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The Gramicidin Channel Ion Permeation Free-Energy Profile: Direct and Indirect Effects of CHARMM Force Field Improvements

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Abstract

A revised CHARMM force field for tryptophan residues is studied as well as the effect of the new peptide torsion-energy cross-correlation correction algorithm (CMAP) using molecular dynamics simulations of gramicidin A (1JNO) embedded in a lipid bilayer (DMPC) with 1 mol/kg NaCl or 1 mol/kg KCl saline solution. The new tryptophan force field produces, in the decomposition, a Na^+ PMF_{Trp} that is consonant with the prediction from the experimental results, analyzed with rate theory by Durrant et al. (2006), in stark contrast to the prediction of the original CHARMM, version 22, tryptophan side-chain force field. However, the effect is lost in the complete PMF due to indirect effects mediated by other components of the system (peptide, lipid, ions, and water). CMAP reduces the excessive translocation barrier. Decomposition demonstrates that this effect is due to effects on the K^+ $\text{PMF}_{\text{H}_2\text{O}}$ rather than on K^+ PMF_{gA} . Both represent examples of indirect effects (i.e., consequences of the force field mediated by “innocent bystander” molecules). The results have been confirmed to be robust using an alternative umbrella potential method.

Keywords

Independent transport coordinate; Relative transport coordinate; Potential of mean force; Highly occupied site; Torsion angles; NPAT; CMAP

Introduction

Molecular dynamics (MD) simulation is an important tool for studying both biological and chemical systems. Its importance comes from its ability to study macromolecular vibrations at the atomistic level, where macroscopic techniques cannot extract some biological phenomena, such as ligand-binding flexibility or details of protein-structural fluctuations.¹

The gramicidin A (gA) channel has a simple well-known structure and well-defined functions.^{2–4} The gA channel is a polypeptide that dimerizes head-head, forming a cation channel in lipid bilayer membranes. It is composed of two right-handed⁵ single-stranded hydrogen-bonded $\beta^{6,3}$ -helices^{6,7} with the amino acid sequence: Formyl-L-Val-L-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp⁹-D-Leu-L-Trp¹¹-D-Leu-L-Trp¹³-D-Leu-L-Trp¹⁵-

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Ethanolamine.⁸ Another interesting protein is the gramicidin M (gM) channel, where the tryptophan residues at positions 9, 11, 13, and 15 of gA channel are replaced with the phenylalanine residues, affording independent assessments of the difference in the side-chain dipole potentials along the channel transport pathway through modeling of experimental channel currents and through MD simulations.

The tryptophan side-chains in the gA channel are very important for the channel organization and functions.^{4,9–23} In addition, their dipole moment affects the cation stability, the cation affinity at the highly occupied site (HOS) (often called Binding Site), the thermodynamic parameters of the cation affinity, and the cation free-energy barriers, which govern the channel conductance.^{3,24–34} Binding site is a term used by pharmacologists to refer to specific key-in-lock vdW–electrostatic–steric complementarity binding; by channelologists to refer to a local minima in the free-energy profile; by biochemists to refer to a region on a protein, DNA, or RNA where some other specific molecules or ions form a chemical bond; and by biologists to refer to a region where an enzyme can attach itself to a compound and react with it. It is hard to know the limits on the use of the term Binding site, and in gramicidin, the term has always been controversial (but still generally used) because the electrostatic factors causing the local minima in the free-energy profile appear to be long-range effects rather than intrinsic protein–ion interaction effects. Consequently, we prefer to use the term HOS instead of the term Binding site because the long-range-electrostatic factors causing the local minima in the free-energy profile are specific in the sense that only one ion can fit at a time and it must be, with a few exceptions,^{35,36} an alkali metal cation or ammonium, but not in the sense of complex ligand-shape complementarity.

A theoretical analysis of experimental results for gA, gM, and heterodimers of the two indicated that the tryptophan dipole potential (relative to the phenylalanine dipole potential) must be bowl-shaped with a depth of ≈ -4.9 kcal/mol,³⁷ whereas version 22 of the CHARMM force field predicts a double-well potential for the static structure.³⁸ Because the ion potential is sensitive to the choice of the biomolecular force field,^{39,40} could the dynamic average ion–Trp potential energy for gA be closer to experimental prediction if we use the newly recommended MacKerell force field⁴¹ for tryptophan residues instead of version 31 of the CHARMM force field?

