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Gramicidin A Backbone and Side Chain Dynamics Evaluated by Molecular Dynamics Simulations and Nuclear Magnetic Resonance Experiments. I: Molecular Dynamics Simulations

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Abstract

Gramicidin A (gA) channels provide an ideal system to test molecular dynamics (MD) simulations of membrane proteins. The peptide backbone lines a cation-selective pore and, due to the small channel size, the average structure and extent of fluctuations of all atoms in the peptide will influence ion permeation. This raises the question of how well molecular mechanical force fields used in MD simulations and potential of mean force (PMF) calculations can predict structure and dynamics as well as ion permeation. To address this question, we undertook a comparative study of nuclear magnetic resonance (NMR) observables predicted by fully atomistic MD simulations on a gA dimer embedded in a sodium dodecyl sulfate (SDS) micelle with measurements of the gA dimer backbone and tryptophan side chain dynamics using solution state ¹⁵N-NMR on gA dimers in SDS micelles. This comparison enables us to examine the robustness of the MD simulations done using different force fields, as well as their ability to predict important features of the gA channel. We find that MD is able to predict NMR observables, including the generalized order parameters (S^2), the ¹⁵N spin-lattice (T_1), spin-spin (T_2) relaxation times, and the ¹H-¹⁵N nuclear Overhauser effect (NOE), with remarkable accuracy. To examine further how differences in the force fields can affect the channel conductance, we calculated the PMF for K⁺ and Na⁺ permeation through a gA channel in a dimyristoylphosphatidylcholine (DMPC) bilayer. In this case, we find that MD is less successful in quantitatively predicting the single-channel conductance.

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SUPPORTING INFORMATION AVAILABLE:

Figures showing: the simulation setup (Supplementary Figure 1), representative gA dimer backbone RMSD traces for P27, P27+CMAP and P27+dCMAP FF simulations (Supplementary Figure 2), the corrected K⁺ and Na⁺ PMFs (Supplementary Figure 3), and the effect of adding the dCMAP and TRP FF extensions (Supplementary Figure 4). This information is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

ion permeation; molecular mechanical force field; NMR observables; potential of mean force; solution NMR; sodium dodecyl sulfate micelles

INTRODUCTION

Gramicidin A (gA) channels represent near-ideal systems to test all-atom molecular dynamic (MD) simulations of membrane proteins and mechanisms of ion permeation¹. gA channels are mini-proteins, formed by two subunits synthesized by the soil bacteria *Bacillus brevis*. The subunits have an alternating L-D amino acid sequence, formyl-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-ethanolamine². The channels form when two subunits from opposing bilayers leaflets dimerize to form a monovalent cation conducting channel^{3,4}. The structure of gA channels has been determined by solution-state nuclear magnetic resonance (NMR)^{5,6} and by solid-state NMR^{7,8}. The structures are in overall agreement, but differ slightly in backbone pitch and in a few amino acid side chain orientations. Some of these differences have been resolved using MD simulations on gA dimers in planar bilayers⁹.

Important for the present study, extensive information is available about the positions of the ion binding sites in the channel. The binding sites were first inferred using the voltage dependence measurements and estimates of the electric distance felt by the permeating ions^{10,11}. Binding site location was determined indirectly from solution-state ¹³C NMR chemical shifts of gA incorporated into lysophosphatidylcholine¹² or dodecylphosphocholine¹³ micelles or solid state ¹³C and ¹⁵N chemical shift anisotropy in oriented multilayers^{14,15}. Tl⁺ ion locations were determined using X-ray scattering on gA incorporated into oriented lipid bilayers¹⁶. MD simulations have been used to calculate the free energy profile along the axes of the gA channel pore¹⁷, for review and analysis of the experimental and MD simulation work see^{18,19}. A consensus emerging from this analysis is that the two ion binding sites are symmetrically disposed and about 20 Å apart, close to each channel entrance, and involve the carbonyl oxygens of Trp9, 11, 13 and 15^{12;13;19}.

In addition to being extensively studied, gA channels are small, with a molecular weight $\approx$ 4 kDa, one to two orders of magnitude less than channels formed by integral membrane proteins. This allows for long MD simulations and predictions of experimental observables in tractable time—calculations that provide for tests of the accuracy of the force fields (FF) used in MD simulations. This feature is important because of the perennial concern in such simulations, namely how well the parametrization of the FF describes the interatomic interactions that underlie the calculations.

