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Parametrization of macrolide antibiotics using the Force Field Toolkit

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Abstract

Macrolides are an important class of antibiotics that target the bacterial ribosome. Computer simulations of macrolides are limited since specific force field parameters have not been previously developed for them. Here we determine CHARMM-compatible force field parameters for erythromycin, azithromycin and telithromycin, using the Force Field Toolkit plugin in VMD. Because of their large size, novel approaches for parametrizing them had to be developed. Two methods for determining partial atomic charges, from interactions with TIP3P water and from the electrostatic potential, as well as several approaches for fitting the dihedral parameters were tested. The performance of the different parameter sets was evaluated by molecular dynamics simulations of the macrolides in ribosome, with a distinct improvement in maintenance of key interactions observed after refinement of the initial parameters. Based on the results of the macrolide tests, recommended procedures for parametrizing very large molecules using ffTK are given.

Keywords

Macrolides; Force Field Toolkit; Ribosome; CHARMM parameters; force field parametrization

INTRODUCTION

Macrolides are powerful antibiotics, which are commonly used for chest and respiratory tract infections, for patients allergic to penicillin, and for penicillin-resistant bacteria. They consist of a large macrolactone ring of 14–16 atoms with attached sugar moieties¹. Some commonly used macrolides are erythromycin², the first discovered macrolide; azithromycin³, a derivative of erythromycin that has better stability and a broader bacterial spectrum; and telithromycin⁴, a recently developed antibiotic for bacteria resistant to erythromycin and azithromycin; (see Scheme 1A for their chemical structures).

Macrolides target bacteria by inhibiting protein synthesis in the ribosome. They bind in the protein exit channel (PET), through which the nascent peptide exists during protein synthesis, by a combination of hydrophobic and hydrophilic interactions^{1,5–13}, inhibiting protein synthesis^{14–16}. Due to structural differences between eukaryotic and prokaryotic ribosomes¹⁷ only bacterial cells are affected.

While macrolides antibiotics have many advantages, resistance to them is a growing problem. The most common cause of resistance is mutation or methylation of specific bases

of the ribosome in the vicinity of the macrolide binding site^{1,18–23}. Although prevention of hydrogen bonding between the macrolide and the ribosome has been suggested as a reason for resistance^{9–12,22}, the differences in the resistance effects for various macrolides are not well understood^{19,24–26}.

Atomistic molecular dynamics simulations could provide more details on the interaction of macrolides with the wild-type ribosome and how these interaction change in a resistant ribosome. In addition, the effects of dynamics and water, which are not typically observed in crystal structures, could be investigated. Unfortunately, there are no force field parameters available for macrolides, and consequently, computational studies of macrolides are limited. A few nuclear magnetic resonance studies and one crystallographic study have used complementary modeling of macrolides with CVFF or Amber force fields^{27–31}. Furthermore, oleandomycin, erythromycin and roxithromycin have been studied in the ribosome with Amber force fields^{32–34}. Finally, a computational study by Small et al. investigated the resistance effects of telithromycin in the ribosome using CHARMM force fields²⁶. The studies with Amber force fields^{32–34} optimized the partial atomic charges, but none of the studies optimized the bonded parameters, taking them instead from a general force field.

Although general force fields are popular for simulating drug-like molecules, these molecules have a large variety of different structures, making accurate coverage of all possible compounds difficult. Therefore, care needs to be taken when using a general force field, and the potential loss of accuracy due to generalization of parameters should be investigated. For example CGenFF³⁵, a general force field for CHARMM-compatible parameters, offers automatic assignment of atom types and parameters through www.paramchem.org^{36,37}, together with the penalties of those parameters. Penalties below 10 mean that the analogy is fair, penalties between 10 and 50 mean that validation of parameters is required and penalties over 50 mean that parameter optimization is required. When we submitted erythromycin, azithromycin and telithromycin to www.paramchem.org, we got penalties between 25 and 100, which means that further optimization/validation of parameters was needed. Since accurate force field parameters are particularly desirable in order to study the subtle chemical differences between the macrolides, as well as the small alternations of the ribosome that lead to resistance, we decided to develop parameters for macrolides from first principles.

We have previously developed Force Field Toolkit (ffTK) for developing CHARMM compatible force field parameters directly from ab initio calculations³⁸. ffTK simplifies many tedious tasks for parameter fitting. It easily identifies missing force field parameters, creates Gaussian³⁹ input files for the required ab initio calculations and reads the data from the Gaussian output files. Furthermore, it provides mathematical algorithms for finding the best fit between the quantum chemical data and the molecular mechanics (MM) simulations. While the instructions on how to use ffTK for smaller molecules are already published³⁸, larger molecules have many more challenges associated with them. Specifically, due to the high cost of ab initio calculations, large molecules need to be broken into several pieces for fitting of the parameters. In addition, the optimization of partial atomic charges in CHARMM is done by fitting the interaction energies and optimal distances of water

molecules to the charges³⁵. This optimization can be challenging in the cases where water molecules can not be placed for certain atoms due to steric clashes with surrounding atoms. Two solutions are possible for addressing these clashes: use of smaller compounds without the clashing groups, or obtaining the charges from restricted electrostatic potential (RESP) fit⁴⁰, instead of from water interactions. Finally, the optimization of dihedral terms is often a challenging and time consuming process. Although an automatic fitting procedure of multiple terms at once has been developed by Guvench and MacKerell⁴¹, and implemented in ffTK³⁸, the resulting optimization problem is often underdetermined and poorly conditioned^{42,43}.