In a related issue, the cation-transport free-energy profiles, predicted by MD simulations, ^{39,40,42–49} yield a significantly higher translocation barrier than is expected from the predicted PMF from the physiological data.^{37,50,51} The inclusion of the long-range-electrostatics corrections^{39,40,44} and the introduction of polarizability^{40,45,46,49,52} in the force field have partly alleviated this problem, but the relative roles of these and other factors are yet to be evaluated. Another improvement in the CHARMM force field, version 31, concerns the peptide backbone treatment, which was improved based on the dipeptide properties of alanine, glycine, and proline.⁵³ The improvement included adding additional optimized ϕ , ψ dihedral parameters to version 22 of the CHARMM force field, which improved treatment of protein-folding problems and protein-structural properties. Although the CMAP corrections are not chirally symmetric like version 22 of the CHARMM force field, we discovered that their use with gA channel (in which alternate residues are of D chirality or glycine) yielded an improvement in the free-energy profile for cation transport. As we will show, the improvements (which admittedly may be fortuitous, in part, due to inaccurate corrections for the dihedrals governing the D-residues) are due to indirect effects, mediated by channel water molecules.

Understanding ion permeation through membrane proteins and its selectivity mechanism is important, and a strong challenge to force field accuracy for interactions, from short-range⁵⁴ to long-range^{31,33}. Therefore, we tested these two improvements on the ion permeation

through the gA channel via umbrella-sampling simulations. Our findings show partial improvements for both issues and highlight the complications presented by indirect effects.

Methods

Model System and Parameters

The initial system consisted of a gA dimer, with the 1JNO structure,³ embedded in 40 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) molecules as a lipid bilayer, as well as 2366 water molecules, and 1 mol/kg NaCl. The 1JNO structure is a relaxed structure, based on solution state NMR results for dimers in sodium dodecyl sulfate micelles,³ and consistently reproduces the solution and the solid-state NMR properties.⁵⁵ A TIP3P model was used as the water model, which was modified in CHARMM from the original model⁵⁶. The terminal residues, ethanolamine and formyl, were given their partial charges and force field parameters based on other similar atomic groups in the force field. The tryptophan residues were first given MacKerell force field parameters⁴¹ and this system was denoted gA_{Mac}. The initial structure [Fig. 1(a)] was constructed, using the VMD software,⁵⁷ in a box with initial dimensions of $40 \times 40 \times 90$ Å in x , y , and z dimensions, respectively.

System Preparation

The total simulation time for system preparation was 25 ns, with different periodic boundary conditions and harmonic restraints, as shown in (Table I). The preparation included conjugate-gradient energy minimization of the initial configuration, followed by heating from 0 to 303.15 K, equilibration in the NVT ensemble, three-stages of annealing (40 ps heating to 1000 K followed by 80 ps cooling to 303.15 K), and progressive release of restraints on the protein over multiple stages of equilibration (Table I). This preparation allowed relaxation of the protein and disordering the lipid molecules, ions, and water molecules [Fig. 1(b)] starting from the crystal-like structure.

Both the lipid deuterium order parameters and its surface area are sensitive to the changes in the bilayer structures due to many factors such as the lipid hydration level, number of lipid molecules, temperature, statistical ensemble, surface tension, and saline-solution concentration.^{58–65} For simulating a pure lipid bilayer in pure water, the appropriate surface tension has been evaluated to be within the range of 30–45 dyn/(cm²-interface).^{61,63,66,67} However, with a more complex lipid bilayer system, such as one that includes salt and protein, the surface tension is unknown. We carried out a test for the suitable surface tension using the Equilibration 3 stage conditions, that is, a fixed protein in the 1JNO structure to avoid protein-surface perturbation effect on the lipid bilayer properties,²¹ two planar harmonic restraints on the lipid phosphorus atoms ($k_f = 10$ kcal/(mol Å²)) at $z = \pm 17.5$ Å to agree with the experimental head-head or phosphate-phosphate thickness of 35.3 Å,^{68,69} and with 2.5 unit increments of the surface tension, γ , in the range of 0–75 dyn/(cm²-interface). Based on accuracy and precision in the lipid molecular surface area consistent with the experimental DMPC molecular surface area,⁶⁹ the optimal surface tension was found to be 40 dyn/(cm²-interface).

The Equilibration 3 stage was then carried out with $\gamma = 40$ dyn/(cm²-interface), a fixed protein in the 1JNO structure, and two planar harmonic restraints on the lipid phosphorus atoms ($k_f = 10$ kcal/(mol Å²)) at $z = \pm 17.5$ Å (Table I). This stage was run for 20 ns, enough time to obtain stable lateral dimensions for the system under study.⁴³ It gave, in the last 10 ns, a lipid surface area of 60.53 ± 0.82 Å² after subtracting 250 Å² as a gA surface area²⁰. The purpose of this stage is to prepare a realistic lipid environment for the peptide channel.

