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An Amber Force Field for *S*-Nitrosoethanethiol That Is Transferable to *S*-Nitrosocysteine

Sanghwa Han

Department of Biochemistry and Institute for Life Sciences, Kangwon National University, Chunchon 200-701, Korea
E-mail: hansh@kangwon.ac.kr
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Protein *S*-nitrosation is common in cells under nitrosative stress. In order to model proteins with *S*-nitrosocysteine (CysSNO) residues, we first developed an Amber force field for *S*-nitrosoethanethiol (EtSNO) and then transferred it to CysSNO. Partial atomic charges for EtSNO and CysSNO were obtained by a restrained electrostatic potential approach to be compatible with the Amber-99 force field. The force field parameters for bonds and angles in EtSNO were obtained from a generalized Amber force field (GAFF) by running the Antechamber module of the Amber software package. The GAFF parameters for the CC-SN and CS-NO dihedrals were not accurate and thus determined anew. The CC-SN and CS-NO torsional energy profiles of EtSNO were calculated quantum mechanically at the level of B3LYP/cc-pVTZ//HF/6-31G*. Torsional force constants were obtained by fitting the theoretical torsional energies with those obtained from molecular mechanics energy minimization. These parameters for EtSNO reproduced, to a reasonable accuracy, the corresponding torsional energy profiles of the capped tripeptide ACE-CysSNO-NME as well as their structures obtained from quantum mechanical geometry optimization. A molecular dynamics simulation of myoglobin with a CysSNO residue produced a well-behaved trajectory demonstrating that the parameters may be used in modeling other *S*-nitrosated proteins.

Key Words: Force field, Molecular dynamics simulation, *S*-Nitrosoethanethiol, *S*-Nitrosocysteine

Introduction

Intracellular nitrogen monoxide and its congeners often react with a cysteine residue converting the latter to *S*-nitrosocysteine (CysSNO). Such *S*-nitrosation has recently been proposed as a mode of NO-mediated cell signaling that has a range of physiological consequences.[1] It is obvious that biological role of *S*-nitrosation can be explained on a molecular level only when the structural perturbation induced by *S*-nitrosation is properly interpreted. Unfortunately three dimensional structures have been solved only for a few *S*-nitrosated proteins.[2-5] Therefore molecular dynamics (MD) simulation with good force field parameters may serve as an alternative method for probing the structural alteration in *S*-nitrosated proteins.

Most MD software packages are equipped with force field parameters only for standard residues. To perform MD simulation of a protein with a modified residue, one has to provide the missing parameters associated with the modified moiety such as the force constants (for bonds, angles, and dihedrals), atomic charges, and van der Waals parameters.[6-9] Atomic charges are calculated according to the procedure adopted in the original development of a particular force field, e.g.; a restrained electrostatic potential approach in the Amber-99 force field.[10] The van der Waals parameters are difficult to obtain so that in most cases they are estimated based on chemical analogy. Force constants for bonds and angles can be obtained by using the empirical equations of the generalized Amber force field.[11,12] Since protein conformation is sensitive to torsional degrees of freedom, however, the torsional force constants must be determined accurately by fitting the experimental or theoretical torsional energy profiles with the energies obtained from molecular mechanics energy minimization.

Theoretical torsional energy profiles of CC-SN and CS-NO in *S*-nitrosothiols can readily be obtained from quantum mechanical geometry optimization. Unlike simple *S*-nitrosoethanethiol (EtSNO), torsional energy profiles of the dihedrals in CysSNO, especially CC-SN, are complicated in shape due to lack of symmetry so that their force constants are not easy to estimate accurately. Interested in redox regulatory proteins, we previously reported MD simulations of *S*-nitrosated thioredoxin[9] in which the CC-SN dihedral lies in between 185 and 289 degrees.[3] Other *S*-nitrosated proteins,[4] however, may well have other values for the dihedral angles so that it is desirable to have a better force field that accounts for the whole range of dihedral angles. In this report we developed an Amber force field for EtSNO that can be transferred to CysSNO reproducing the CC-SN and CS-NO torsional energies of CysSNO in both α and β-conformations for the whole range of CC-SN and CS-NO dihedrals.