In this paper we investigated how to handle the challenges noted above while using ffTK. Multiple approaches for the fitting of charges and dihedral parameters were tested. In addition, we parametrized three commonly used macrolides: erythromycin, azithromycin and telithromycin and made the force field parameters available for public use. Finally, we simulated these macrolides in the ribosome for validation of our parameters.

METHODOLOGY

Fitting of Force Field Parameters

The potential energy function in the CHARMM force field consists of bonded and non-bonded terms, see Eqs 1 and 2, where the force constants K ; the equilibrium values of the bonded terms; b_0 , θ_o , and ϕ_o ; the dihedral phase δ ; the partial atomic charges q ; and the Lennard-Jones parameters ϵ and σ need to be determined⁴⁴. The fitting of partial atomic charges, bonds, angles and dihedral terms was done using ffTK³⁸, while Lennard-Jones parameters and impropers were taken by analogy from CHARMM's CGenFF and sugar force fields^{35,45}. All non-identical atoms except hydrogens were assigned a unique atom type. We used the parametrization order as specified in the general CHARMM procedure³⁵, where the partial atomic charges were optimized first, followed by bonds and angles, and finally the dihedrals. The Gaussian09 program³⁹ was used for all quantum mechanical (QM) calculations and the optimization of all parameters was done using the molecular geometry obtained by energy minimization at the MP2(6–31G*) level of theory. An exception was made for the pyridine-imidazole part of telithromycin, where cc-DVZ basis set was used instead of 6–31G* for all MP2 calculations. This change of basis sets was done because the 6–31G* optimized geometry of pyridine-imidazole was not completely planar, having an angle of 20° between the pyridine and the imidazole rings. Vibrational analysis was done at the same level of theory as the geometry optimization for all geometries and showed no imaginary frequencies.

$$V_{\text{bonded}} = \sum_{\text{bonds}} K_b(b - b_o)^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_o)^2 + \sum_{\text{dihedrals}} \sum_{n=1}^{n=6} K_{\phi,n}(1 + \cos(n\phi - \delta)) + \sum_{\text{impropers}} K_\varphi(\varphi - \varphi_o)^2 \quad (1)$$

$$V_{\text{nonbonded}} = \sum_{\text{pairs}_{ij}, \text{charges}} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} + \sum_{\text{pairs}_{ij}, \text{Lennard-Jones}} \epsilon_{ij} \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - 2 \left(\frac{\sigma}{r_{ij}} \right)^6 \right] \quad (2)$$

Aliphatic and aromatic non polar hydrogens in CHARMM are automatically assigned the standard charges of 0.09 and 0.15, respectively, while aromatic carbons not adjacent to a heteroatom are assigned the standard charge of -0.15^{35} . The charges for the other atoms are fitted to the interactions with water molecules³⁵. The QM energies and distances for these interactions are calculated by placing water molecules, one at the time for each atom and optimizing its distance at the HF(6-31G*) level of theory³⁵. In ffTK all of the water accessible atoms in the molecule are classified as hydrogen bond donors, acceptors or both. For each of these atoms, a complex between the water molecule and the compound is constructed, where the water molecule is ideally oriented for the hydrogen bonding³⁸. For generality, we optimized both the distance between the water and its interaction partner as well as the rotation angle of the water about that bond. Although the latter is recommended to be fixed when parametrizing for the CHARMM force field (personal communication), this requires some intuition on the part of the user. In order to compensate for under-polarization of compounds in gas phase compared to solution, the QM interaction distances are shifted by -0.2 Å and the QM interaction energies are scaled by a factor of 1.16 for neutral compounds, while no energy scaling is used for charged compounds^{35,38}. The obtained QM energies and distances are fitted to the minimized molecular mechanics (MM) values using a TIP3P water molecule^{35,46}. The MM charges that give the best fit to the scaled QM data are chosen for the subsequent optimization of bonded parameters.