In this study we explore this question by examining the sensitivity of a number of experimental NMR observables to changes in the FF. The experimental observables include the generalized order parameters (S^2), the ¹⁵N spin-lattice (T_1) and spin-spin (T_2) relaxation times and the ¹H-¹⁵N nuclear Overhauser effect (NOE) determined in solution-state NMR experiments on ¹⁵N-labeled gA incorporated into perdeuterated sodium dodecyl sulfate (SDS) micelles²⁰. We also monitored how the changes in the FFs affect the potential of mean force (PMF) of an ion along the channel axis, which is one of the key quantities controlling the rate of permeation. The simulations were done using the CHARMM program^{21,22} with the CHARMM FF and parameter set 27 (P27)²³. A number of enhancements to the P27 FF were considered. First, a dihedral-based correction map (CMAP), which was developed to improve the treatment of the peptide backbone by adding an extension to the potential energy function that constrains the peptide backbone ϕ , ψ

dihedral cross terms²⁴. CMAP is chirality specific and the original version was designed only for L-amino acids. A modified version has been introduced (A. D. MacKerell, personal communication), which includes corrections for both L- and D-amino acids (referred to as dCMAP, as to not confuse it with the uncorrected CMAP). Second, a refined tryptophan FF (TRP)²⁵, in which the partial atomic charges, Lennard-Jones parameters, and force constants were revised to be consistent with NMR experiments²⁶. We are thus able to explore how these relatively recent enhancements to a modern FF improve the prediction of peptide backbone and amino acid side chain dynamics.

As an alternative strategy to evaluate the different FFs, we calculate the PMF for ion movement through gA channels, where the Trp residues are likely to play an important role due to long-range ion-dipole interactions²⁷⁻³¹.

METHODS

gA-SDS micelle molecular dynamics simulations

The gA-SDS micelle simulations were set up to approximate the conditions used in the solution-state NMR experiments in the accompanying article²⁰. The system (Figure 1) consists of: one gA dimer (two head-to-head-associated gA monomers), 65 dodecyl sulfates, ~11165 water molecules (ranging from 11150 to 11181 in the different simulations, depending on the water box overlay), and 65 sodium ions (total SDS concentration 0.28 M), totaling ~36840 atoms in the simulation cell. The simulation setup was adapted from reference 9, with the DMPC bilayer substituted for a SDS micelle. The system was built in the following manner, see also Supplemental Figure 1. First, for the gA dimer, the PDB ID: 1JNO⁶ coordinates were used and the dimer pore was filled with water molecules. Second, a dodecyl sulfate (DS) micelle was built around the gA dimer by randomly placing 65 pseudo-detergent particles, constrained to be on an imaginary sphere surrounding the dimer, excluding the areas right in front of the pore (Supplemental Figure 1a). Third, the pseudo-detergent particles were randomly replaced with explicit DS, oriented with their acyl chains facing the gA dimer (Supplemental Figure 1b). A similar procedure was used by MacKerell³² to build a pure SDS micelle. The DS were picked from a library of 600 pre-equilibrated dodecyl sulfate molecules. The library was generated from the coordinates of 60 SDS molecules from a pre-equilibrated micelle³², and each DS was subjected to further dynamics in vacuum with a cylindrical constraint, and the structures were saved every 10 ps. Fourth, the imaginary sphere (containing the gA dimer and all the DS molecules) was incrementally shrunk while running energy minimization to a final radius of 24 Å (Supplemental Figure 1c). Fifth, a previously energy-minimized box of water molecules was overlaid on the gA-DS micelle in a hexagonal periodic boundaries box, which was 76.8 Å high and wide (Supplemental Figure 1d), and all water molecules closer than 2.4 Å to other molecules were removed. Sixth, 65 Na⁺ were randomly placed in the aqueous phase (5.4 Å dist away from the gA-dodecyl sulfate micelle), to establish electroneutrality. (In the NMR experiments, apart from having one Na⁺ per DS, concentration 0.50 M, there were also ~2 perdeuterated trifluoroethanol molecules and 0.09 K⁺ mono- and diphosphate per SDS²⁰, which were not included in the simulations.) Finally, energy minimization and dynamics were run while slowly removing all constraints.

The simulations were run using the CHARMM program version 33a^{21;22}. Constant pressure and temperature (NPT) was maintained using a Nose-Hoover chain thermostat^{33;34} with 3000 kcal ps² coupling and the Andersen constant pressure algorithm^{35;36} set to a temperature of 330 K (the NMR experiments were done at 328 K²⁰) and a piston weight of 750 amu. The particle mesh Ewald (PME) method³⁷ was used for the long range electrostatics, and the SHAKE algorithm^{38;39} was used to constrain bond vibrations involving hydrogen atoms.

Five different FF combinations were tested: P27, P27+CMAP, P27+dCMAP, P27+TRP and P27+dCMAP+TRP where P27 is the CHARMM27 parameters force field²³ with the Na⁺ R_{min} parameter changed to 1.41075 Å, cf. reference 40 (CMAP, dCMAP and TRP are defined above). The TIP3P water model⁴¹ was used for all simulations. Duplicate 20-50 ns long simulations were run for each FF; the simulations were run with a time step of 2 fs, and trajectories were saved every 200 fs. The combined simulation time totaled over 250 ns. For each simulation the first two ns after simulation setup were excluded from the analysis to ensure gA-SDS micelle equilibrium.

