Parametrization of Synthetic Amino Acids

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Received: April 30, 2003; In Final Form: September 25, 2003

C(α)−C(α)dialkylglycines, sarcosine, *O*-methyltyrosine and *β*-(imidazol-1-yl)-alanine are noncoded amino acids with a large pharmaceutical potential. We have developed a set of parameters for these amino acids, consistent with the AMBER force field. Several dipeptide and tripeptide models were built to simulate the different possibilities for insertion of the noncoded amino acids in a peptide backbone. Bonding parameters were obtained from both existing force fields and quantum calculations. The Coulombic parameters have been determined using a multiconformational weighted approach and a restricted electrostatic potential fitting, at a 6-31G* ab initio level. Molecular dynamics simulations have been carried out on the model peptides to validate the parameters obtained. The characteristic geometry features such as the planarity of peptide bonds have been conserved on these models. The peptide models, which included monosubstituted residues, have revealed considerable backbone flexibility. The conformational flexibility of the peptides containing disubstituted residues has been significantly restricted by the length and volume of their side chains.

Introduction

Research on bioactive peptides is a growing field of bioorganic chemistry, as they play an important role in living systems.[1] Many illnesses are related to excess production of some bioactive peptides.[2] In many cases, the administration of appropriate antagonists can prevent the undesired action of the biomolecules produced in excess, by blocking the respective receptors.[1] On the other hand, the deficient secretion of many peptides may also cause illnesses.[3] One possible therapy currently in use for this type of illnesses is the administration of their agonists usually produced by synthetic means.[1]

One of the most important and higher commercial value field in peptide chemistry concerns the design of new synthetic peptide agonists and antagonists to be used as drugs.[4] Peptide drugs cannot be administrated orally, as they are decomposed by digestive enzymes.[5] Consequently, the patients must be injected, which is highly undesirable, particularly in chronic illnesses.[5] A recent approach to circumvent this problem is to create peptomimetics that present affinity to living substrates but are poorly recognized by digestive enzymes.[1,6] These drugs have high commercial value once they are suitable for oral administration and can be obtained by strategic substitution of one or more natural amino acids by noncoded synthetic amino acids.[6] Some of us have been involved in the development and synthesis of this type of amino acids, such as C(α)−C(α)-dialkilglycines.[7−9]

Mutations of coded amino acids by noncoded ones can create peptomimetic drugs with high pharmaceutical potential.[10] A further insight into biological properties of peptomimetic drugs can be provided by computer-aided molecular modeling and simulation techniques.[11−18] The main limitation to this approach is that the well-established biomolecular force fields such as CHARMM,[19,20] AMBER,[21−23] and GROMOS[24] do not have parameters for noncoded amino acids. This work reports on the parametrization and validation of a set of parameters, committed to the AMBER force field, for a series of noncoded amino acids.

Parameter Development

The main purpose of this work is to develop and validate a set of appropriate parameters suitable for the AMBER force field, for a series of noncoded amino acids. Therefore, the well-established functional form of this force field has been adopted.[23,25]

The noncoded amino acids which have been parametrized are presented in Figure 1. These synthetic amino acids are intended to be used in substitution of natural amino acids, both in terminal and nonterminal positions, with the objective of producing peptomimetic drugs. With this purpose in mind, appropriate two and three peptide models have been modeled to simulate the different possibilities for noncoded amino acid insertion in a peptide backbone (N-terminal, nonterminal, and C-terminal forms have been considered). The general form of the peptide models is presented in Figure 2.

The new parameters are intended to contribute as an extension of the AMBER force field parameters.[23,25,26] Therefore, the atom types have been adopted according to this force field convention.

Only one new atom type was introduced (NM, nitrogen), with VDW parameters from nitrogen atoms in AMBER.

Atomic charges have been obtained considering the conformational flexibility of the synthetic amino acid side chains. For this purpose, a systematic conformational analysis has been performed to search for the conformational minima on each model (N-terminal, nonterminal, and C-terminal models). The conformational space has been defined by fixing the dihedral

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10.1021/jp030544y CCC: $25.00
Published on Web 11/27/2003