Unfortunately, in the case of bulky molecules, a placed water molecules may sterically clash with other parts of the compound as shown in Figure 1A, resulting in it moving very far away from the interacting atom and giving meaningless interaction energies. While this clash can be solved by dividing the molecule into smaller parts, the accuracy of the fit may be compromised due to losing the effects of neighboring functional groups or ring strain. The clash problem is generally caused by a CH₃ group and if this group is bound to another carbon, it can be replaced with an aliphatic hydrogen with only a small change to the chemistry of the compound. The rest of molecule can then be optimized, while the replaced CH₃ group is given the total charge of 0.0 from CGenFF force field, maintaining the integer charge of the whole molecule. However, in some cases the CH₃ group causing the clash is connected to an oxygen or a nitrogen; replacing such a group with a hydrogen would significantly alter the chemistry of the whole compound. Therefore, we have developed a novel approach for charge optimization of bulky molecules with minimal reduction in size and used it on the sugars in macrolides. In this approach, the clashing group is replaced with a smaller, chemically similar group or a hydrogen. As shown in Table S2 in the Supporting Information, this replacement has a small impact on the interaction energies of the atoms far away from the replaced group. For desosamine, the molecule was divided into two integer charge groups (see Scheme 2A). The first group does not contain sterically hindered atoms and was optimized using the original compound and the second group with the sterically hindered atoms was optimized using the reduced compound. Since changing the partial charge on an atom can affect the interaction energies of the neighboring atoms, the charge optimization of both groups was done iteratively until convergence. For cladinose we had to use two reduced compounds and two charge groups, as shown in Scheme 2B, while using the same iterative charge optimization approach as with desosamine. Although we used two

reduced compounds, the optimized charge groups were identical to their corresponding groups in cladinose.

RESP fitting was done according to general procedures⁴⁰, using AmberTools14⁴⁷. Non-polar hydrogens and aromatic carbons not adjacent to a hetero atom were constrained to the same charges as in the CHARMM procedure to retain some compatibility with the CHARMM force field. The electrostatic potential was optimized at the HF(6–31G*) level of theory as in the standard RESP optimizations⁴⁰. The same optimized geometries as in CHARMM fitting procedure were used. Alternative geometries were not added to the fitting, as is often done in RESP, in order to directly compare the charges with those from the CHARMM procedure. The bonded terms were reoptimized for the RESP charges in the same way as for the water-fitted charges, described below.

Bond and angle equilibrium values were optimized through comparison with the energy minimized QM geometry. The force constants were optimized using the Hessian calculated in the internal coordinates at the MP2(6–31G*) level of theory³⁵. The Hessian is a matrix of the secondary derivatives of the potential energy as function of input coordinate pairs. Hence, the QM potential energy surface (PES) sampled by the distortion of bonds and angles can be constructed from the Hessian. The analogous MM PES can be calculated for trial force field parameters using also small distortions of each internal coordinate. Bond and angle parameters are refined during fitting until the best match with the QM geometry and PES is found³⁸.

The dihedral PES was calculated with QM from relaxed dihedral scans at MP2(6–31G*) level of theory³⁵. The optimization of dihedral parameters was done by the simulated annealing protocol developed by Guvench and MacKerell⁴¹ and implemented in fTK³⁸; the PES cutoff for the fitting was 10 kcal/mol. Care needs to be taken when setting the multiplicities n and the phase δ from Eq 1 during the fit. Although using multiple multiplicities for the same dihedral and allowing the δ value to be fitted to either 0° or 180° tends to give a better agreement with the QM PES, using too many multiplicities may result in unphysical behavior and the loss of transferability of the parameters. The dihedral fitting problem is highly undetermined as the QM PES of the rotation around one bond is often used to fit multiple dihedrals. Thus, several different sets of dihedral parameters may fit the same QM PES. In addition, the dihedral parameter for a set of four atoms often consists of a sum of several dihedral terms with different multiplicities n , (see Eq. 1), further increasing the number of possible sets.

Furthermore, the dihedral fitting problem is often ill-conditioned and unrealistically high force constants may arise because the fitting algorithm attempts to fit a small peak in the PES or because two dihedrals' constants counteract each other⁴². These fitting problems are referred to as over-fitting and can cause unphysical behavior of the fitted molecules in simulations. Several approaches for fitting the dihedral parameters were investigated and are further described in the Results section.

Since frequency calculations at the MP2 level of theory, the bottleneck for QM calculations for force field parameters, are feasible for only up to around 40 atoms, the macrolides,

which contain 120–140 atoms, needed to be divided for parametrization. In our division, shown in Figure S1B in Supporting Information, the macrolide sugars were optimized separately, while the large macrolacton ring was divided into three parts. This division was chosen to preserve the ring structure of the smaller rings and to provide all fragments with an integer charge. During reconstruction of the whole molecule from fragments, the charges on the edge atoms were modified by absorbing the charges of the removed hydrogens³⁵. In the case of overlapping atoms between the sugars and the connecting part of the large macrolactone ring, the charges from the sugar optimization were taken. To ensure an integer charge on whole molecule, these overlapping groups were constrained to a neutral charge during the optimization of the macrolactone ring. The missing bonds, angles and dihedrals involving multiple fragments were optimized using model linking compounds constructed such that they contained at least three carbon atoms with attached functional groups from each linking part.