The NMR observables were calculated using the NMR analysis module of CHARMM, described in²¹. The NMR module follows the convention of references 42,43 defining the spectral densities ($J(\omega)$) as:

$$J(\omega) = \int_0^\infty \cos(\omega t) C(t) dt \quad (1)$$

where ω is the frequency and $C(t)$ the internal correlation function. The integration of the fast and slow component of $J(\omega)$ is done separately:

$$J_{\text{fast}}(\omega) = \int_0^{T_{\text{MAX}}} \cos(\omega t) (C(t) - C_{\text{plateau}}) \exp(-t/R_{\text{tumble}}) dt \quad (2)$$

$$J_{\text{slow}}(\omega) = \int_0^\infty \cos(\omega t) C_{\text{plateau}} \exp(-t/R_{\text{tumble}}) dt \quad (3)$$

where T_{MAX} is the integration time for the fast component, C_{plateau} the plateau value reached by the internal correlation function and R_{tumble} is the tumbling time of the molecule/complex in question. For further details on the NMR analysis module and the formulas used for calculating T_1 , T_2 , NOE, and the order parameters (S^2) see the on-line CHARMM NMR module documentation (<http://www.charmm.org/html/documentation/c33b2/nmr.html>).

For the calculations, we used the experimentally determined gA-SDS micelle $R_{\text{tumble}} = 6.6$ ns, the default gyromagnetic ratio/gamma parameters (see Table 2.1 in⁴⁴), and a magnetic field of 11.74 T (corresponding to a 500 MHz for a proton). The fast part of the spectral density was integrated for 1 ns ($T_{\text{MAX}} = 1$ ns) and all atoms within 5 Å of each ¹⁵N were considered in the calculation of $J(\omega)$. A sample script for how we used the NMR module to calculate T_1 , T_2 , NOE, and S^2 is included in the Supporting Information.

PMF calculations

The PMF calculations of K⁺ and Na⁺ along the pore axis were carried out using CHARMM²² and the umbrella sampling method⁴⁵ according to a previously established procedure⁴⁶. Briefly, the gA dimer was embedded in a bilayer comprising 20 dimyristoylphosphatidylcholine (DMPC), which corresponds to about one shell of phospholipids surrounding the dimer (Figure 2). The KCl or NaCl concentration was set to 1 M, to provide better sampling of the ions in the bulk region. The calculations were done using hexagonal periodic boundaries, with the respective *xy*-translation lengths being 32.1 Å. The periodic box was fixed in the *xy* dimensions and pressure coupling was employed in the *z* direction, parallel to the normal of the membrane. The average *z* length of the box is about 75 Å. PME³⁷, SHAKE³⁸ and constant pressure and temperature algorithms³⁶ were employed in all simulations, pressure and temperature were kept at 1 atm and 330 K, respectively. To test the effects of dCMAP and the new tryptophan FF (TRP), we employ

four of the five FF combinations that were examined in the gA-SDS micelle simulations: CHARMM27 only (P27); CHARMM27 + dCMAP (P27+dCMAP); CHARMM27 + new Trp (P27+TRP); CHARMM27 + dCMAP + new Trp (P27+dCMAP+TRP). The TIP3P water model⁴¹ was used for all simulations. The K⁺ and Na⁺ ion models were taken from reference 40, with the Lennard-Jones parameters $E_{\min} = -0.0870$ kcal/mol, $R_{\min} = 1.76375$ Å for K⁺ and $E_{\min} = -0.0469$ kcal/mol, $R_{\min} = 1.41075$ Å for Na⁺.

The z coordinate of the ion along the channel axis is used as the reaction coordinate; this has been shown to provide a good representation of the permeation processes⁴⁶⁻⁴⁸. In the umbrella sampling simulations, the z position of the ion is biased by a harmonic “window” potential function, $w_i(z) = 0.5K_i(z-z_i)^2$, with force constant $K_i = 10$ kcal/mol/Å² centered on a series of z_i positions spaced every 0.5 Å in the interval from -20 to +20 Å, relative to the channel center. A flat-bottom cylindrical restraint of radius 8 Å and force constant of 10 kcal/mol/Å² was introduced to restrict the lateral displacements of the ion^{46,49} and using a sphere of diameter L (28 Å) to define the single-ion occupied pore region, cf. reference 46. The umbrella sampling simulations were unbiased using the weighted histogram analysis method (WHAM)⁵⁰, with a bin width of 0.01 Å and a convergence criterion for variation between two iterations of < 0.0005 kcal/mol. Configurations taken from previous PMF calculations of K⁺ in a gA channel based on the P27 force field⁴⁶⁻⁴⁸ were used as to initiate the umbrella sampling MD simulations. For each FF combination, each window was equilibrated for 40 ps, followed by production trajectories of 2 ns to collect data for the PMF calculations. Convergence of the umbrella sampling computations was assessed from the raw non-symmetrized PMFs, and the final results were symmetrized for visual display, as described previously⁴⁶.

The maximal conductance ($g_{\max}$) of the singly-occupied channel was calculated as described in reference 46. In the limit of low membrane potentials and under symmetric concentrations the conductance is expressed as:

$$g_{\max} = \frac{e^2}{k_B T L^2} \left\langle D(z)^{-1} e^{+W(z)/k_B T} \right\rangle^{-1} \left\langle e^{-W(z)/k_B T} \right\rangle^{-1} \quad (4)$$

where k_B and T are the Boltzmann's constant and temperature in Kelvin, e the elementary charge of the permeating ion, L the length of pore region as defined above (28 Å). The diffusion coefficient within the channel ($D(z)$) is estimated as ~2/3 of bulk diffusion coefficient⁴⁶, the brackets are spatial averaging over the length L , and $W(z)$ is the 1D PMF.