Methods

Softwares. Chimera[13] was used to build the initial structures of EtSNO and the tripeptide ACE-CysSNO-NME. All quantum mechanics (QM) calculations were performed by using Gaussian 03 Rev. E.01.[14] Gromacs 4.0.5[15] was employed in all molecular mechanics (MM) calculations. Amber-99 force field parameters were translated into the Gromacs format according to Sorin and Pande[16] (also visit http://chemistry.csulb.edu/ffamber). Topologies obtained from the Antechamber module of Amber 9 MD software package[17] were converted into the Gromacs formats by using a perl script amb2gmx.pl.[18] R.E.D.-III tools[19] (also visit http://q4md-forcefieldtools.org/RED/) were used to aid the RESP charge derivation.

Calculation of partial atomic charges. To be compatible with the Amber-99 force field, partial atomic charges of EtSNO and CysSNO were derived by using a restrained electrostatic potential (RESP) approach.[10] Both α and β-conformers of the tripeptide ACE-CysSNO-NME were included in the charge calculation. Due to scarcity of crystal structures of *S*-nitrosated proteins the amide dihedrals ϕ and ψ could not be determined from predominant conformations. We therefore adopted the ϕ/ψ values of unmodified residue (CysSH) that were used in the original Amber-99 force field development,[10] i.e.; −60/−40 for the α and 206/141 degrees for the β-conformation. The values of χ_1 were also adopted from CysSH and set to −76 and 172 degrees for the α and the β-conformation, respectively. QM torsional energy profile of CC-SN had a minimum at ~90 and ~270 degrees for the α and β-conformer, respectively, so that we used these values for the initial structures. The CS-NO dihedral was set to 0 degrees (i.e. syn conformation) for both α and β-conformer. The initial structures were subjected to geometry optimization at the level of HF/6-31G* with the amide dihedrals frozen at the initial values. Other coordinates were allowed to vary during optimization. The resulting optimized structures were incorporated in a multi-conformation RESP charge derivation using the RESP module of Amber 9.0 with the help of R.E.D.-III tools. In order to obtain the charges of the central fragment, total charges of ACE and NME were restrained to zero and charges of the peptide bond atoms C, O, N, and H were set, respectively, to 0.5973, −0.5679, −0.4157, and 0.2719 in accordance with the Amber-99 force field.[19]

Determination of missing force field parameters. Based on chemical analogy, the atom types of S, N, and O in the nitrosyl group were assigned to S, NC, and O, respectively, that were included in the Amber-99 force field. As such the van der Waals parameters of these atoms need not be newly defined. Force constants for bonds (S-NC and NC-O) and angles (CT-S-NC and S-NC-O) in EtSNO were obtained by running the Antechamber unit of Amber 9.[11,12] The remaining two parameters, X-CT-S-NC and CT-S-NC-O, were estimated by fitting the QM torsional energy profiles with the corresponding torsional energies obtained from MM energy minimization. We first generated conformers of EtSNO by varying the CC-SN dihedral from 0 to 360 degrees at the interval of 10 degrees. The CS-NO dihedral was kept at 0 degrees. These initial structures were optimized at the level of HF/6-31G* with the CC-SN dihedrals fixed at the initial values. We next performed a single point energy calculation at the level of B3LYP/cc-pVTZ on each optimized structure to obtain the torsional energy profile. For the CS-NO torsional energies the initial value of the CC-SN dihedral was set to 90 degrees, the position of an energy minimum, and the CS-NO dihedral was scanned from 0 to 360 degrees. The QM torsional energy profiles of CC-SN and CS-NO were fitted separately with the corresponding MM energies to determine the torsional force constants. For MM torsional energies we minimized the energy of a conformer with a fixed dihedral angle by using a conjugate gradient method. We changed the torsional force constants until the MM energies reproduced the QM energies. These parameters for EtSNO were transferred to ACE-CysSNO-NME without modification. The QM torsional energy profiles of ACE-CysSNO-NME were calculated at the level of B3LYP/cc-pVTZ//HF/6-31G*. The initial value of the CS-NO dihedral was 0 degrees for both α and β-conformers for the calculation of the CC-SN torsional energy profile whereas the CC-SN dihedral was initially set to 90 and 270 degrees for the α and β-conformer, respectively, for the CS-NO torsional energy profile.

