play an important role in stabilizing an ion in the GA channel. As the polarizable models allow larger values of dipole moments compared with the nonpolarizable ones, inclusion of polarizability should help to ameliorate the barrier problem in the GA channel.

The past experience with polarizable models of water has taught us that inclusion of polarization starting from a nonpolarizable model is fraught with difficulties. A better approach would be to use the ab initio methods (e.g., Car and Parinello 1985) as a guide in coming up with a polarizable force field. GA certainly offers one of the simplest systems for such a purpose.

Conclusions

In this paper, we have reported PMF calculations for permeation of potassium ions across the GA channel using the AMBER force field, thus completing the test of the three major force fields commonly used in MD simulations of biomolecules. All three force fields result in rather high central barriers that are incompatible with the experimentally observed conductance values. These results suggest that the traditional force fields need to be reassessed for applications to membrane proteins. It is argued that lack of polarizability is the most likely source of this discrepancy. We are currently working on a polarizable force field and will repeat the PMF calculations in the GA channel with this force field. If a reduction in the barrier height is found, this will reinforce the importance of polarization effects in MD simulations of peptides in membrane environments, and hopefully instigate more effort into the development of polarizable force fields.

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