During the subsequent equilibrations, the restraints on the protein and the lipid phosphorus atoms were progressively reduced without significant changes in the system structure. These

simulations in the NPAT ensemble (Equilibrations 4–10) utilized a tetragonal box having a lateral dimension of 38.22 Å, the final value after the Equilibration 3 stage. Both the Equilibration 10 stage and the routine 10 ns production run (done in parallel with the umbrella sampling simulations) were performed with the protein center-of-mass (COM) as the only restraint. Considering that the lipid molecules immediately adjacent to the gA channel have different properties than those farther away from the channel due to protein-surface perturbations,²¹ and that 16 lipids per leaflet has previously been shown sufficient bulk-like lipid surroundings for the gA dimer,⁴⁹ our final system has a reasonable size.

A second system was derived from the gA_{Mac} system, by replacing the MacKerell force field parameters⁴¹ for tryptophan residues with version 31 of the CHARMM force field parameters, using the final frame of the Equilibration 9 stage structure for the Equilibration 10 stage. This system was denoted gA_{C31}.

Another system was derived from the gA_{C31}, replacing all Na⁺ ions with K⁺ ions, using the Equilibration 10 stage structure as the starting point. This system was used for potential of mean force (PMF) studies.

The gM system was derived from the gA system, replacing each tryptophan residue with a phenylalanine residue, and equilibrated in the same way as the gA_{Mac} system, using all of the stages in (Table I).

Molecular Dynamics Simulations

Simulations were performed with NAMD software,⁷⁰ version 2.6, using version 31 of the CHARMM force field.^{53,71–75} Trajectory files were written every 1 ps for both the long test-ion-free production run and the umbrella sampling production runs using a time step of 2 fs and the NPAT ensemble. The test-ion-free production run was for 10 ns. A smoothing function was applied to both the electrostatic and van der Waals forces at a distance of 8 Å with a switching cutoff distance of 12 Å, a pair list distance of 14 Å, and all bonds with hydrogen being rigid using the ShakeH algorithm. The non-bonded interactions parameters (switching, spherical cutoff, and pair list distances) were carefully chosen to achieve realistic macromolecular simulations.⁷⁶ The Particle Mesh Ewald algorithm (PME) was used for electrostatic calculations with an interpolation function of order 5, and PME box grid size of 64, 64, 128 in *x*, *y*, and *z* direction, respectively. Langevin dynamics was used to maintain the temperature at 303.15 K with a damping coefficient of 5 ps⁻¹, whereas the pressure in the *z* direction was maintained at 1 atm using the Nosé–Hoover Langevin piston method with an oscillation of 1 ps and a damping time of 0.1 ps.^{77,78} All the analysis scripts were composed locally using both VMD and Tcl⁷⁹ commands.

Umbrella Sampling Simulations

All the umbrella sampling simulations were performed using the final Equilibration 10 structure of the system under study as an initial structure. In these simulations, two umbrella-potential methods were used: 1), Relative Transport Coordinate (RTC), in which the protein COM is restrained to within 0.1 Å of the origin ($k_f^{\text{COM}} = 1000 \text{ kcal}/(\text{mol} \cdot \text{Å}^2)$) and the test ion, selected from the bath ions, in the *z* direction, is restrained relative to the protein COM ($k_f^{\text{RTC}} = 15 \text{ kcal}/(\text{mol} \cdot \text{Å}^2)$); 2), Independent Transport Coordinate (ITC), in which the protein COM is restrained to within 0.1 Å of the origin ($k_f^{\text{COM}} = 1000 \text{ kcal}/(\text{mol} \cdot \text{Å}^2)$) and the test ion, selected from the bath ions, is restrained to a plane normal to the *z* axis ($k_f^{\text{ITC}} = 20 \text{ kcal}/(\text{mol} \cdot \text{Å}^2)$). All k_f symbols designate force constants (i.e., harmonic spring constants).

The protein COM was strongly restrained to the origin to gain better convergence as deduced from earlier work.^{46,47,49} In the RTC method, the transport coordinate (traditionally called the reaction coordinate) was chosen to be the distance of the test ion relative to the protein COM in the z direction. An equal but opposite force [Eq. (2)] should be distributed over all the channel atoms; however, this was not implemented due to its negligible effect compared with the presence of the restraining force on the protein COM. In the ITC method, the transport coordinate was chosen to be the position of the test ion relative to the origin of the simulation box (0, 0, 0) in the z direction. The restraining force on the protein COM is not a component of the transport coordinate in the ITC method, but rather a structural restraint designed to establish a reference point for the coordinate system, whereas it is in the RTC method. In both cases, the PMF is not expected to be affected by the structural restraining force⁸⁰ as long as the system is well equilibrated and the PMF is calculated with respect to the bulk. The ITC approach has the advantages of being practically easier to implement in NAMD compared with the RTC method and producing the same results as the RTC method. We do not anticipate that the ITC approach would be affected by the presence of the restraining force on the protein COM,⁸⁰ which, in any case, is the same as in the RTC method.