Validation of the force field parameters. Our new force field parameters were validated by comparing the QM geometry optimized structures of ACE-CysSNO-NME with the corresponding structures obtained from MM energy minimizations in vacuo. Same starting structures were used for both QM and MM calculations: the initial values of ϕ, ψ, χ_1, CC-SN, and CS-NO were set respectively to −60, −40, −76, 90, and 0 for the α-conformer and 206, 141, 172, 270, and 0 for the β-conformer. Both ϕ and ψ were fixed at the initial values but all other internal coordinates were allowed to vary during QM (at the level of HF/6-31G*) and MM energy minimization. Chimera was used to compare a QM geometry optimized structure with the corresponding MM energy minimized structure.

Molecular dynamics simulation of *S*-nitrosated myoglobin. Structures of myoglobin (Mb) with ^{10}CysSNO residue were extracted from 2nrm.pdb.[4] Iron was removed from the heme and the pyrrol nitrogens were deprotonated. Atomic charges of the resulting demetallated heme were calculated according to a RESP protocol. GROMACS 4.0.1 software package[15] in conjunction with the Amber-99 force field and TIP3P water model was used in MD simulations. The initial structure was immersed in a periodic water box of truncated octahedron shape (10 Å thickness) and electrically neutralized by adding a Na^+ ion. The particle mesh Ewald method[20] was used to calculate the electrostatic energy. Cutoff distances for the calculation of the Coulomb and van der Waals interaction were 10 and 12 Å, respectively. After energy minimization using a steepest decent method, the system was subjected to equilibration at 300 K and 1 bar for 100 ps under the conditions of position restraints for heavy atoms and LINCS constraints[21] for all bonds. A velocity rescaling thermostat[22,23] and Parrinello-Rahman barostat[24,25] were used, respectively, for temperature and pressure coupling. Finally the position restraints were removed in the production MD calculations while keeping all the other conditions unaltered. Since the heme was demetallated we froze the distal and proximal histidines and the heme. The results were analyzed using the standard softwares that were included in the GROMACS package.

Results and Discussion

Protein *S*-nitrosation is common in cells under nitrosative stress. Physiological consequences of *S*-nitrosation can fully be understood when structural alteration induced by *S*-nitrosation is characterized at a molecular level. Crystal structures are available only for a few *S*-nitrosated proteins, however, and thus we need an alternative structural probe such as MD simulation. We developed force field parameters for EtSNO and CysSNO that are compatible with the Amber-99 force field.[26]

Partial atomic charges of EtSNO and CysSNO. To be compatible with the Amber-99 force field, atomic charges of EtSNO were calculated by using a restrained electrostatic potential

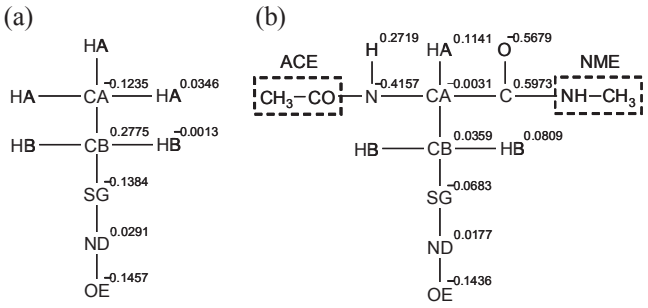

Figure 1. Partial atomic charges of (a) *S*-nitrosoethanethiol and (b) a capped tripeptide containing *S*-nitrosocysteine. Atom names are assigned according to the conventions of ffamber99, the Amber-99 force field in Gromacs format.