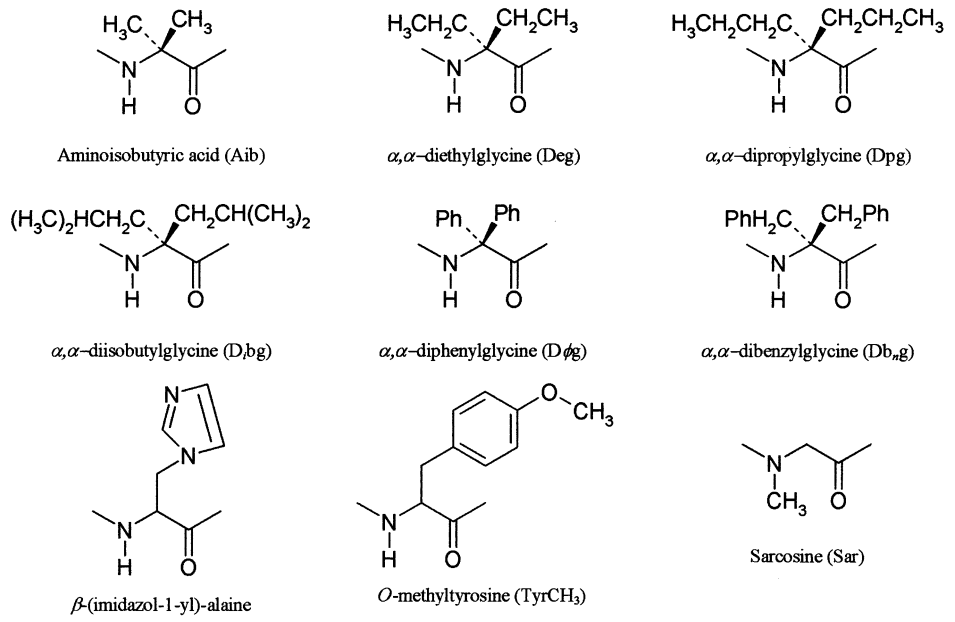

Figure 1. Noncoded amino acids parametrized in this work.

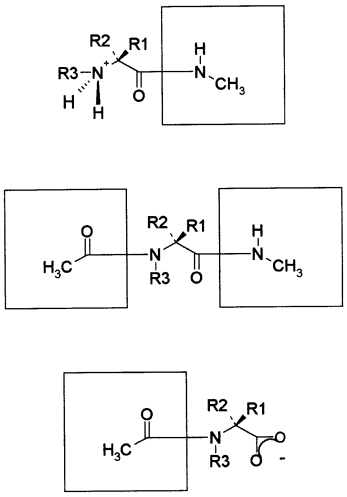

Figure 2. General form of the models used to simulate the different possibilities for insertion of a noncoded amino acid, on a peptide backbone, during the quantum mechanics calculations.

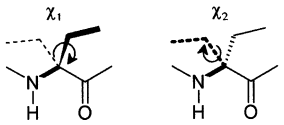

Figure 3. Schematic representation of geometrical parameters used to define the conformational space in the models of C(α),C(α)-dialkylglycines.

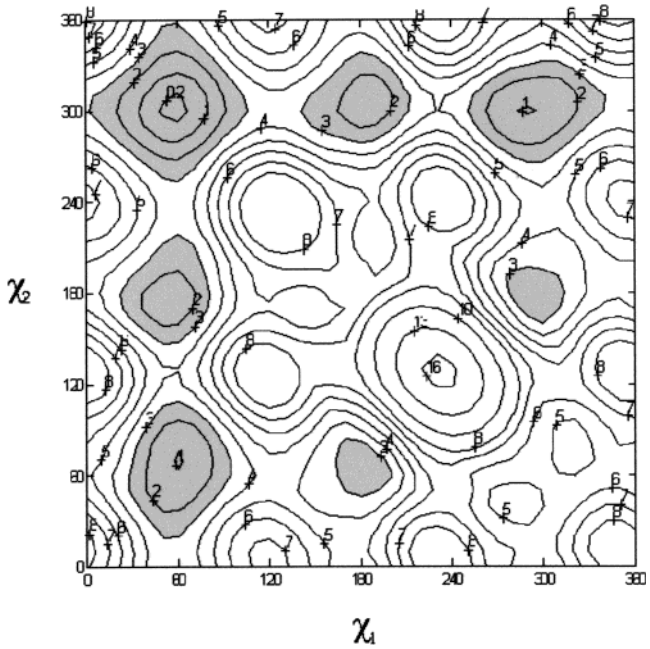

Figure 4. Conformational map, obtained for the Deg amino acid in C-terminal position. Values of the dihedral angles χ_1 and χ_2 are presented in degrees and the relative energy values are presented in kcal mol^{-1} (shaded areas are within 3 kcal mol^{-1} of the global minimum).