Simulations

All MD simulations were done with NAMD⁴⁸, using CHARMM36 protein⁴⁹ and nucleic⁵⁰ force fields for the ribosome and the TIP3P model for water⁴⁶. For ParamChem simulations of macrolides the parameters were obtained from paramchem.org website^{36,37}. Periodic boundary conditions were used, and the temperature and the pressure were kept constant at the biologically relevant values of 310 K and 1 bar, respectively, using the Langevin thermostat and piston. All covalent hydrogen bonds were kept rigid at the equilibrium length specified in the parameter file, which allowed for integrating the equations of motion with a 2-fs time step. Short-ranged electrostatic interactions were evaluated every step, while a long-range electrostatic evaluation was done every second step. The cutoff for non-bonded interactions was 12 Å and smoothing functions were applied starting from 10 Å to ensure a smooth decay to zero. The non bonded interactions were excluded for 1–2 and 1–3 terms.

Each simulation system was constructed from a corresponding PDB, namely 3OFR for erythromycin¹², 3OHZ for azithromycin¹¹, and 3OAT for telithromycin¹². While 3OFR and 3OAT are from *Escherichia coli*, 3OHZ is from *Thermus thermophilus*. Thus, azithromycin was modeled into the *Escherichia coli* ribosome based on the structure with erythromycin; the binding sites of the two in 3OFR and 3OHZ, respectively, are practically identical. Because the focus of our studies is on the exit tunnel and PTC, only portions of the ribosome within 25 Å of these regions were maintained. The resulting reduced system is similar to that used in a previous study of SecM in the exit tunnel, although smaller as the the full tRNAs were no longer retained⁵¹. Mg²⁺ ions were added at points of electrostatic potential minima and their octahedral solvation shells were completed; this was followed by the addition of bulk water using solvate plugin in VMD and 0.1 mol/L of KCl ions. Ribosome residues within ~10 Å of the boundary were restrained to their crystal structure positions using a force constant of 10 kcal/mol·Å² for all simulations. Although solvation of a closed of binding pocket can be an issue²⁶, the number of water molecules within the vicinity of macrolides increased from the initial configuration and fluctuated about a mean during the MD simulations (see Table S1), suggesting that water in the binding site could equilibrate within ~30 ns.

Since the system was shown to be sensitive to the initial conditions, minimization was done in two steps and equilibration was done in four steps. In the first minimization step, all of the hydrogens in the system were minimized and in the second step, the macrolide, water and ions were minimized. The equilibration was done in four steps of 1 ns each: first, only the solvent was equilibrated, second, the macrolide and the side chains of residues within 10 Å of the macrolide were equilibrated. In the third step, the backbone outside the frozen region was restrained by soft constraints, using a force constant of 2 kcal/mol·Å², while everything else was allowed to equilibrate. In the final fourth step, the force constant on the backbone was decreased to 1 kcal/mol·Å², using the same restraints as in the third step. The test runs for the different dihedral parametrization strategies were 30 ns, which was sufficient to see the conservation of the important interactions, while the other production runs were 150 ns. Only the frozen region was restrained during the test and production runs.

RESULTS AND DISCUSSION

Since the parametrization of the desosamine sugar is responsible for the two most important interactions with the ribosome (see Scheme 1B) the results of its parametrization are discussed in detail below. In particular, two methods for determining the partial atomic charges: ffTK and RESP are compared to the charges from www.paramchem.org^{36,37}. Ideally, parametrization of sugar molecules should be based on CHARMM36 carbohydrate parameters instead of CHARMM general forcefield. Unfortunately, the former force field presently lacks parameters for amines and is not supported by www.paramchem.org, making it difficult to determine the best analogous parameters for desosamine. Therefore, the general CHARMM force field parameters obtained from www.paramchem.org were used as a starting point for desosamine in this work. Multiple schemes for determining dihedral parameters were tested before settling on the approach used for the rest of the macrolides. It should be noted here that www.paramchem.org parameters are only meant to be a starting point for refinement; their high penalties are an indication that they are not meant to be used for production simulations. Nonetheless, in order to have a common reference point of comparison, the www.paramchem.org parameters are used below.

Partial Atomic Charges

Table 1 compares the partial atomic charges for polar atoms of desosamine, obtained from www.paramchem.org, ffTK fitting and RESP fitting, as well as the corresponding errors in the interaction energies with water, computed at the HF(6-31G*) level of theory. The ParamChem charges have very high errors, up to > 2 kcal/mol, for the oxygen atoms, a moderate error for H1 and very small errors for H2 and Cn from the amine group of desosamine. The ffTK optimized charges are significantly smaller in magnitude than the ParamChem charges, especially for N and O2, and dramatically reduce the errors in the interaction energies to below 0.4 kcal/mol for all polar atoms. The presence of a positively charged amine group on the desosamine sugar affects the interaction energies of the atoms close to it, which is not accounted for in the charges given by www.paramchem.org. Thus, with ffTK it is possible to re-optimize the charges and improve the interaction energies with water.