(RESP) approach.[10] As shown in Fig. 1a, atoms S and O in the SNO moiety had a negative charge whereas N had a small positive charge. The charge of S (−0.1384) in EtSH was significantly larger than that in CysSNO. Dipole moments of EtSH, EtS^-, and EtSNO calculated at the level of B3LYP/cc-pVTZ//HF/6-31G* were 1.64, 4.35, and 2.66 D, respectively. Therefore *S*-nitrosation of ethanethiol increases or decreases the polarity depending on the protonation state of the thiol. Similar changes in local polarity are expected for the CysSNO residue in an *S*-nitrosated protein although one should consider the conformational variation in the amide backbone.

Atomic charges of an amino acid residue in the original Amber-99 force field were obtained by a multi-conformation RESP fitting that took predominant conformations into calculation.[10] We could not deduce any preferred conformations, however, because 3D structures are available only for a few *S*-nitrosated proteins.[2-5] We therefore adopted the values of ϕ, ψ, and χ_1 (i.e. C-N-CA-CB dihedral) from the conformation of unmodified CysSH that is listed in the Amber-99 force field.[26] Optimization at the level of HF/6-31G* was performed on the initial structures in which the value of CC-SN was set to 90 and 270 degrees for the α and β-conformer, respectively, and CS-NO was set to 0 degrees. Optimized structures of both conformers were taken into RESP fitting and the results are shown in Fig. 1b. The sum of the charges of S, N, and O was less negative in CysSNO (-0.194) than in EtSNO (−0.255).

Determination of the force constants associated with the nitroso group in EtSNO. We need to add two new force constants for bonds (S-NC and NC-O) and two for angles (CT-S-NC and S-NC-O) associated with the nitroso group in EtSNO. These force constants were conveniently obtained by running the Antechamber module of Amber 9, which is based on the empirical generalized Amber force field (GAFF).[11,12] Since the equilibrium bond lengths and angles from Antechamber were comparable to those from QM optimized structures, we used the GAFF parameters for bonds and angles as obtained (see Table 1).

We next turn to force constants for the dihedrals CC-SN (X-CT-S-NC) and CS-NO (CT-S-NC-O). Torsional force constants should be estimated accurately because they are an important determinant of protein conformation. Although an experimental value of the CS-NO torsional barrier in CH_3SNO has been reported,[27] a full torsional energy profile is not available for either CC-SN or CS-NO. Therefore we calculated the torsional energy profiles theoretically at the level of B3LYP/cc-pVTZ//HF/6-31G*. As shown in Fig. 2, both CC-SN and CS-NO torsional energy profiles showed a two-fold symmetry reflecting the molecular structure of EtSNO. The CC-SN torsional energy had a maximum at 0 degrees with value of 6.5 kcal/mol and minima at 100 and 260 degrees (Fig. 2a, closed circles). There was a small barrier of 1.0 kcal/mol at 180 degrees. The CS-NO torsional energy profile showed a large barrier at 90 and 270 degrees (Fig. 2b, closed circles). The barrier height (14.8 kcal/mol) is comparable to a reported value (12.9 kcal/mol) for CH_3SNO.[27] The ante conformation at 180 degrees was slightly less stable (0.9 kcal/mol) than the syn conformation at 0 degrees in agreement with a previous report.[27] The ab initio result is consistent with the X-ray crystal structure in which syn conformer predominates.[3]

We next examined if the GAFF torsional force constants obtained from Antechamber could reproduce the QM torsional energy profiles. As shown in Fig. 2a, the CC-SN torsional energy profile (dashed line) calculated from the GAFF parameters deviated from the theoretical profile (closed circles) especially in the central region where theoretical profile predicted a barrier while MM profile had a global minimum. The MM torsional energy profile for CS-NO (dashed line in Fig. 2b) reproduced the shape of the theoretical profile but the barrier height was much lower. This indicates that the torsional force constants obtained from Antechamber are not accurate enough to account

Table 1. Newly introduced force constants for the nitroso group of *S*-nitrosothiol[a]