angles χ_1 and χ_2 and optimizing all the other parameters (see Figure 3). The starting points of the analysis were the conformations with $\chi_1 = 180°$ and $\chi_2 = 180°$. The used amplitude for the χ_1 and χ_2 dihedrals was from 0° to 360°. Each conformation was obtained from the previous optimized one, with increments of ±10° for the dihedral angles. The conformational maps generated with this procedure have 1369 points for each parametrized disubstituted glycine residue. For the other parametrized amino acids, the minima search was simpler as they only have one (or no) side chains. All calculations have been performed at the AM1 semiempirical level.[27] The conformational minima obtained have been further optimized, at the Hartree−Fock/6-31G(d) level.[28] This particular combination of quantum mechanical level and basis set has been chosen in agreement with the parametrization followed in AMBER, to ensure an equilibrium between the newly parametrized noncoded residues and the already existing coded ones.[23] All the calculations have been carried out with the Gaussian 98 package[29] on an Alpha Digital Personal Work Station 600au.

An electrostatic potential grid has been generated for each conformational minimum. The atomic charges have been calculated combining a restrained electrostatic potential (RESP) fitting with a multiconformational approach,[30−32] using normalized Boltzman populations as weight factors for each minimum.

Most bonding parameters are directly available in the AMBER force field. The missing parameters have been obtained from quantum calculations and other similar force fields.[20,24]

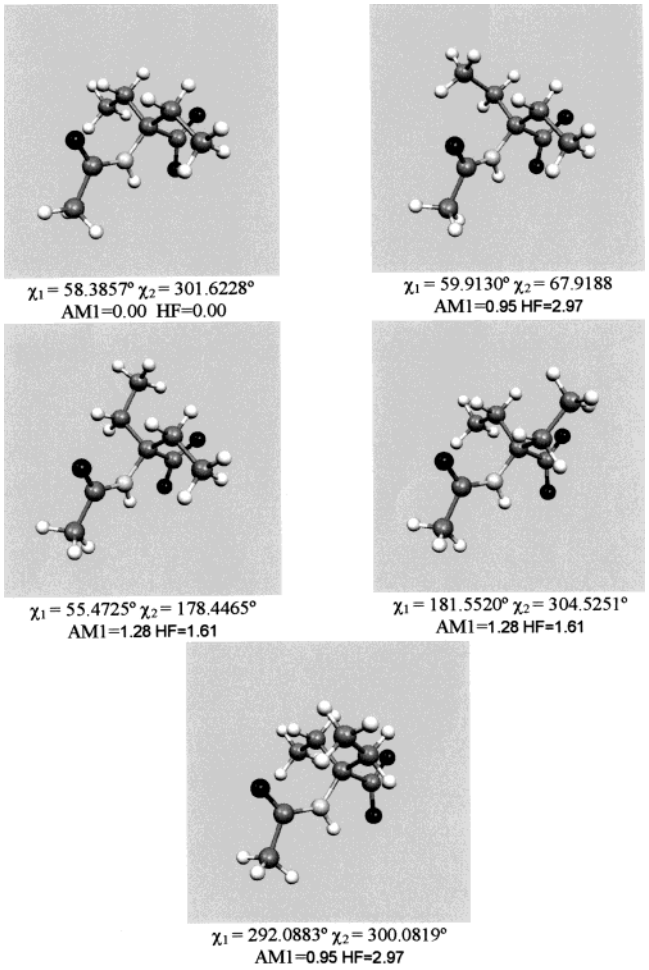

Figure 5. Structures of the optimized stored minima for the Deg residue in C-terminal position. Both values of calculated relative energies (AM1 and HF/6-31G*) are shown in kcal mol^{-1}.

The quantum-derived parameters have been fitted to the AMBER force field functional form. In particular, two dihedral angles parameters, not found in the AMBER force field, have been parametrized using Hartree−Fock/6-31G(d) calculations[28] with the Gaussian 98 program.[29] These diedrals potentials were fitted to the theoretical energy profile, to have a good agreement between the AMBER and the theoretical calculated amplitudes and periodicities.

Molecular Dynamics

The behavior of the peptides in aqueous solution was simulated to validate the force field parameters. Several peptide models were created (di- and tripeptides), each having one synthetic amino acid bonded to glycines, simulating the three possible insertions in a peptide backbone (Gly−XXX−Gly sequence for nonterminal position, Gly−XXX for C-terminal position, or XXX−Gly for N-terminal positon, XXX is the synthetic amino acid). Each initial structure was minimized until the root-mean-square energy gradients were lower or equal to 0.001 kcal mol^{-1}, using 1000 steps of steepest descent and performing the remaining steps by the conjugate gradients method.