The RESP charges for desosamine improve the interaction energies for the oxygens and H1, compared to ParamChem charges, although the errors are still higher than for ffTK charges. Additionally, the errors for the atoms of the amino group are greatest for the RESP charges. This behavior of errors can be explained by examining the charges in Table 1. Most RESP charges are slightly smaller in magnitude than the ParamChem charges, which leads to improvement in the interaction energies just as with ffTK charges; however, the charges for N and Cn are dramatically different and change sign, -0.456 to 0.023 and 0.152 to -0.059 , respectively. The slightly positive charge on the nitrogen is unrealistic and is most likely an artifact of RESP optimization scheme, in which the charges of the buried atom are often underdetermined. Nevertheless, the RESP charge optimization scheme is faster and easier to use than the ffTK charge optimization and offers significant improvement in the interaction energies compared to the ParamChem charges. However, a different approach is needed to determine the charges on buried atoms when a RESP optimization scheme is used.

Dihedral Optimization

In order to avoid unnecessary over-fitting, while still maintaining a good fit to the quantum mechanical (QM) potential energy surface (PES), several approaches for selecting the multiplicities and the phases of the fitted dihedrals were tested for azithromycin with ffTK charges by simulating the macrolide in the ribosome. These approaches were judged through evaluation of azithromycin geometries, RMSD of the macrolide and conservation of the interactions with the ribosome, namely the hydrogen bond between the OH of desosamine and base A2058 and the ionic interaction between the amine-group of desosamine and the phosphate of G2505, (see Scheme 1B⁹⁻¹²).

The MM PES of selected dihedral sets are compared to the QM PES for desosamine in Figure 2, which shows that the dihedral set taken from www.paramchem.org^{36,37}, referred to as ParamChem, results in high deviations from the QM PES for several configurations. In our first parametrization of the dihedrals, referred to as n-123, we aimed to get the MM PES as close to the QM PES as possible. Therefore, δ was allowed to be either 0 or 180° and at least three multiplicities, $n = 1, 2, 3$, were used on all dihedrals as well as $n = 4, 6$ for the dihedrals containing a carbonyl carbon. Since ParamChem parameters had 4-fold ($n = 4$) dihedral terms for ring oxygens in the sugars, these terms were added in n-123 parametrization if they significantly improved the PES fit. Using this dihedral set in simulations resulted in low RMSD and good conservation of the important interactions with the ribosome. However, the hydrogens in the macrolide showed undesired behavior, often adopting eclipsed conformations instead of the chemically correct staggered conformations (see Figure 3A and C). This incorrect behavior of hydrogens was likely due to over-fitting of the dihedrals.

Therefore, we decided to reduce the number of fitting parameters for the dihedrals by taking the phase and the multiplicity for all dihedrals from www.paramchem.org and just refitting the force constants; this dihedral set is referred to as ParamChem-n. The resulting force constants were of lower magnitude, which is generally desirable⁴²; however, the deviations from the QM PES were higher than for the n-123 fit. Additionally, the simulations of azithromycin using these parameters resulted in high RMSD from the crystal structure and

breaking of the interactions with the ribosome. Furthermore, frequent flipping between chair and envelope conformations was observed for the macrolide sugars (see Figure 3B).

Since envelope conformations are not generally stable for 6-membered pyranose sugars, we suspected that ParamChem-n parameters still suffered from over-fitting. Examination of the atom types assigned to the macrolide sugars by www.paramchem.org showed that the ring oxygens were assigned the same atom types as the ether oxygens in dioxane. In addition, the dihedrals consisting of these oxygens and the carbons of the sugar ring backbone were given multiplicities $n = 1, 2, 3, 4$, although it has been shown that multiplicities of $n = 3$ are sufficient for these dihedrals in order to reproduce the QM PES of pyranose sugars⁴⁵. To test if over-fitting was causing the sugar flipping problem, we re-parametrized the desosamine sugar taking the dihedral multiplicities from the previous CHARMM fitting of sugars⁴⁵. We used $n = 3$ and $\delta = 0^\circ$ for all dihedrals except the ones containing the OH group or the non-cyclic acetal oxygen, where $n = 1, 2, 3$ and $\delta = 0, 180^\circ$ were used. Indeed, with these new parameters the desosamine sugar stayed in the chair configuration, which is the most favorable one for pyranose sugars. Unfortunately, the interactions with the ribosome were still not reliably conserved.