During umbrella sampling with the test ion, a spherical restraint ($k_f = 20 \text{ kcal}/(\text{mol} \cdot \text{\AA}^2)$) was applied on all other ions to keep them more than 14 $\text{\AA}$ away from the origin and a cylindrical restraint ($k_f = 20 \text{ kcal}/(\text{mol} \cdot \text{\AA}^2)$) was applied on the test ion to keep it within 5 $\text{\AA}$ of the z axis. The purpose of the cylindrical restraint is to assure a bounded sampling region outside the channel for the test ion, whereas the purpose of the spherical restraint is to assure a one-ion PMF.^{39,40,44}

We used 201 windows with a window width of 0.2 $\text{\AA}$ to cover the transport coordinate $-20 \leq z \leq 20 \text{ \AA}$. For each window, the z coordinate of the test ion was set to the window position and its x and y initial values were set to zero before performing minimization for 3000 steps, heating from 0 to 303.15 K, equilibration in the NPAT ensemble for 1 ns, and production in the NPAT ensemble for 1 ns. We used the weighted histogram analysis method (WHAM) to unbias the umbrella potential on the test ion and extract the PMF.⁸¹ The code is available for free download from the web.⁸² The mathematical details for calculating or extracting the PMF were discussed elsewhere.^{39,81,83,84}

The decomposed potential of mean force (PMF_i) was calculated by integrating the z component of the mean force $\langle F_i(z_{ion}) \rangle$ applied by the interesting group (i) on the test ion over the transport coordinate,⁵²

$$PMF_i(z_w) = PMF_i(z_o) - \int_{z_o}^{z_w} \langle F_i(z_{ion}) \rangle dz_w, \quad (1)$$

where the $PMF_i(z_o)$ is an arbitrary reference value, generally assumed to be zero at z_o , z_w is the window-reference position, and $\langle F_i(z_{ion}) \rangle$ is the average equilibrium force applied by group (i) on the test ion at window w . The force $F_i(z_{ion})$ was extracted via the pair-interaction-calculation method available in the NAMD software package. This method analyzes the available dynamic trajectory (ours was sampled every 1 ps for the ensemble average) and calculates the forces, vdW and electrostatic, from the second group, group (i), on the first group, the test ion.

The potential of mean restraint (PMR) was used as a test for the WHAM results. The PMR can be extracted as follows:⁸⁰

$$F^{\text{RTC}}(z_{\text{ion}}, z_{\text{COM}}) = -k_f^{\text{RTC}}(z_{\text{ion}} - z_{\text{COM}} - z_w), \quad (2)$$

$$F^{\text{ITC}}(z_{\text{ion}}) = -k_f^{\text{ITC}}(z_{\text{ion}} - z_w), \quad (3)$$

$$PMR^{\text{RTC}}(z_w) = PMR^{\text{RTC}}(z_o) + \int_{z_o}^{z_w} \langle F^{\text{RTC}}(z_{\text{ion}}, z_{\text{COM}}) \rangle dz_w, \quad (4)$$

$$PMR^{\text{ITC}}(z_w) = PMR^{\text{ITC}}(z_o) + \int_{z_o}^{z_w} \langle F^{\text{ITC}}(z_{\text{ion}}) \rangle dz_w, \quad (5)$$

where z_{ion} is the test ion position in the z direction (sampled every 2 fs for the ensemble average), z_{COM} is the protein COM position in the z direction (sampled every 2 fs for the ensemble average), z_w is the window-reference position, $F^{\text{RTC}}(z_{\text{ion}}, z_{\text{COM}})$ is the instantaneous restraint force applied in the z direction to the test ion under the RTC method, and $F^{\text{ITC}}(z_{\text{ion}})$ is the instantaneous restraint force applied in the z direction to the test ion under the ITC method. The symmetrization of our results was obtained by averaging the free-energy values at positive and negative values of the transport coordinate. The Composite Simpson's Rule was used as a numerical integration method.⁸⁵

Results and Discussion

Preparation and Simulation Methods Stability

The preparation process successfully produced a stable and disordered system compared with the initial crystal-like structure [Fig. 1(a)]. On the basis of simulating two other gA systems (data not shown), which vary in the number of lipids molecules (i.e., one with 16 and the other one with 280 DMPC lipid molecules), we concluded that three-lipid shells surrounding the protein are required to provide the bulk lipid structural properties at the periodic boundaries.