All molecular dynamics of the peptides in aqueous solutions were performed using periodic boundary conditions. Each peptide was placed in a near 30 Å cubic box, and overlapping TIP3P water molecules[33] were deleted if the distance between one of their atoms and any of the solute atoms was less then the sum of the respective VDW radii. A total number of between 1213 and 1787 water molecules were included in the simulations. All simulated systems were initially equilibrated at 300 K for 50 ps in a canonical (NVT) ensemble, using the Berendsen temperature coupling method[34] with a coupling constant of 1.0 ps. Production simulations were then performed at 300 K and 1 atm in the NPT ensemble using the Berendsen thermostat and barostat[34] with coupling constants of 1 and 0.2 ps (pressure relaxation time), respectively.

All molecular simulations were performed using periodic boundary conditions. The integration of the equations of motion was carried out using the Verlet leapfrog algorithm,[35] with a time step of 1 fs, and SHAKE[36] constrains were applied to all bonds between hydrogen and heavy atoms. The particle mesh Ewald (PME)[37,38] method was used to calculate the Lennard-Jones and electrostatic interactions, with a cutoff distance of 12 Å. Instant values of thermodynamic and structural quantities were stored every 50 steps (0.050 ps) for analysis. All calculations were carried out in a Pentium IV computer, with the Amber 6 package.[39]

TABLE 1: Bonding Parameters Added to the Amber Force Field

bonds	source	K_r (kcal mol^{-1} Å^{-2})[a]	r_{eq} (Å)[b]
C−OS	CHARMM	365.0	1.38
NM−CR	G98/AMBER	410.0	1.40
CV−NM	G98/AMBER	410.0	1.40
CV−CV	G98/AMBER	512.0	1.41
CT−NM	G98/AMBER	337.0	1.427

angles		K_θ (kcal mol^{-1} rad^{-2})[c]	θ_{eq} (deg)[d]
C−OS−CT	CHARMM	61.0	108.0
CA−C−OS	CHARMM	55.0	120.0
H4−CV−CV	G98/CHARMM	23.0	129.44
NM−CV−CV	G98/CHARMM	65.0	106.1
NM−CV−H4	G98/CHARMM	25.0	122.4
NM−CR−H5	G98/CHARMM	25.0	122.6
NM−CR−NB	G98/CHARMM	100.0	111.4
CV−NM−CR	G98/CHARMM	70.0	106.5
CV−CV−NB	G98/CHARMM	65.0	110.0
H1−CT−NM	G98/AMBER	50.0	109.2
H1−CT−N3	AMBER	50.0	109.5
CT−NM−CV	G98/CHARMM	70.0	127.0
CT−NM−CR	G98/CHARMM	70.0	126.4
CT−CT−NM	G98/CHARMM	46.5	114.5
CA−CT−C	CHARMM	70.0	109.47
CA−CT−CA	CHARMM	70.0	109.47
N−CT−CA	CHARMM	70.0	111.6

dihedrals		no. of paths[e]	$V_n/2$ (kcal mol^{-1})[f]	γ (deg)[g]	n[h]
X−C−OS−X	G98	2	0.850	180.0	2
X−NM−CV−X	AMBER	4	6.00	180.00	2
X−CT−NM−X	G98	6	1.725	0.00	2
X−NM−CR−X	AMBER	4	9.30	180.0	2
X−CV−CV−X	AMBER	4	14.50	10.00	2

[a] Bond length force constant. [b] Equilibrium bond length. [c] Bond angle force constant. [d] Equilibrium bond angle. [e] Factor by which torsion barrier $V_n/2$ is divided. [f] One-half of the torsion barrier magnitude. [g] Phase. [h] Periodicity. When G98/force field is presented in the source column, this means that a comparison between G98 results and the existing parameters was performed to choose the best existing force constant.