Hence, we decided to further reduce the number of dihedral constants in the fit by taking the values of all the hydrogen dihedrals with penalties less than 10 from www.paramchem.org, as suggested by Vanommeslaeghe et al.⁴². Most other dihedrals were fitted with $n = 3$ and $\delta = 0^\circ$. For the dihedrals containing non-cyclic acetal, ether, hydroxyl or ester oxygens $n = 1, 2, 3$ was used, while for the dihedrals containing a carbonyl center $n = 1, 2, 3, 4, 6$ was used, δ was allowed to be either 0 or 180° in both cases. For the aromatic part of telithromycin, $\delta = 180^\circ$ and $n = 2$ were used, while for planar dihedrals involving an ester or an amide group $n = 1, 2$ were used. For the cases where a good QM PES fit could not be obtained, additional multiplicities were added, or phase restrictions were removed, as needed. The values of the hydrogen-containing dihedrals were added to the parameter file before the fitting. This dihedral fitting scheme is referred to as n-3. Though the resulting dihedral constants were smaller than in the previous fits, almost close to 0 in some cases, the fit to QM PES had more deviations than both n-123 and ParamChem-n but fewer than the initial ParamChem dihedrals. Surprisingly, the simulations showed a good conservation of the interactions between the ribosome and the macrolide, in spite of the less optimal PES fit. In addition, correct conformations of the sugar ring and methyl hydrogens were observed (see Figure 3C). Therefore, the n-3 dihedral fitting scheme was used for the final fitting of both fTK and RESP charge sets.

Simulations of Macrolides—Due to the lack of experimental data on isolated macrolides for validation, the parameters were evaluated by simulating each macrolide in the ribosome exit tunnel for at least 150 ns. RMSD and conservation of the two interactions with the ribosome from the crystal structure, the hydrogen bond between OH of desosamine and A2058 and the ionic interaction between desosamine amino group and the phosphate of G2505, displayed in Scheme 1B⁹⁻¹², were used as benchmarks. Unfortunately, large fluctuations were observed for these interactions in the ribosome. Therefore, the simulations were repeated at least once for each parameter fitting scheme in order to distinguish the effects of the parameters on the interactions from the equilibration effects and the dynamic

fluctuations of the macrolide. Because the crystal structures were of relatively low resolution (3.10Å) and at low temperatures, some deviations from these structures during MD simulations are to be expected. The MD simulations done for this work are summarized in Table 2.

Four parameter approaches were tested. Parameters taken from www.paramchem.org, referred to as ParamChem, were compared to two sets of parameters derived from QM calculations: ffTK and RESP parameters, where the partial atomic charges were derived from water interactions and RESP fitting, respectively, followed by optimization of the bonded parameters to the QM data using the n-3 dihedral fitting scheme. Finally, we investigated the importance of refitting the bonded parameters after changing the charges by testing the ffTK/ParamChem approach, in which the charges were fitted with ffTK, while the bonded parameters were taken from www.paramchem.org.

Figure 4 and Figure 5 compare RMSD and conservation of interactions with the ribosome for erythromycin and the four different approaches. As previously discussed, all of the approaches show breaking of interactions with the ribosome to some degree. Both of the ParamChem simulations show low RMSD, but also frequent breaking of the interactions with the ribosome. The ffTK parameters result in better conservation of the macrolide's interaction with the ribosome, in spite of the somewhat high RMSD. RESP simulations show low RMSD and the best conservation of the hydrogen bonding out of four approaches; however, there is almost no conservation of the ionic interaction. The loss of the ionic interaction could be due to the error in the charges of the ionic $\text{NH}(\text{CH}_3)_2$ group of desosamine, discussed above in the charge optimization section. The same trends of high conservation of the hydrogen bond with the ribosome but no conservation of the ionic interactions were also seen in RESP simulations of the other macrolides (see Figures S3 and S5 in Supporting Information). The mixed ffTK/ParamChem approach does not improve the interactions with the ribosome for erythromycin, compared to the ParamChem parameters (Figure 4 and Figure 5). The ffTK/ParamChem simulations of azithromycin and telithromycin show a decrease of interactions with the ribosome (see Figures S3 and S5 in Supporting Information Figures). As the bonded terms often depend strongly on the partial atomic charges, re-optimization of the bonded terms is needed if these charges are changed significantly.

RMSD and interactions of azithromycin with the ribosome for ffTK and ParamChem parameters are displayed in Figure S2 in Supporting Information. The advantage of the ffTK parameters is less evident for the azithromycin, as the ionic interaction is somewhat better conserved, while the hydrogen bonding is far less conserved. However, investigation of the simulation trajectories showed that in Sim2 with ffTK parameters azithromycin is adopting a slightly different position in the ribosome, (see Figure 3D for comparison of positions), in which the macrolide is hydrogen bonded to A2059 instead of A2058. Therefore, hydrogen bonding to the ribosome is still maintained in Sim2.