Bonds[b]	K_r[d]	r_{eq}[e]		
S-NC	331.41	1.656		
NC-O	789.90	1.209		
Angles[b]	K_θ[d]	θ_{eq}[e]		
CT-S-NC	65.90	96.4		
S-NC-O	68.40	115.3		
Torsions[c]	no. of paths[f]	$V_n/2$[g]	γ[h]	n[i]
X-CT-S-NC	3	3.63	180.0	1
	3	1.21	180.0	3
CT-S-NC-O	1	0.49	180.0	1
	1	8.18	180.0	2
	1	0.82	180.0	3

[a]Amber-99 force field is based on the following energy function.

$$E_{\text{total}} = \sum_{\text{bonds}} K_r (r - r_{eq})^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_{eq})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] + \sum_{i<j} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\varepsilon R_{ij}} \right]$$

[b]Values calculated according to the generalized AMBER force field. [c]Newly parametrized in this work. [d]Force constant for bonds in kcal/mol·Å^2 or for angles in kcal/mol·rad^2. [e]Equilibrium distance in Å or equilibrium angle in degrees. [f]Number of bond paths that the total $V_n/2$ must be divided by. CT is bonded to two H's and one C so that the number of bond paths is equal to 3. [g]Torsional potential in kcal/mol. [h]Phase offset in degrees. [i]Periodicity of the torsion.

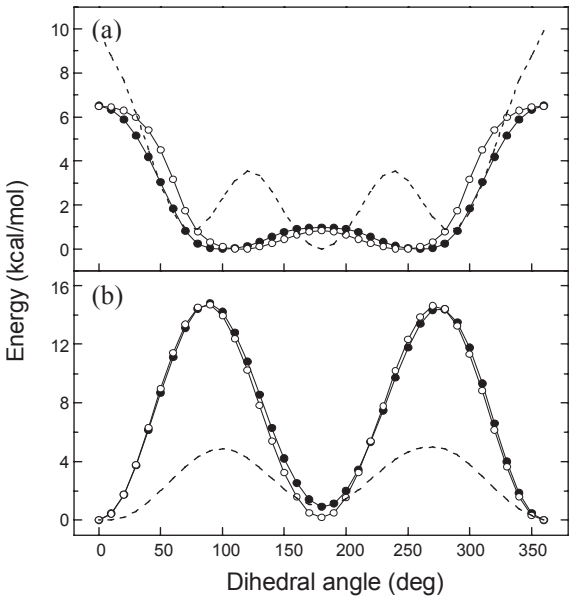

Figure 2. Quantum mechanical (closed circles) and molecular mechanical (open circles) energy profiles of *S*-nitrosoethanethiol as a function of the dihedral angle (a) CC-SN and (b) CS-NO. QM energies were calculated at the level of B3LYP/cc-pVTZ//HF/6-31G* and MM energies were calculated with the force constants given in Table 1. Dashed lines are MM energies calculated with GAFF parameters from Antechamber.

for the QM torsional energy profiles. We therefore varied the torsional force constants and calculated the MM torsional energy profiles until the latter fitted the QM torsional energy profiles. Parameters for the best fit are presented in Table 1. As shown in Fig. 2, the MM energies (open circles) agreed very well with the QM energies (closed circles) for both CC-SN and CS-NO dihedrals.

Transferrability of the torsional parameters of EtSNO to CysSNO. In this study we calculated QM torsional energies at the level of B3LYP/cc-pVTZ//HF/6-31G* which was higher than the level used in the previous study. As shown in Fig. 3a and 3b (closed circles), QM energies of the CC-SN dihedral in CysSNO showed a complicated shape due to a low symmetry in its molecular structure. In addition the torsional energy profile for the α-conformer was quite different from that for the β-conformer. Theoretical values for the dihedral angles between 330 and 370 degrees for the β-conformer (Fig. 3b) could not be calculated because the SNO moiety is so close to the amide backbone that the terminal O atom is transferred to a backbone atom in QM optimized structures.

By using the same force constants that fitted the QM torsional energy profiles of EtSNO, we could reproduce reasonably well the CC-SN torsional energy profiles for CysSNO in terms of heights of the barriers and the positions of the extrema. In particular the global minimum at 90 degrees for the α-conformer and 260 degrees for the β-conformer in the QM torsional energy profiles were accurately predicted by the MM energies. Interestingly the discontinuity at 310 degrees in the CC-SN torsional energy profile of the β-conformer (Fig. 3b, closed circles) was also evident at 300 degrees by the MM calculations (Fig. 3b, open circles).