Results

Typical results of the conformational analysis of the peptide models, obtained for the Deg residue in a C-terminal position at the AM1 level, are presented as an example in Figure 4. All

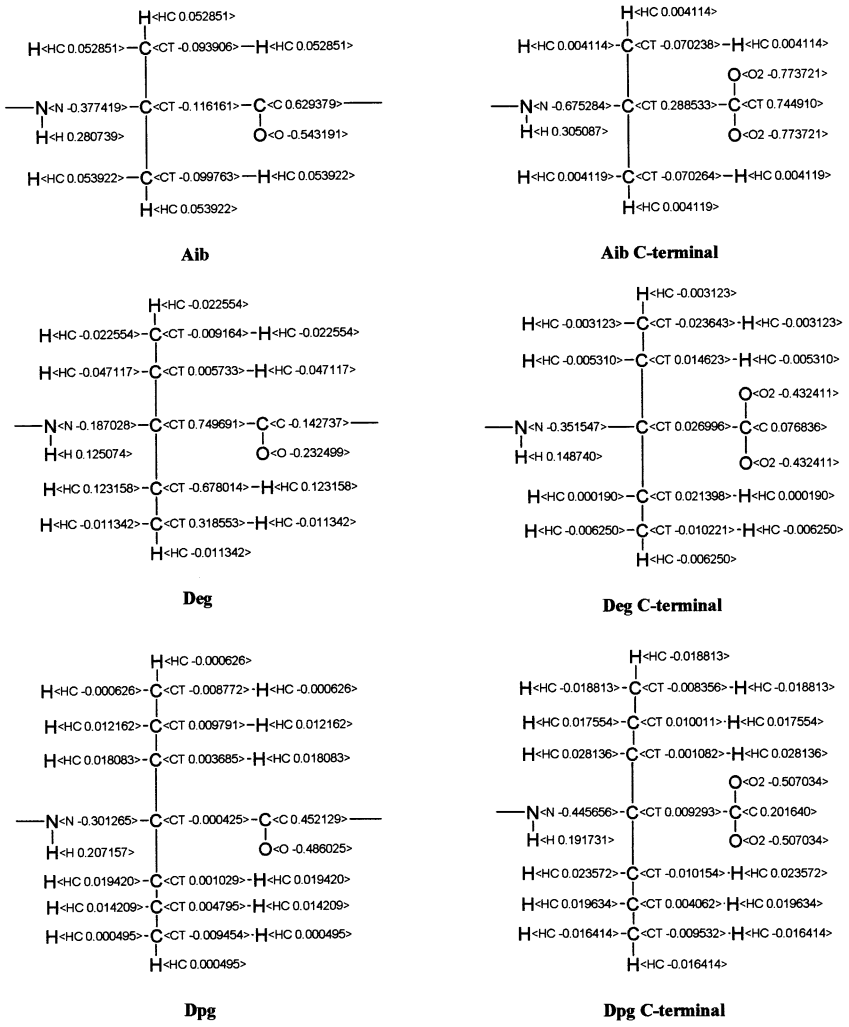

Figure 6. Atom types and atomic charges for the Aib, Deg, and Dpg, both in nonterminal and in C-terminal positions.

the local minima obtained within a window of 3 kcal mol^{-1} relative to the global minimum have been stored for posterior analysis.

For each peptide model, all stored minima have been further fully optimized at the Hartree−Fock/6-31G(d) level. The results obtained with the semiempirical and the ab initio calculations are in good agreement. In fact, geometries and relative energies are essentially conserved. The optimized minima obtained for the Deg amino acid in a C-terminal position are presented in Figure 5.

For each conformational minimum, atomic charges have been initially fitted to the restrained electrostatic potential at a grid of points generated according to the Merz−Kollman method.[40] The charges have been obtained using Hartree−Fock/6-31G-(d) calculations because these types of Coulombic parameters are appropriate to describe interactions with empirical TIP3 water models with implicit polarization effects included.[30] The final charges have been obtained using a multiconformational approach, using normalized Boltzman populations as weight factors for each conformational minimum obtained at ab initio level. The obtained results are shown in Figures 6−8.

The set of new parameters developed within the Amber force field are presented in Table 1. The dihedral parameters X−C−OS−X and X−CT−NM−X have been derived from Hartree−Fock/6-31G(d) calculations using a euristic fitting to the AMBER force field functional form (Figures 9 and 10).

The molecular dynamics simulations carried out for the peptide models in aqueous solution have revealed that the developed parameters are stable. All the systems have equilibrated very quickly (around 10 ps) as can be seen in Figure 11. Furthermore, characteristic geometric features such as the planarity of the peptide bonds have been very well preserved during the simulations (see Figure 12 as an example). For each model, values of the dihedral angles ϕ and ψ have been monitored as a function of the simulation time. Considerable flexibility associated with the occurrence of conformational transitions has been observed in the models, which included dialkylglycines with the shorter side chains Aib, and in Dϕg (this last one only used in a C-terminal position; see Figure 13). In the peptide models which included this type of disubstituted residues with large side chains, the values of the mentioned dihedral angles have been essentially preserved at 180° during the whole simulation (see Figure 14 as an example). Monosubstituted amino acids have revealed a considerable flexibility regarding these backbone parameters (see Figure 15 as an example).