The simulating annealing, based on the deuterium order parameters [Fig. 2(a)], was sufficient to produce disordered lipids in gA_{Mac}, gA_{C31}, and gM systems. The deuterium order parameters of the deuterium-labeled segment was calculated from: 58, 60, 62, 64

$$S_{CD}^{(i)} = \frac{1}{2} \langle (3\cos^2\theta_i - 1) \rangle, \quad (6)$$

where θ_i is the angle between the i th C–H vector and the z axis (bilayer normal), and the angle brackets denote an average over both acyl chains from all lipid molecules, all C–H vectors associated with the i th carbon, and time. Our results excellently agree with both the experimental^{86–88} and the simulation⁶⁴ results, except in the region close to the head group, where the order remains high in experiments,^{86–88} but commonly declines in simulation.⁶⁴

The root-mean-square displacement (RMSD) shows reasonable protein structural stability within the production run time scale except for slight excesses in the gA_{Mac} and gM systems after 5 ns [Fig. 2(b)]. The density profiles of those systems (data not shown) show a good distribution for all atoms. One contribution to the slight drifting in the RMSD is the mobility of the ethanolamine residues, which is higher in the gA_{Mac} and the gM systems than in the gA_{C31} system [Fig. 2(c)].

The conformational stability of the side-chains can be represented by their torsion angles (dihedral angles) such as χ_1 and χ_2 angles. For tryptophan and phenylalanine side-chains, the χ_1 angle is the counterclockwise rotation of the $C_\beta-C_\gamma$ bond around the $C_\alpha-C_\beta$ bond in $N-C_\alpha-C_\beta-C_\gamma$ chain of atoms, whereas χ_2 angle is the counterclockwise rotation of the $C_\gamma-C_{\delta 1}$ bond around the $C_\beta-C_\gamma$ bond in $C_\alpha-C_\beta-C_\gamma-C_{\delta 1}$ chain of atoms.⁸⁹ This definition gives a positive torsion angle, whereas a negative torsion angle requires rotation in the opposite direction.

Both χ_1 and χ_2 angles for the side chain residues at positions 9, 11, 13, and 15 of the protein as a function of time are presented in (Fig. 3). Trp⁹ starts and mostly stays in a different rotameric state, and drifts a bit [Figs. 3(a-d)], compared with other positions, consistent with previously reported simulation results.⁵⁵ The reason for this difference is that all the tryptophan residues except Trp⁹ are close to the membrane surface, therefore, they have stronger interactions with the water molecules and the lipid head groups via hydrogen bonding, which stabilize them at their positions.⁵⁵ On the basis of the Lovell et al. rotamer library nomenclature,⁸⁹ our results show that the Trp⁹ residues maintain the **t90°** rotamer state, whereas the Trp^{11, 13, 15} residues maintain the **m-90°** rotamer state through the simulation time scale. Our results agree with those data extracted from the experimental results in sodium dodecyl sulfate micelles leading to the 1JNO structure,³ but conflicts with the data extracted from those experiments in DMPC bilayers leading to the solid-state NMR results giving the 1MAG structure,⁵⁰ where Trp⁹ residues assumes the **m-90°** rotamer state. Based on the Lovell et al. database,⁸⁹ the most common rotamer state for tryptophan is **m95°** with 32% observations, whereas **t90°** and **m-90°** rotamer states were observed with 18% and 5%, respectively.

For the gA_{Mac} system, both χ_1 and χ_2 angles have reasonable and stable values throughout the production run time scale [Fig. 3(a,b)]. Clearly, the lipid head groups dramatically stabilize the side-chains because in vacuum their simulations show numerous rotamer changes on the ns time scale.⁹⁰ In the gA_{C31} system, except for some temporary fluctuations in χ_1 for Trp^{11,R}, and in χ_2 for Trp^{9,R}, the tryptophan side-chain positions are generally stable throughout the production run time scale [Fig. 3(c,d)], as is also true for the subsequent umbrella sampling runs. Both χ_1 and χ_2 angles for phenylalanine residues on gM are shown in [Fig. 3(e,f)], respectively. Using the same nomenclature,⁸⁹ Phe^{9,R} residue generally maintains the **t80°** rotamer state, whereas the rest of phenylalanine residues maintain the **m-85°** rotamer state through the simulation time scale. This is the first report of MD simulations of the gM channel allowing assessments of the conformational stability of the phenylalanine side-chains. They show good conformational stability on this time scale, with drifting of Phe⁹ residue in some cases and a temporary perturbation of Phe^{11,L} residue in other cases. According to Lovell et al. database,⁸⁹ the common rotamer state for phenylalanine is **m-85°** with 44% observations, whereas **t80°** rotamer state was observed with 33%. The phenylalanine rotamer states in our gM simulations primarily reflects the initial conformation.

The conformational variants for the tryptophan and the phenylalanine side-chains in the gramicidin channels as well as the slight excesses of the RMSD for the gramicidin channels are not expected to produce much asymmetry or error in the Na⁺ PMFs, because they do not occur in the umbrella sampling runs, which are generally similar to the first two ns of the production runs, except for minor fluctuations induced by the test ion.