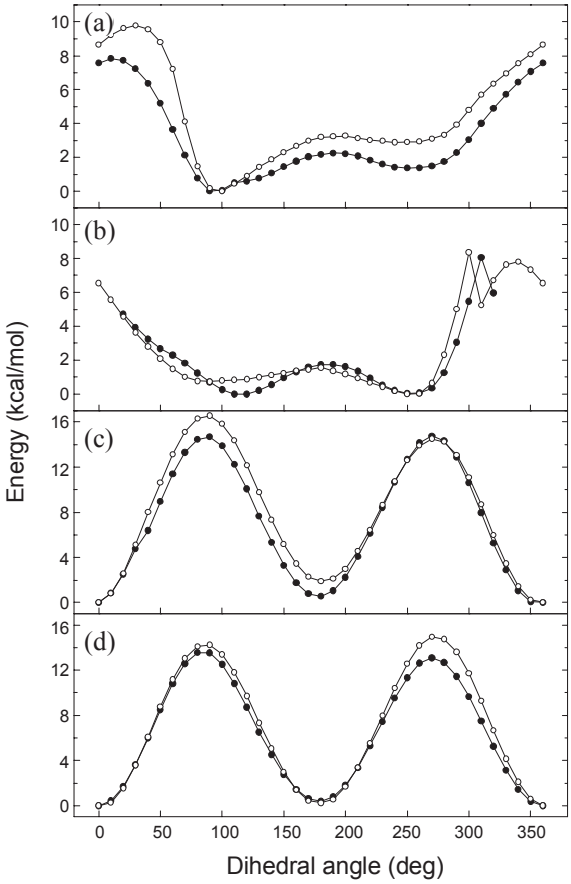

Figure 3. Quantum mechanical (closed) and molecular mechanical (open) torsional energy profiles of the dihedral angle CC-SN (a,b) and CS-NO (c,d) for α (a,c) and β-conformer (b,d) of the tripeptide ACE-CysSNO-NME. As in Fig. 2, QM energies were calculated at the level of B3LYP/cc-pVTZ//HF/6-31G* and MM energies were calculated with the force constants given in Table 1.

The force field parameters for EtSNO reproduced the theoretical CS-NO torsional profiles of CysSNO quite accurately. As shown in Fig. 3c and 3d, the MM profiles agreed well with the QM profiles for both α and β-conformer although the energy of the ante-conformation (dihedral angle of 180 degrees) was slightly overestimated for the α-conformer and the shape was slightly skewed for both α and β-conformers.

Validation of the force field parameters. We tested the validity of our newly introduced parameters by comparing the structures obtained from QM geometry optimization and MM energy minimization. Initial values of CC-SN and CS-NO were set respectively to 90 and 0 degrees for the α-conformer and 270 and 0 degrees for the β-conformer in the starting structures of ACE-CysSNO-NME that were used for both QM and MM calculation. These structures corresponded to the minima of the QM torsional energy profiles of the α and β-conformer (see Fig. 3a and 3b). All internal coordinates except ϕ and ψ were allowed to vary during QM and MM energy minimization in vacuo. As shown in Fig. 4, the QM optimized structures of the α and β-conformers agreed reasonably with the structures obtained from the MM energy minimization. The root-mean-square deviation was 0.191 and 0.137 Å for the α and β-conformer, respectively. This proves that the force field parameters

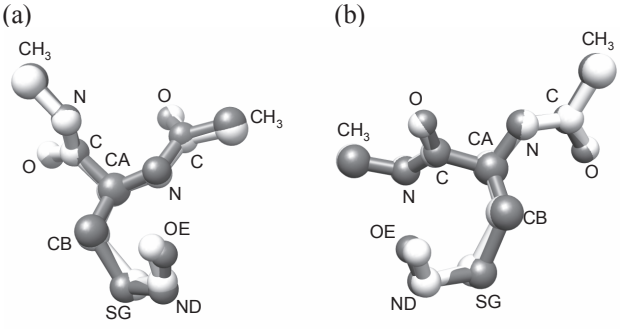

Figure 4. Comparison of the structures obtained from quantum mechanical geometry optimizations (dark) and molecular mechanical energy minimizations (light) of capped tripeptides ACE-CysSNO-NME in (a) α and (b) β-conformation. Only heavy atoms are shown for clarity.