RESULTS

gA-SDS micelle stability

Except for the gA-SDS simulations using the P27+CMAP FF, the gA-SDS micelle system was stable in all simulations, no matter what FF that was used. Except for the P27+CMAP FF simulations, the gA dimer was stable—with small, short-lived excursions in the gA backbone RMSD, which represent one of the ethanolamine groups “losing” its hydrogen bond to the peptide backbone. It thus “sticks up” for then to relax back down (Figure 3a and Supplemental Figure 2a). In simulations using the P27+CMAP FF, the gA dimer begins to break up (Supplemental Figure 2b). This is expected because the standard CMAP corrections map is chirality specific and therefore not compatible with the D-amino acids in the gA sequence. Supplemental Figure 2b shows gA dimer backbone RMSD for representative P27, P27+CMAP and P27+dCMAP FF simulations together with snapshots of the gA dimer structure from the simulations.

For the four FF combinations (excluding simulations with unmodified CMAP) the average gA backbone RMSD varied little, 0.77 ± 0.01 (mean $\pm$ SD), (shown for one P27 FF simulation, Figure 3a). The Lindemann disorder parameter is based on the Lindemann criterion⁵¹, and is used to classify solid vs. liquid materials, by comparing the amplitude of atomic fluctuation with the lattice constant of the material (the most-probable non-bonded near-neighbor distance). We calculated the Lindemann disorder parameter for the gA backbone of 0.171 ± 0.003 (mean $\pm$ SD), estimated as described in reference 52 with a most-probable non-bonded near-neighbor distance (a') of $4.5 \text{ \AA}$ ⁵². A value of 0.17 for the gA backbone indicates that it is fairly rigid but still above the threshold value (0.10-0.15) usually considered to separate solid-like from liquid-like behavior⁵².

The stability of the gA-SDS micelle simulations was checked by monitoring the gA backbone RMSD and the energetic coupling between the gA dimer and the SDS micelle. Figure 3 shows results from one of the P27 FF simulations (the other simulations gave similar results). Though the individual SDS molecules are dynamic, the micelle itself is stable and retains its average shape. The normalized average positions of different SDS micelle atom groups (sulfur and carbon 1, 6 and 12, numbered consecutively after the sulfur atom) relative to the gA dimer center of mass were calculated every 2 ns, see representative results in Figure 3c. The average position of the different atom groups did not change over the course of any of the simulations (results not shown). The distribution of the SDS micelle atom groups in the gA-SDS micelle simulations is broader than those for a pure SDS micelle³²—consistent with the less spherical, more toroidal-shaped gA-SDS micelles. In about half the simulations, one SDS molecule (two molecules in one simulation) dissociated from the micelle and diffused out in the aqueous phase; in about half those cases, the SDS molecule reintegrated back into the micelle later in that simulation.

The stability of the gA dimer was also monitored by calculating the dimer's average number of amino acids per turn and the average axial rise per turn (Figure 3d). (The decrease in the residues per turn over time, Figure 3d top left panel, is due to fluctuations; it reverses later in the simulation and was not seen in the other simulations.) The average number of residues per turn, 6.23-6.27 with no significant difference among the FFs, is in good agreement with the 1JNO PDB structure, 6.3 residues/turn⁶, and simulations of gA dimers in planar bilayers, 6.25 ± 0.04 residues/turn⁹. Similarly, the average rise per turn, 4.77-4.80 $\text{\AA}$, does not vary among simulations done with the different FFs—and is in good agreement with the 1JNO PDB structure⁶, where the rise was 4.7 $\text{\AA}$ /turn, and X-ray diffraction studies in oriented bilayers⁵³, where the rise was $4.7 \pm 0.2 \text{ \AA}$ /turn, as well as the bilayer simulations in reference 9, where the rise was $4.84 \pm 0.07 \text{ \AA}$ /turn.

NMR observables

Figure 4 shows the calculated NMR observables for simulations using the four different FF combinations (P27; P27+dCMAP; P27+TRP; P27+dCMAP+TRP) superimposed on the experimental NMR results from the accompanying article²⁰. The NMR experimental data and calculated NMR observables are shown for all backbone N-Hs (one for each gA monomer amino acid, numbered 1 through 15) and for the Trp indoles (amino acid 9, 11, 13 and 15), except for Figure 4a where there is no experimental data for the Trp indoles. Figure 4a shows the generalized order parameter (S^2); Figure 4b and c show the ^{15}N spin-lattice (T_1) and spin-spin (T_2) relaxation times, respectively; Figure 4d the steady-state ^1H - ^{15}N NOE's. All four tested FF combinations predict observables for the peptide backbone that are in good agreement with each other and with the NMR experimental results. In the case of the Trp indoles, however, there are differences among the observables predicted using different FFs—and with the experimental results (discussed further below). For the backbone, the differences between the predicted and observed results are quantified in Figure 5. Simulations with the dCMAP correction (P27+dCMAP) and with both

enhancements (P27+dCMAP+TRP) provide slightly better agreement with the gA dimer backbone experimental NMR observables, as compared to the P27 and P27+TRP simulations. The differences among the Trp indoles were not quantified because repeats with the same FF varied greatly (as evident in the large standard deviations in Figure 4).