Tryptophan Force Fields

How the two types of the force field and the side-chain residue species affect the shape and the amplitude of the Na⁺ PMF_(side chains) are shown in [Fig. 4(a)]. As stated in the Methods section [Eq. (1)], this is calculated from the *z* component of the mean force exerted by the

side-chain on the test ion, starting with the β -carbon. The CHARMM force field, version 31, for phenylalanine has a double-welled shape with a minimum of -3.2 kcal/mol and the Trp potential contributes to the Na^+ PMF in a double-welled shape with a minimum of -5.7 kcal/mol. In contrast, the new MacKerell force41 for tryptophan produces a bowl-shaped potential with a minimum of -7.1 kcal/mol.

The MacKerell force field result for the free-energy difference (Na^+ PMF_{Trp} minus Na^+ PMF_{Phe}) [Fig. 4(b)], when compared to the experimentally derived gA-gM free-energy-difference profile,³⁷ has the same bowl-shape. The minimum in the computed side chain PMF is -6.1 kcal/mol, within the simulation and experimental uncertainties, which is similar to the experimental minimum of ≈ -4.9 kcal/mol. Therefore, apparently, the MacKerell force field is superior to the CHARMM force field, version 31, in treating tryptophan electrostatic properties, producing a more accurately shaped, albeit somewhat deeper, profile.

Since the new Trp potential has a shape and minimum different from that of the older one, could we see the long-sought reduction in the Na^+ translocation barrier due to this change in the tryptophan force field? The answer to this question is discussed in the next section.

Force Fields Effects on the Na^+ PMF

The Na^+ PMF in gA_{Mac} and gA_{C31} systems is presented in [Fig. 5(a)]. Surprisingly, there is very little difference between the two curves in spite of change in the tryptophan force field. Both force fields produce HOSs at ± 9.1 Å in agreement with those at ± 9.2 Å extracted from ^{13}C chemical shift anisotropy experiments³⁸ and differ dramatically from previous simulation result⁴⁹ with regard to the absolute magnitude of the translocation barrier, ≈ 4 kcal/mol versus ≈ 15 kcal/mol.

One way to explore why changing the tryptophan side-chain force field has no impact on the Na^+ PMF is by decomposing it into its components [Fig. 5(b)]. The Na^+ PMF_{Trp} most affects the larger components of the Na^+ PMF in the translocation region ($-12.5 \leq z \leq 12.5$ Å), but not in the expected ways. Rather than making the Na^+ PMF_{gA} a bowl-shaped potential, as one would expect from the direct electrostatic effect [Fig. 4(b)], the MacKerell force field causes the ion-protein interaction to be less favorable throughout the translocation region. From this, it is clear that effects of the tryptophan side-chains on the ion, mediated by the protein backbone or other side-chains, must have more impact than the direct electrostatic effects. We will refer to this phenomenon as an *indirect* effect, that is, an effect of the modified force field on the free energy profile mediated by induced changes in the positions or dynamics of other atoms in the system. Other indirect effects, mediated by the lipids, the other ions, and the water molecules, are also remarkably large. Ultimately, there is a complete compensation between two groups, the Na^+ PMF_{gA} and Na^+ PMF_{lipids} versus the Na^+ PMF_{H₂O} and Na^+ PMF_{ions}. Under the MacKerell force field, the first group has a higher Na^+ PMF; than with the CHARMM force field, version 31, within the channel region, $-12.5 \leq z \leq 12.5$ Å, whereas the second group has a lower one. This behavior is consistent with and explains previous MD simulations showing that the gA channel structurally responds to external perturbations such as an ion in the pore.^{39,52}

For completeness, we show the Na^+ PMF in the gM system [Fig. 5(c)]. The phenylalanine residues have small partial charges compared with tryptophan residues in the empirical force field, which should give a Na^+ PMF with a higher-central-translocation barrier in the gM system compared with that in the gA_{C31} system. This prediction is supported by the results of the kinetic analysis of the homo- and heterodimer conductance data,³⁷ which indicate that the translocation step is ≈ 100 -fold faster in gA than in gM. It is also supported by the side-chain PMFs from our simulations, which show the Na^+ PMF_{Phe} with a higher

maximum-energy compared with that of Na^+ PMF_{Trp} [Fig. 4(a)]. However, the Na^+ PMF has net translocation free-energy barriers of 6.7 and 8.7 kcal/mol for gA_{C31} and gA_{Mac} [Fig. 5(a)], respectively, which are larger than the net translocation free-energy barrier of 5.7 kcal/mol for gM [Fig. 5(c)], rather than a broad peak, exceeding that in gA by 2.0-3.0 kcal/mol at the center of the channel as envisioned in the kinetic analysis of heterodimers³⁷ and perturbations of gM function⁹¹. We suppose that the difference between our results and the experimental prediction reflects inaccuracies in the force fields like those suggested by the results presented above for tryptophan side-chains.