Final Remarks

We are interested in the modeling, simulation, and syntheses of new biomimetic peptides with noncoded amino acids as potential drugs. However, the well-established force fields do not include all the parameters for these synthetic residues.

In this work, we have presented the development of appropriate parameters, consistent with the AMBER force field, for a

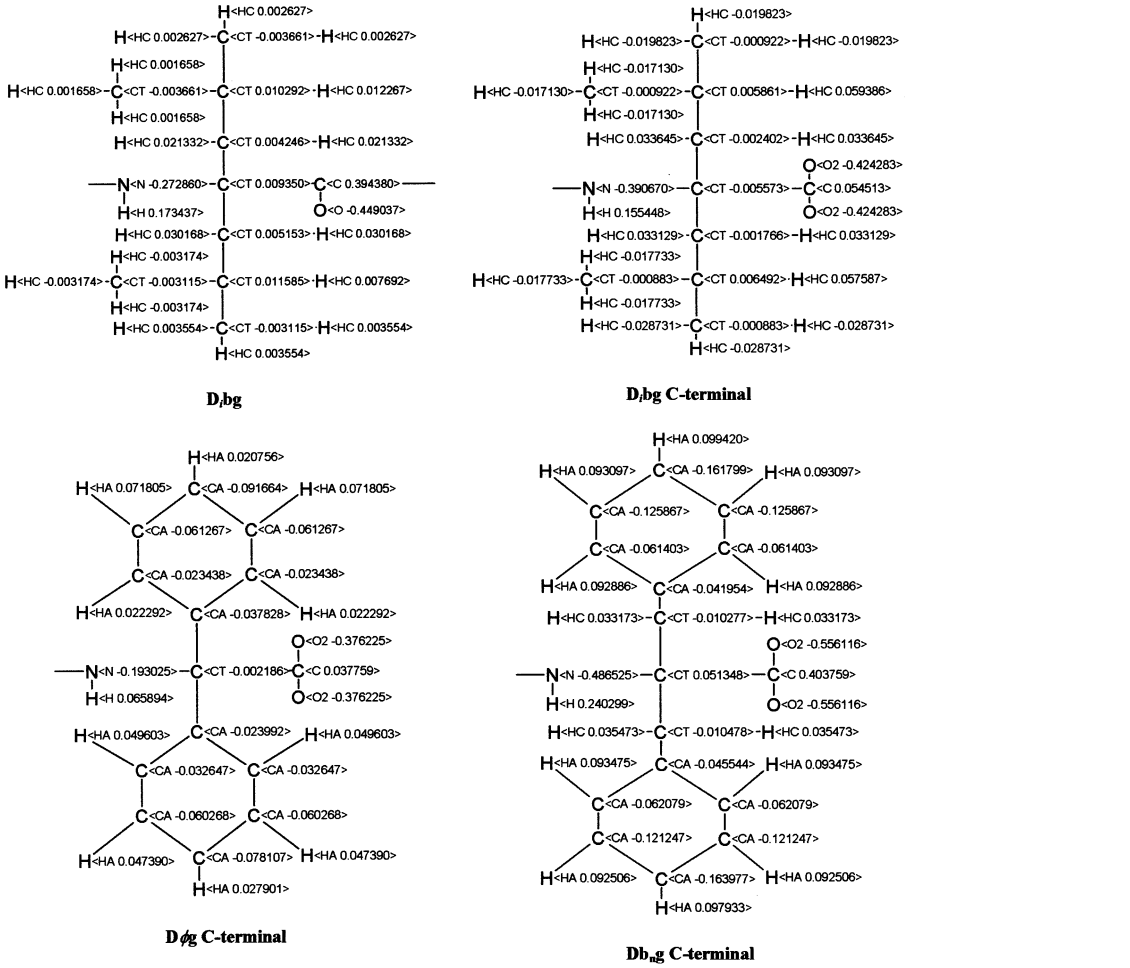

Figure 7. Atom types and atomic charges for the D$_i$bg (in both nonterminal and in C-terminal position), Dϕg, and Db$_n$g (both in C-terminal position).