To investigate the variability in the predicted Trp indole observables, and the lack of agreement with experimental results, we examined the N-H dipoles' internal correlation functions and Trp side chain rotameric states (Figure 6). The internal correlation functions for the N-H dipoles are the main contributors to the T_1 and T_2 signal and the sole contributor to the NOE signal. Figure 6a shows the correlation functions for one gA monomer from one of the P27 simulations. For the majority of the N-H dipoles, the correlation function decays to a plateau within a few ps; the main exceptions being the Trp indoles (the other simulations show similar behavior, results not shown). To better quantify this behavior, we calculated the range of the correlation functions after 1 ns for all N-H dipoles and FF combinations (Figure 6b). Even after 1 ns, the Trp indole N-H dipole correlation functions have not decayed to a plateau, a behavior that is especially pronounced for Trp9. The slowly decaying and variable correlation functions are indicative of slow time-scale motions that have not converged⁵⁴. We conclude that the Trp side chain movements are not fully sampled in the 20 ns simulations. (We did a few simulations lasting 50 ns; these simulations showed similar behavior as is seen in Figure 6.)

We further explored the Trp side chain dynamics and average orientation by examining the Trp dihedral angles χ_1 and χ_2 (Figure 7). Figure 7a shows the χ_1 and χ_2 angles with time from a P27 simulation for Trp (amino acids 9, 11, 13 and 15) and both gA monomers (Figure 7a, left and right panels). Similar behavior (rotameric states occupied and number of transitions between states) was observed for all the other (20 ns long) simulations; whatever the FF used. Only a handful of transition between Trp rotameric states were observed; for Trp11, 13 and 15 there were less than one transition per Trp in each simulation, and the side chains occupied the stable $\chi_1 \approx -60^\circ$, $\chi_2 \approx -80^\circ$ rotameric state for over 90% of the simulation time. In agreement with previous simulations in planar bilayers⁹, Trp9 exhibited the greatest mobility. The Trps change rotameric states on the ns time scale, often enough to observe occasional transitions during a 20 ns simulation and thus influencing the internal correlation function, but too slow for proper sampling. Simulations extended to 50 ns did not improve the sampling sufficiently; the slow time scale motions of Trp11, 13 and 15 require 1-2 orders of magnitude longer simulations. Similar lack of convergence has been observed for low frequency undulations in other membrane proteins, e.g.⁵⁵.

To represent the Trp rotameric states, the combined χ_1 - χ_2 results from all the simulations were pooled (Figure 7b), as there were no obvious differences among the results obtained with the different FFs. The results show the presence of at least four distinct Trp rotameric states for all four Trps, in agreement with calculated adiabatic energy density of Trp side chain configurations⁹. (Given the likely limited sampling of the different rotameric states—at least for Trp11, 13 and 15—this question was not explored further.)

We also determined the Na^+ occupancy in the pore, by constructing probability histograms of the position of Na^+ along the pore axis. Figure 8 shows the results from -30 to $+30$ Å. Na^+ binds in several distinct locations that are separated by ~ 1.6 Å, similar to the L-D-dipeptide spacing. (The ions beyond ± 12.5 Å are outside the pore proper; the broad distribution between ± 12 Å and ± 20 Å presumably reflects the enrichment of aqueous Na^+ by the negative charges on the SDS.) The positions of the most favorable locations (i.e., the binding sites) are in good agreement with experimental results (for reviews see references 18 and 19) and the local binding sites are in excellent qualitative agreement with early MD simulation results obtained by Roux and Karplus¹⁷ based on a much simpler model system

with the extended PARAM19 FF. We explore this point further below, where the density profile for Na⁺ along the pore axis is used to calculate a PMF for ion movement through the pore.

PMF calculations

Based on the agreement between the predicted and experimental NMR observables, we are able to conclude that MD simulations indeed provide for an accurate representation of the fast dynamics of the channel backbone. To examine this issue further, we calculated the PMF for K⁺ and Na⁺ along the axis of the gA channel incorporated in a bilayer using the various FF combinations. Figure 9a,b shows the PMFs for K⁺ (Figure 9a) and for Na⁺ (Figure 9b), calculated using the four FF combinations.