Why then do we have a much lower translocation-energy barrier for the gA channel [Fig. 5(a)] compared with prior results,^{42,49} even before any long-range-electrostatic corrections^{39,40,44} and with a non-polarizable force field? If changing the tryptophan force field has no effect on the Na^+ PMF, what could be the reason for this acceptably low-translocation barrier? To answer this question we first reproduced the K^+ PMF results, because of extensive work that has been done using that cation, searching for a possible explanation for this result. The next section shows that it was the inclusion of the CMAP algorithm in the CHARMM force field, version 31 that reduced the central-barrier height.

CMAP Impact and Umbrella Potential Methods Effect

The K^+ PMF using the CHARMM force field, version 31, with and without the CMAP algorithm is shown in [Fig. 6(a)]. In the former case, simulations were carried out with two different umbrella-potential methods. The K^+ PMF without the CMAP algorithm (red curve) is similar to other previously reported K^+ PMFs^{39,43,44,48,49}, demonstrating that previous computations are reproducible. We also compared these results to those obtained with the PMR method [Eq. (4)], which match qualitatively and quantitatively to within statistical errors.

The RTC method, both with and without the CMAP algorithm, shows excellent convergence. That is, PMFs based on increasingly complete fractions of the umbrella sampling trajectory (0.25, 0.5, 0.75, and 1.0 ns) converged to within 0.5 kcal/mol over the entire extent of the integration, and to within 0.5 kcal/mol of the reference energy (0 kcal/mol at $z = -20$ Å) at the opposite z position ($z = 20$ Å). The non-symmetrized PMFs suggest that the energy uncertainty due to structural variations is on the order of ≈ 1 kcal/mol.

Further tests are desirable to understand why CMAP lowers the energy barrier and whether symmetrization for chirality would further modify the free-energy profile for gramicidin cation transport. In this paper, we illustrate the breadth of the issue by decomposing the K^+ PMF, obtained with the current version of CMAP, into four major components, K^+ PMF _{i} [Eq. (1)], where i denotes the mean force applied on the test ion by the other ions, the water, the peptide, or the lipid. We compare the component K^+ PMFs, K^+ PMF _{i} , from our simulations in the presence and in the absence of the CMAP algorithm using the RTC method [Fig. 6(b)]. The direct effect of the CMAP algorithm, seen in K^+ PMF _{gA} , raises the translocation barrier by 8.8 kcal/mol. With the CMAP algorithm, the K^+ PMF _{H_2O} is lower in energy than without. This means that there is a stronger attraction between water molecules and the test ion in the channel when using the CMAP algorithm. This may indicate the water molecules inside the channel can more easily flip to aid in solvating the test ion when using the CMAP algorithm. Apparently, CMAP affects the peptide motions or structure, or water interaction in a way that facilitates the stabilization of the test ion by the single-file waters, and allows a decrease in mean force they exert on the ion as it translates through the channel. We suggest that this may be a crucial aspect of solving the high translocation barrier problem, and suspect that it will persist with chirality symmetrization of the CMAP corrections, which hopefully will provide further improvements in the backbone structure.

In the CMAP calculations, 53 backbone torsion energies were taken from quantum mechanical predictions for Ala, Gly, and Pro with empirical corrections needed for Ala and Gly. Perhaps the generalization of the corrected L-Ala parameters to both L- and D-stereoisomers of Leu, Val, and to L-Trp in gA, could cause misleading results. However, we expect that this would not be a major problem and that our results would be qualitatively correct. Also, we note that CMAP was refined based on the protein folding problem,⁵³ but was not tested for other problems like ion transport through membrane channels.

The ITC method produces results essentially identical to the RTC method [Fig. 6(a)], including the quality of convergence. Because ITC is easier to apply under some circumstances, we will use the two methods interchangeably in this and related future publications.

Future work will be required to balance force field issues (direct and indirect, including polarizability) and to assess whether channel conductance estimated from simulations accurately predicts the full range of measurements now available for this model system.

Conclusions

The results indicate that the original CHARMM force field, version 31, for tryptophan differs from that predicted by data-fitting, being double-welled, and that the MacKerell force field is consistent with data-fitting, being bowl-shaped and -6.1 kcal/mol deep at the center of the channel. However, the benefit of the improved partial charges is completely ablated by indirect effects, apparently a victim of small differences of large components judging from the decomposition, so the Na^+ PMF for gA does not differ appreciably from that for gM and does not predict the observed differences in current-voltage-concentration relationships between these two channels.