The results of telithromycin simulations with ffTK and ParamChem parameters are shown in Figure S4 in Supporting Information. Both parameter sets produce simulations with low RMSD and good conservation of interactions, but also simulations with high RMSD and

significant breaking of the interactions with the ribosome. In general, ffTK parameters appear to be better at maintaining the ionic interaction, while ParamChem parameters appear to be better at maintaining the hydrogen bond. The breaking of interactions with the ribosome is often correlated with the breaking of π -stacking between the imidazole-pyridine arm of telithromycin and A752 (see Figure 3E for the differences in arm position). Previous simulations of telithromycin by Small et al.²⁶ showed a greater conservation of the ionic interaction, hydrogen bonding and π -stacking with the ribosome than our simulations. Force field parameters are not a likely cause for the differences in these simulations, because our ParamChem parameters that are very similar to the ones used by Small et al. also often show breaking of interactions in the simulations. The two studies used slightly different models of the ribosome around the macrolide, which could explain the differences in the simulation results. While we used the whole protein exit tunnel, including the residues within 25 Å of the tunnel, Small et al. used a spherical model of the ribosomes, where the residues within 40 Å of the center of telithromycin were included. Another possible reason for the discrepancy in the simulation results is the length of simulations. Small et al. did five 30-ns-long simulations, while we did two or three 150-ns-long simulations. Both approaches have advantages and disadvantages; while many shorter simulations provide better statistics on short term behavior, our longer simulations show that long term behavior can differ from that on the short term. For example, the breaking of interactions with the arm occurs after 32 ns in Sim2 and would not have been observed in a short simulation. It is unclear if the arm movement observed in our simulations is the accurate longterm dynamical behavior of telithromycin or an artifact of the force field limitations in describing π -stacking interactions.

CONCLUSIONS

Multiple approaches for parametrization of macrolide antibiotics were tested and evaluated based on their ability to maintain the primary two interactions with the ribosome (see Scheme 1B) observed in the crystal structures^{9–12}. The derivation of partial atomic charges from either water interactions using our Force Field Toolkit (ffTK) fitting scheme, from our modified RESP fitting⁴⁰, or from www.paramchem.org^{36,37} was investigated. The RESP fitting scheme showed some improvement in water interaction energies, except for the charges of amino group that is responsible for the ionic interaction, and a significant improvement in conserving the hydrogen bond interaction between the macrolide and the ribosome compared to the ParamChem charges. Unfortunately, the ionic interaction was mostly broken for the RESP charges, likely due to the errors in the charges for the amino group. ffTK charges showed great improvement in the interaction energies with water, a small improvement in maintaining the hydrogen bond to the ribosome, and a moderate improvement in maintaining the ionic interaction to the ribosome. Note, it was shown for the ffTK charges that the bonded terms needed to be re-optimized in order to obtain any improvement in the simulation results over www.paramchem.org parameters. Based on our simulations, the ffTK derived charges offers the best improvement in conserving both of the interactions with the ribosome. Fitting of RESP charges is an easier and faster procedure, which can give an improvement over ParamChem charges in terms of both interaction energies with water and maintenance of the hydrogen bond in the simulations. Further

development is needed, however, in tackling the buried atoms with RESP optimized charges.

Several approaches for parametrizing the dihedral terms were tested. It was shown that the best QM PES fit does not necessarily lead to the most accurate simulation results. Instead, it is often advantageous to minimize the number of parameter constants being fit, while still maintaining a reasonable fit to the QM PES. We showed that both can be achieved by taking the dihedrals for all of the hydrogens with low penalties from paramchem.org and using only multiplicity of $n = 3$ for dihedrals containing only aliphatic carbons. The choice of multiplicities for the remaining dihedrals needs to be carefully chosen. As it was shown that www.paramchem.org sometimes guesses these multiplicities incorrectly, readers are advised to search the scientific literature, including examining other parts of the CHARMM force field, before fitting the dihedrals.

Our parametrization approach for large molecules is described in detail in the Methods section. Briefly, it is recommended to divide the molecule in smaller fragments of 40 atoms or less, for the ease of quantum chemical (QM) calculations. The division should be done such that each fragment is expected to have an integer charge. Charges and bonded parameters for each fragment should be optimized as described. When the whole molecule is reconstructed from these parts, charges on the edge atoms are adjusted by absorbing the charges of the removed hydrogens to ensure that the whole molecule has an integer charge. The missing bonds, angles and dihedrals involving multiple parts of the molecule can be parametrized from linking compounds, constructed from pairs of separate fragments, that contain all the missing dihedrals for the linking of the two fragments.

As validation of our approach, simulations of macrolides in the ribosomal exit tunnel showed that interaction with the ribosome were improved with our new optimized parameters, compared to the initial ones, although some breaking of interactions was observed in almost all simulations. Furthermore, different orientations of the macrolides in the ribosome, not observed initially, were found with our optimized parameters. Several simulations of telithromycin resulted in high RMSD and breaking of the interactions, probably due to breaking of π -stacking interactions between the aromatic imidazole-pyridine arm of telithromycin and A752, and consequent movement of this arm. Although experimental data suggest higher flexibility for the arm of telithromycin⁵², improved force field description of π -stacking interactions is needed in order to confirm that this movement may lead to breaking of other interactions with the ribosome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Illustration of the water clash problem for desosamine, the water molecule and the interacting oxygen of desosamine. A) In the original desosamine molecule the placed water is sterically clashing with a $N - CH_3$ group. B) Optimized interaction of water with the same oxygen in the reduced desosamine molecule, where the clashing CH_3 group was replaced with a hydrogen.