For each FF combination, the PMF shows minor minima separated by ~1.6 Å, similar to the separation of the peaks in the Na⁺ distribution (Figure 8). Based on the PMFs there are two major ion binding sites in each subunit: an outer binding site at $z = \pm 11.1 - 11.3$ Å; and an inner binding site at $z = \pm 9.3 - 9.5$ Å. The positions of the ion binding sites are in good agreement with earlier MD simulations¹⁷, as well as experimental results e.g. the X-ray scattering results of Olah et al.¹⁶, the ¹³C NMR chemical shifts of gA incorporated into lysophosphatidylcholine¹² or dodecylphosphocholine¹³ micelles, and the solid-state NMR results of Smith et al.¹⁴ and Tian et al.¹⁵ (see references 41 and 18 for a detailed reconciliation of the solid-state NMR results). The simulations with the TRP FF enhancement had no discernible effect on the positions of the energy barriers or the binding sites in the PMFs, as compared to the simulations with the old Trp FF, but both dCMAP and TRP altered the depths of the binding sites and the heights of the barriers.

The new tryptophan parameters (P27+TRP) yield the deepest binding sites, whereas dCMAP (P27+dCMAP) yields the shallowest outer binding sites. CHARMM27 alone (P27) and the incorporation of both dCMAP and TRP (P27+dCMAP+TRP) yield intermediate results.

The maximum barrier, relative to the bulk solution, calculated with P27+TRP is 8.2 kcal/mol for K⁺ and 9.3 kcal/mol for Na⁺, and is the lowest of all the four FF combinations. The maximum barrier calculated with P27+dCMAP is 12.4 kcal/mol for K⁺ and 13.6 kcal/mol for Na⁺ (it is the highest of them all); P27 and P27+dCMAP+TRP yield intermediate values 9.5-9.6 kcal/mol and 10.4-12.4 kcal/mol for K⁺ and Na⁺, respectively. (The changes in the peak barrier relative to the energy wells that form the outer and inner binding sites are less than the changes in the peak barriers relative to the bulk solution.) For K⁺ and Na⁺ permeation through gA channels, the energy barrier increases by several kcal/mole when the dCMAP is included in the FF. The new Trp parameters lower the barrier by a few kcal/mol, as compared to PARAM27 with the old Trp parameters (P27). As a result, the PMF calculated with the combination P27+dCMAP+TRP yields a free energy barrier similar to the one calculated with P27. The net effect of the various FF combinations on the rate of K⁺ and Na⁺ movement through the gA channel is shown in Table 1. The maximum conductances ($g_{\max}$) are calculated using Eq. 4 (see Methods) from the (uncorrected) PMFs in Figure 9 and from the corrected PMFs (Supplemental Figure 3); where the PMFs in Supplemental Figure 3 have been adjusted for simulation size artifacts and lack of bilayer polarizability^{47;48}.

The positions of the energy minima in the PMFs are in good agreement with the peaks in the ion distribution (Figure 8), but the increase in the overall energy barrier is larger than would have been predicted from the Na⁺ distribution (Figure 8), even after correcting the PMFs for simulation size artifacts and lack of bilayer polarizability (see Supplemental Figure 3). This can be seen in Figure 10, where the PMF extracted from the probability density of Na⁺ along

the channel axis for a gA embedded in SDS micelle ($-k_B T \ln[P(z)]$) is compared with the PMF calculated by umbrella sampling with a gA incorporated into a planar DMPC bilayer. The two PMF are very similar regarding the position of the local ion binding sites along the channel axis. However, the central barrier relative to the two main binding sites at the channel's entrance appears to be higher for the gA channel in the bilayer than for the gA dimer in the micelle (even after correcting for simulation size artifact and bilayer polarizability artifacts). This lower central barrier for the gA channel embedded in the SDS micelle is expected because the complex is surrounded by a high dielectric aqueous phase, which contributes to stabilize the cation at the center of the dimer. In contrast, the gA channel incorporated into a lipid bilayer experiences an environment that corresponds to a low dielectric planar slab.

DISCUSSION

MD simulations on gA dimers incorporated in SDS micelles result in stable micellar assemblies and dimer structures (Figure 3), in good agreement with simulations in oriented bilayers⁹. The simulations were stable for all force field combinations tested—except for simulations with the unmodified CMAP. This finding is understandable because CMAP has chirality specific elements that result in improper constraints on the D-amino acids, which result in breakdown of the gA dimer structure (Supplemental Figure 2b). Any conclusions about gA channels that are obtained in MD simulations using the unmodified CMAP must be regarded as questionable.

The calculated NMR observables for the four FF combinations provide good agreement with the NMR experimental results for the backbone atoms but not as good for the Trp indoles (Figure 4). The overall agreement for the backbone atoms is somewhat expected, as the simulated NMR observables were calculated using the experimentally determined gA-SDS micelle tumbling time (6.6 ns); and furthermore the gA dimer $\beta^{6.3}$ -helical structure is likely to cause the peptide backbone to be fairly rigid with relatively small, fast fluctuations. The calculated Lindemann disorder parameter⁵² for the gA backbone atoms is 0.17—above the threshold value of 0.10-0.15 that is usually is considered to demarcate the transition from solid-like to liquid-like behavior⁵².