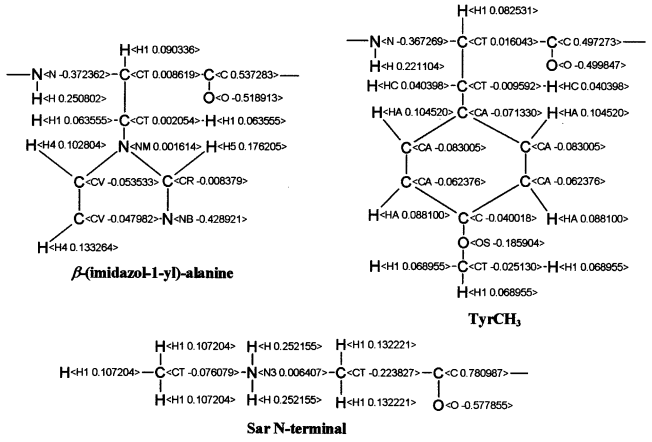

Figure 8. Atom types and atomic charges fitted to the β-(imidazol-1-yl)-alanine and TyrCH$_3$, in nonterminal positions. The Sar residue, in a N-terminal position is also shown. Note that in the β-(imidazol-1-yl)-alanine residue, the nitrogen atom of the imidazol ring that connects to the C(β), has a new atom type; this was done just to differentiate it from a common NA atom type.

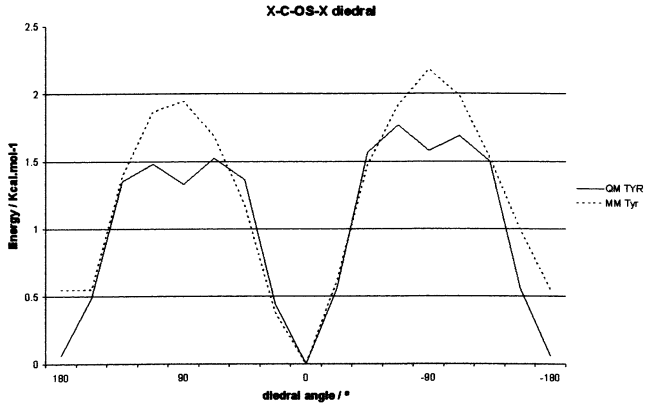

Figure 9. Comparison between the QM (HF/6-31G*) energy profile and the MM (AMBER) obtained with the new dihedral parameters for the X−C−OS−X dihedral.

set of noncoded amino acids. For this purpose, appropriate peptide models have been used. The new parameters have been obtained by fitting the results of Hartree−Fock calculations to the AMBER force field empirical energy function. Strong emphasis has been placed in the parametrization of ab initio derived point charges, because they are essential to an accurate representation of the electrostatic interactions. A multiconformational weighted approach has been used to reduce the conformational dependence of RESP charges. Molecular dynamics, carried out on the peptide models, have demonstrated the stability of the developed parameters.

The preservation of an active conformation could confer specificity to a bioactive peptide relative to a receptor. On the other hand, conformational flexibility can be eventually important to accommodate the nonspecific parts of a bioactive molecule. Our molecular dynamics simulations have strongly suggested that C(α)−C(α)dialkylglycines with large side chains are appropriate to restrict the flexibility of the peptide backbone,

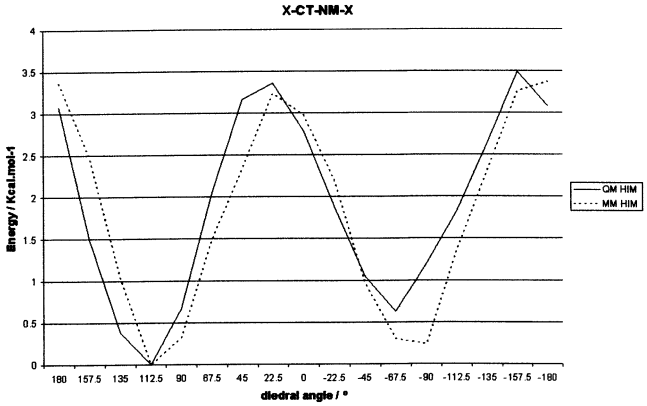

Figure 10. Comparison between the QM (HF/6-31G*) energy profile and the MM (AMBER) obtained with the new dihedral parameters for the X−CT−NM−X dihedral.