Figure 2. Potential energy surface of desosamine. QM energies (black) are compared to MM energies (colored) for several sets of dihedral parameters. ParamChem MM energies were calculated using ffTK charges.

Figure 3.

Snapshots from simulations, non-important atoms omitted for clarity. A. Incorrect, eclipsed conformation of hydrogens in the methyl group of desosamine sugar when using n-123 parameter set, the sugar itself is in the correct chair conformation. B. Envelope conformation of desosamine sugar, frequently observed with ParamChem-n dihedrals. C. Correct chair conformation and staggered conformation of the methyl group in desosamine with n-3 parameter set. D. Overlap of azithromycin positions in Sim1 (blue) and Sim2 (red) from Figure S2 in Supporting Information. Both the macrolactone ring and the desosamine sugar shift their position in Sim2. E. Overlap of telithromycin positions in Sim1 (blue) and Sim2 (red) from Figure S4 in Supporting Information. While the positions of both the macrolactone ring and the desosamine sugar are similar for both simulations, the position of the arm is significantly different.

Figure 4.

RMSD (top) and conservation of the two most important interactions of the ribosome: the hydrogen bond to A2058 (middle) and the ionic interaction with G2505 (bottom) for erythromycin with ffTK (left) and ParamChem (right) parameters.

Figure 5.

RMSD (top) and conservation of the two most important interactions of the ribosome: the hydrogen bond to A2058 (middle) and the ionic interaction with G2505 (bottom) for erythromycin with RESP (left) and mixed ffTK/ParamChem (right) parameters.

Scheme 1.

A) Structures of macrolides that are parametrized in this paper. B) Interactions of desosamine sugar, present in all macrolides, with the ribosome.

A Desosamine Optimization Scheme

Original Compound

Optimize Charge Group 1

Reduced Compound

Optimize Charge Group 2

B Cladinose Optimization Scheme

Original Compound

Reduced Compound 1

Optimize Charge Group 1

Reduced Compound 2

Optimize Charge Group 2

Scheme 2.

Schematic representations of the charge optimization scheme used on bulky atoms for desosamine and cladinose sugars. The sterically hindered groups are shown in blue, the molecule parts that are different for the original and reduced compounds are shown in red and the charge group optimized in each structure is encircled. (A) Charge optimization of desosamine sugar. Since the OH group is sterically hindered on both sides, the molecule was divided into two charge groups, where Group 2 contained the hindered O-H and the adjacent C-H, while Group 1 contained the rest of the molecule. In the optimization scheme Group 1 was optimized using the original compound while Group 2 was optimized using the reduced compound. (B) Charge optimization of cladinose sugar. The oxygens connecting both methyl groups and the hydroxyl oxygen are sterically hindered; hence, two reduced compounds and two charge groups were constructed. The two reduced compounds were used for charge optimization; however the optimized charge groups (one from each) were identical to the corresponding groups in cladinose.

Table 1

Comparison of charges and errors for water interaction energies for the polar atoms in desosamine sugar (see Scheme 1B for atom names). The errors in the interaction energies were calculated as $E_{MM}-E_{QM}$ and the distances of the water molecule were optimized separately for E_{MM} and E_{QM} . The reduced desosamine compound was used to calculate the interaction energies for O2 and H1. In the case of Cn, the interaction energies with water were calculated for the bound hydrogen, not for the carbon.

Atom	Partial atomic charge			Water interaction energy error		
	ParamChem	fTK	RESP	ParamChem	fTK	RESP
O1	-0.35	-0.17	-0.341	-2.68	-0.016	-1.742
O2	-0.658	-0.424	-0.613	-2.783	-0.387	-0.373
O3	-0.384	-0.317	-0.297	-1.143	-0.031	-0.505
H1	0.42	0.39	0.429	0.352	0.1	0.177
H2	0.309	0.238	0.266	-0.023	-0.045	-1.284
Cn	0.152	0.056	-0.069	0.026	0.139	1.1
N	-0.456	-0.196	0.023	-	-	-

Table 2

List of all MD simulations (total of 4.2 μ s) performed in this study.

Macrolide	Charge Optimization	Dihedral Optimization	Simulations	Time/simulation (ns)
azithromycin	ffTK	n-123	2	30
azithromycin	ffTK	ParamChem-n	2	30
azithromycin	ffTK	n-3	2	30
azithromycin	ffTK	n-3	3	150
azithromycin	RESP	n-3	2	150
azithromycin	ffTK	ParamChem	2	150
azithromycin	ParamChem	ParamChem	2	150
erythromycin	ffTK	n-3	3	150
erythromycin	RESP	n-3	2	150
erythromycin	ffTK	ParamChem	2	150
erythromycin	ParamChem	ParamChem	2	150
telithromycin	ffTK	n-3	3	150
telithromycin	RESP	n-3	2	150
telithromycin	ffTK	ParamChem	2	150
telithromycin	ParamChem	ParamChem	2	150