Our results thus suggest, at least for the gA dimer backbone, that simulations using the CHARMM27 (P27) FF provide mechanistically meaningful predictions of the backbone dynamics. The dCMAP and new Trp parameters have rather modest effects on the predicted NMR observables for the backbones. Indeed the new Trp parameters (P27+TRP) have no effect, whereas the dCMAP (P27+dCMAP) or combination (P27+dCMAP+TRP) show slight improvements in the predictions of the backbone dynamics (Figure 5).

The relative poor performance, with respect to the predictions of the Trp side chains NMR observables (Figure 4), reflects the slow convergence of the internal correlation function for the Trp indole rings (Figure 6), cf.⁵⁴. The slow (inadequate) convergence of the correlation function reflects the slow transitions among the rotameric states of the Trp side chains (Figure 7). Much longer simulations would be required for proper determination of the Trp indole NMR observables. The poor sampling of the Trp indoles suggests that one to two orders of magnitude longer simulation times are required to observe proper sampling of rotameric states of Trp residues. (We obtained no significant improvement when the simulations were extended to 50 ns.) This slow convergence also may have consequences for PMF calculations. Namely, if the distribution among the rotamer states varies as a function of ion position, the rotamer variations may not be adequately sampled in the umbrella sampling calculations.

The positions of the local free energy minima—whether extracted from the probability density of Na^+ along the channel axis in the gA-SDS micelle or from the PMFs calculated with umbrella sampling for gA incorporated in a planar bilayer—are in excellent agreement with both experimental results and previous MD simulations (see^{18;19}). This situation has essentially not changed for 20 years, e.g.¹⁷⁻¹⁹.

When we estimate the maximum conductance, following⁴⁶⁻⁴⁸, we find that the inclusion of dCMAP (P27+dCMAP) causes even larger discrepancies between the predicted and the observed conductances for K^+ than is the case for the reference CHARMM27 (P27) FF (Table 1). (See the PMFs in Figure 9 and Supplemental Figure 3.) For Na^+ the inclusion of dCMAP causes an overall increase in the PMF barrier height (Figure 9) but little effect on the Na^+ conductance (Table 1)—because the “binding sites” are shallower such that the relative barrier height is similar. This higher energy barrier with dCMAP stands in contrast with recently reported simulation results that used the uncorrected CMAP for the gA channel’s L-amino acids, which suggested a dramatic (6 – 8 kcal/mole) decrease in the ion translocation barrier⁵⁶. The new Trp FF (P27+TRP), nevertheless, leads to predicted conductances that are in better agreement with experiments (cf. reference 47,57). Indeed, dCMAP and the new Trp parameterization have opposite effects on the gA channel K^+ and Na^+ PMFs. Compared to CHARMM27 (P27), the dCMAP yields shallower binding sites and a higher free energy barrier, whereas the new Trp parameterization yields deeper binding sites and a lower barrier (see Supplemental Figure 4).

Somewhat surprising, the changes in the PMF introduced by the enhancements to P27 depend on the “background” in which the enhancement was introduced, as well as the identity of the permeating ion (Supplementary Figure 4). In the middle of the pore, whether the permeating ion is Na^+ or K^+ , $\text{PMF}_{\text{P27+dCMAP}} - \text{PMF}_{\text{P27}}$ (where the subscripts denoted the FF used to calculate the PMF) is about 2 kcal/mole larger than $\text{PMF}_{\text{P27+dCMA+TRP}} - \text{PMF}_{\text{P27+TRP}}$. For K^+ the difference becomes smaller toward the pore entrances, whereas for Na^+ the difference tends to persist. Similarly, $\text{PMF}_{\text{P27+TRP}} - \text{PMF}_{\text{P27}}$ is about 2 kcal/mole larger than $\text{PMF}_{\text{P27+dCMA+TRP}} - \text{PMF}_{\text{P27+dCMAP}}$. Again, for K^+ the differences tend to decrease toward the pore entrances, but not for Na^+ . These results mimic those reported earlier⁵⁶, and provide evidence for a coupling between the side chain and peptide backbone dynamics.

CONCLUSIONS

Our results show that the CHARMM27 force field (P27), using 20 ns simulations, provides good predictions of the gA channel backbone dynamics. The Trp side chain dynamics must be regarded as questionable as they were not properly sampled on the 20 ns (or 50 ns) timescale and are likely to require 1-2 orders of magnitude longer simulation times in order to adequately sample the rotameric states. The addition of the new Trp parameterization (TRP) had no apparent effect on the peptide backbone dynamics, whereas the dCMAP did show slight improvements. For the PMF calculations, none of the FF enhancements caused major changes in the shape of the predicted PMF; dCMAP caused increased deviations from experiments; TRP some improvement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Snapshots of the gA-SDS micelle system. (a) A cut-out view of the gA-SDS micelle showing the side of the gA dimer. (b) A top view looking down the gA channel pore. In both snapshots, the gA dimer is colored yellow and sodium ions green; the SDS molecules have cyan acyl chains, yellow sulfur, and red oxygen atoms. The black scale bar is 10 Å.

Figure 2.

Snapshots of the gA-DMPC planar bilayer system. The gA dimer is colored yellow, sodium ions green and chlorine ions gray; the DMPC molecules have cyan carbons, brown phosphorus, blue nitrogen, and red oxygen atoms. The black scale bar is 10 Å.