The CMAP algorithm lowers the translocation energy barrier to a reasonable level in the upgraded CHARMM force field, version 31. However, the PMF for gM channels does not have the higher translocation barrier expected from experimental studies. Again, indirect effects of force field changes, especially those mediated by channel water molecules, were shown to be complex and unpredictable.

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Figure 1. gA_{Mac} system snapshots: (a) Initially and (b) Equilibration 10 stage. gA dimer using VMD's New Cartoon drawing method (green and red for left (L) and right (R) monomers, respectively); tryptophan side-chain atoms using Licorice drawing method: C (cyan), H (white), and N (blue); DMPC bilayer atoms using Licorice drawing method: C (dark cyan), H (silver), O (dark red), N (dark blue), and P (green sphere); Na^+ (yellow sphere); Cl^- (cyan sphere); Water atoms: O (red) and H (white), nine water molecules inside the channel are shown using the CPK drawing method.

Figure 2.

System stability: (a) Deuterium order parameters for the DMPC tails in gA_{Mac} (black — line), gA_{C31} (grey — line), and gM (black - - line) systems. (b) Root-mean-square displacement versus time for the protein in gA_{Mac} (black line), gA_{C31} (grey line), and gM (red line) systems. (c) Density profile in the z direction for the oxygen atom of the ethanolamine residues in: gA_{Mac} (black line), gA_{C31} (grey line), and gM (red line) systems.

Figure 3.

The torsion angles, χ_1 and χ_2 , versus time for the side-chain residues during long production runs. The superscript notations on the side-chain residue abbreviation to the upper right of each plot represent the residue position and the protein monomer, respectively.

Figure 4.

Symmetrized one-dimensional Na^+ PMF_{*i*} as a function of the transport coordinate from the force of the side-chains on the test ion. (a) The tryptophan effect is shown from both the MacKerell (heavy black — line) and version 31 of the CHARMM (grey — line) force fields, whereas the phenylalanine effect is shown from version 31 of the CHARMM force field (thin black - - line). (b) The tryptophan effect relative to the phenylalanine effect (after subtracting the phenylalanine profile from the tryptophan profiles) is shown for both the MacKerell (black — line) and version 31 of the CHARMM (grey — line) force fields.

Figure 5.

Symmetrized one-dimensional Na^+ PMF and symmetrized one-dimensional Na^+ PMF_i as a function of the transport coordinate using the ITC method. (a) The Na^+ PMF is shown for both gA_{Mac} (black line) and gA_{C31} (grey line) systems. (b) The Na^+ PMF_i for both gA_{Mac} (— line) and gA_{C31} (— — line) systems is shown from the force of ions (green), H_2O (blue), gA (black), and lipid molecules (red) on the test ion. (c) The Na^+ PMF in the gM system.

Figure 6. One-dimensional K^+ PMF and one-dimensional K^+ PMF_i as a function of the transport coordinate. (a) The Symmetrized K^+ PMF is shown from RTC without CMAP (red line), RTC with CMAP (grey line), and ITC with CMAP (black line) implementations. (b) The K^+ PMF_i for both RTC with CMAP (— line) and RTC without CMAP (--- line) implementations is shown from the force of ions (green), H_2O (blue), gA (black), and lipid molecules (red) on the test ion.

Summary of System Preparation Approach

Process	Ensemble ^a	Restraints (k _B ^b , kcal/(mol·Å ²))		Steps			
				Lipids ^c		Protein ^d	
		P	bb	non bb	com		
Minimization	NVT	200	F	F	F		5000
Heating	NV	200	F	F	F		20,000
Equilibration 1	NVT	200	F	F	F		25,000
Annealing ^e	NV	200	F	F	F		200,000
Equilibration 2	NPAT	20	F	F	F		100,000
Equilibration 3	NP γ T	10	F	F	F		10,000,000
Equilibration 4	NPAT	2	100	2	2		100,000
Equilibration 5	NPAT	1	40	1	1		100,000
Equilibration 6	NPAT		10				100,000
Equilibration 7	NPAT		2				100,000
Equilibration 8	NPAT		0.4				100,000
Equilibration 9	NPAT		0.1				400,000
Equilibration 10	NPAT					1000	1,250,000

^aThe symbols in this column stand for; N: constant number of atoms, V: constant volume, T: constant temperature, P: constant pressure, A: constant area, γ : constant surface tension

^bk_B: force constant

^cThe symbol in this column stands for; P: phosphorus atoms

^dThe symbols in this column stand for; bb: backbone, com: center-of-mass, F: fixed atoms

^eIt consists of three stages with each stage: 40 ps heating to 1000 K followed by 80 ps cooling to 303.15 K