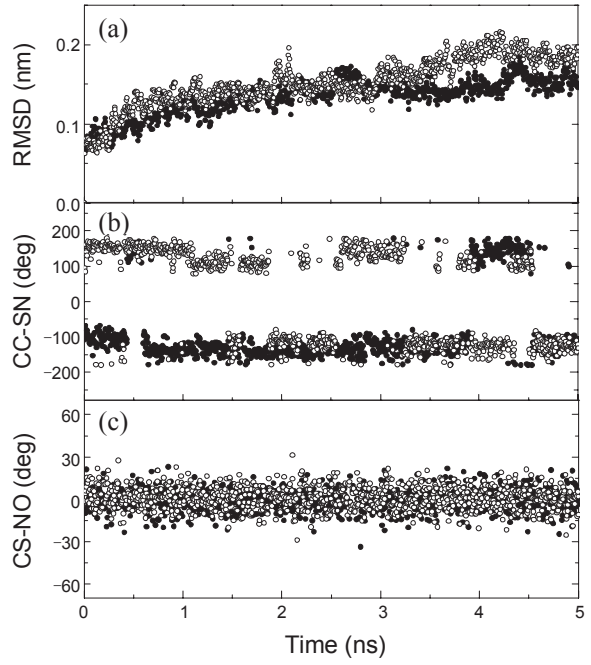

Figure 5. Trajectory of (a) root-mean-square deviation of the alpha carbon, (b) the CC-SN dihedral angle, and (c) the CS-NO dihedral angle during a 5 ns molecular dynamics simulation of myoglobin with ^{10}CysSNO (2nrm.pdb). Initial CC-SN dihedral was −88 (closed) or 124 degrees (open).

developed here are appropriate for MD simulation of a protein containing CysSNO.

Molecular dynamics simulation of *S*-nitrosated myoglobin. Structures were extracted from 2nrm.pdb, a crystal structure of *S*-nitrosated blackfin tuna myoglobin (Mb). For the sake of computational efficiency, Fe was removed from the heme and the proximal His89, distal His60, and the heme were frozen during a production MD simulation. In fact Cys10, the site of *S*-nitrosation, lies far from the heme so that the freezing itself is not expected to affect the conformations of ^{10}CysSNO. To account for the two conformations of ^{10}CysSNO,[4] we performed MD simulations of Mb with ^{10}CysSNO whose initial CC-SN dihedrals are −88 and 124 degrees. As shown in Fig. 5a, the root-mean-square deviations of the the α-carbons produced well-behaved trajectories for both conformations, demonstrating that our parameters are appropriate for MD simulations of *S*-nitrosated proteins. The CC-SN dihedrals showed occasional jumps to the other conformation regardless of the initial conformation (Fig. 5b). This is not unexpected considering the shallow barriers between the two conformations (see Fig. 3a and 3b). The CS-NO dihedral stayed close to syn-conformation although it fluctuated by as much as ± 30 degrees with an average of 0 degrees and standard deviation of 8 degrees (Fig. 5c). Barriers between the syn and anti conformations are so high (Fig. 3c and 3d) that crossing over to the other side is prohibited.

Conclusions

We developed AMBER-99 compatible force field parameters for *S*-nitrosoethanethiol that can be directly transferred to *S*-nitrosocysteine. Molecular mechanical energy minimization using these parameters reproduced the quantum mechanically optimized structures of the capped tripeptide ACE-CysSNO-NME in both α and β-conformation. The parameters were also suitable for molecular dynamics simulations of *S*-nitrosated myoglobin.

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