Figure 3. Stability of the gA-SDS micelle complex, shown for one of the P27 force field simulations; the other simulations gave comparable results. (a) RMSD of gA dimer backbone. (b) Instantaneous interaction energy between the SDS micelle and the gA dimer. (c) The normalized positions of different atom groups relative to the center of mass of the gA dimer. (d) The average number of residues per turn of the gA dimer (top panels) and the average axial rise, in Å, per turn of the gA dimer (bottom panels).

Figure 4.

Calculated NMR observables for the gA-SDS micelle simulations superimposed on the NMR experiments; results for the gA backbone and Trp indoles for all four force field combinations (mean $\pm$ sd, $n = 4$) plus experimental data (mean $\pm$ sd)²⁰. (a) Generalized order parameter (S^2), (b) ^{15}N spin-lattice (T_1) relaxation time, (c) spin-spin (T_2) relaxation time, and (d) steady-state ^1H - ^{15}N NOEs. Note the difference in scale between the gA backbone (left) and Trp indoles (right) graphs.

Figure 5.

Errors of the predicted NMR observables. The errors were estimated as the average normalized squared deviation between the experimental NMR observable and values calculated from the simulations:

$$\left(\sum_i^n \left[(\text{Sim}_i - \text{Exp}_i)^2 / \text{Exp}_i^2 \right] \right) / n$$

for each gA dimer backbone residue (i). The minimum deviation, due to experimental error alone is represented with the dashed lines. The black and orange dashed lines denote the average $\pm$ standard deviation of repeated ($n = 100$) random sampling from a Gaussian distribution, using the average and standard deviation of the experimental results, and calculating the average normalized square deviation.

Figure 6.

Calculated N-H dipole correlation times for the gA SDS micelle simulations. (a) N-H dipole correlation times of a gA monomer (fifteen backbone N-H dipoles plus the four Trp indoles) from a representative P27 FF simulation. (b) Mean and standard deviation of the change in correlation function after the first 1 ns for all four FFs (two simulations for each FF and two subunits each simulation).

Figure 7.

The Trp dihedral angles χ_1 and χ_2 . (a) The χ_1 and χ_2 dihedral angles of the gA dimer's four tryptophans (amino acids 9, 11, 13 and 15), for both gA monomers (left and right panels) in a P27 FF simulation (χ_1 (red) and χ_2 (blue)). (b) Plot of χ_1 vs. χ_2 showing the rotameric states of the Trps, combined data from all simulations. The contours/colors represent the number of counts per 2° square bin on a log10 scale.

Figure 8.

Probability distribution $P(z)$ of Na^+ along the z axis along the gA channel. The distribution was calculated by including only those ions that were within a 6 Å radius cylinder, parallel to the channel pore, by pooling Na^+ occupancy data from the trajectories from all four different FF combinations (totaling over 200 ns of simulation time) and symmetrized around the dimer center.

Figure 9. PMF comparison. One-dimensional K^+ (a) and Na^+ (b) ion PMFs using the four force field combinations. For all the PMFs the reaction coordinate z corresponds to the distance of an ion along the channel pore, relative to the channels center of mass.

Figure 10.

The PMF corresponding to the Na^+ distribution in Figure 8 (black line) is compared to the corrected one-dimension Na^+ ion PMF, using the P27+TRP FF (green line). The PMF corresponding to the Na^+ probability distribution $P(z)$ is calculated as $-k_B T \ln[P(z)]$, where k_B is the Boltzman constant and T the absolute temperature; an arbitrary offset value is chosen to match the PMFs at 11 Å. The PMF calculated in the lipid bilayer was corrected for simulation size artifacts and the lack of bilayer polarizability as described in references 47 and 48. The PMFs reaction coordinate z corresponds to the position of an ion along the channel pore, relative to the channels center of mass.

Table 1Calculated maximum K⁺ and Na⁺ conductances^a

force field	uncorrected K ⁺ g _{max} / pS	corrected K ⁺ g _{max} / pS	uncorrected Na ⁺ g _{max} / pS	corrected Na ⁺ g _{max} / pS
P27	0.0127	1.17	0.0006	0.074
P27+TRP	0.0436	3.75	0.046	3.27
P27+dCMAP	0.0015	0.12	0.0015	0.141
P27+dCMAP+TRP	0.0319	1.95	0.020	1.03

^a Maximum conductances (g_{max}) are calculated from the PMFs as described in references 47 and 48, where the corrected g_{max} is adjusted for simulation size artifacts and lack of bilayer polarizability in the MD simulations. Experimental single-channel conductances, determined in the limit of low potentials (25 mV), for gA channels in diphytanoylphosphatidylcholine (DPhPC)/*n*-decane planar bilayers (1M NaCl or 1M KCl at 25°C) are 12.4 ± 1.5 pS (mean ± sd) for Na⁺ and 23.8 ± 2.7 pS (mean ± sd) for K⁺ (unpublished results by O.S. Andersen).