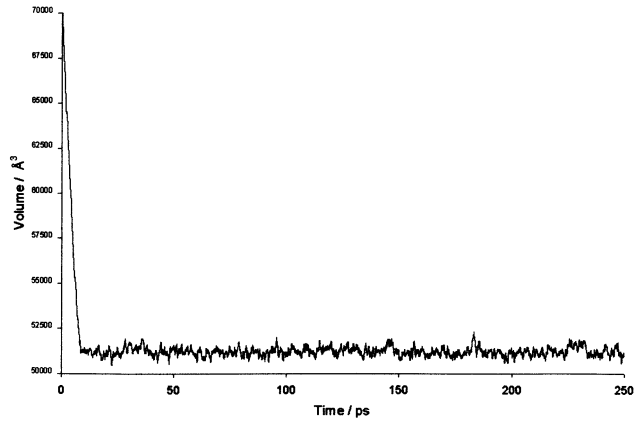

Figure 11. Evolution of the system volume as a function of time. This plot is from the simulation of the Gly−Dpg−Gly peptide.

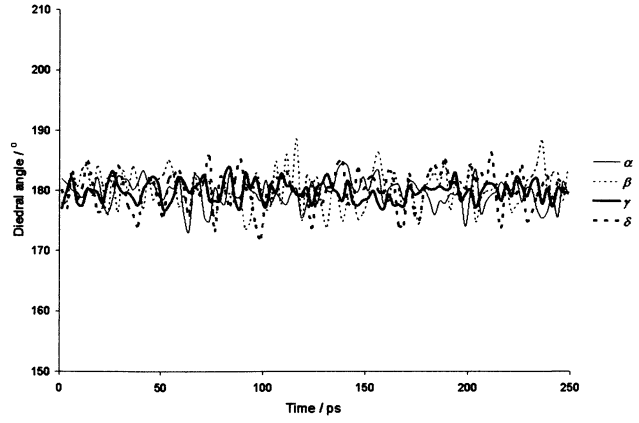

Figure 12. Behavior of peptide bond dihedrals during the simulation run. The shown data was averaged in 50 fs windows. α is the ω_1 dihedral angle of the Aib residue in the Gly−Aib−Gly peptide; β is the ω_2 dihedral angle of the Aib residue in the Gly−Aib−Gly peptide; γ is the ω_1 dihedral angle of the $D_i$bg residue in the Gly−$D_i$bg−Gly peptide; δ is the ω_2 dihedral angle of the $D_i$bg residue in the Gly−D_i-bg−Gly peptide.

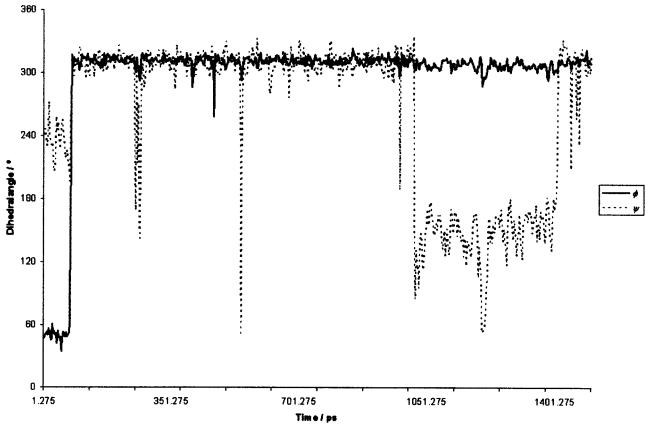

Figure 13. Behavior of the ϕ and ψ dihedral angles of the Aib amino acid, during the molecular dynamics simulation of the Gly−Aib−Gly peptide. The presented data was averaged in 50 fs windows.

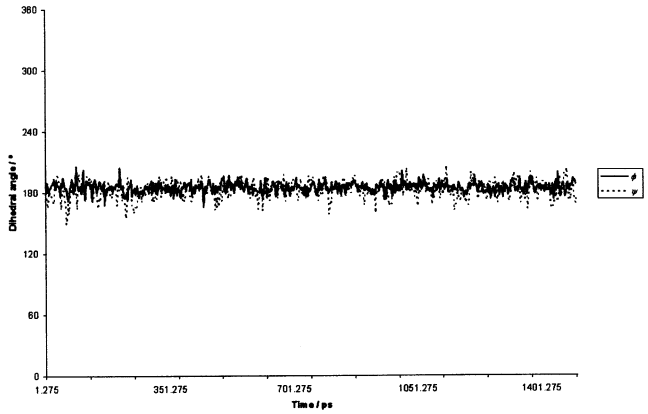

Figure 14. Behavior of the ϕ and ψ dihedral angles of the Deg amino acid, during the molecular dynamics simulation of the Gly−Deg−Gly peptide. The presented data was averaged in 50 fs windows.

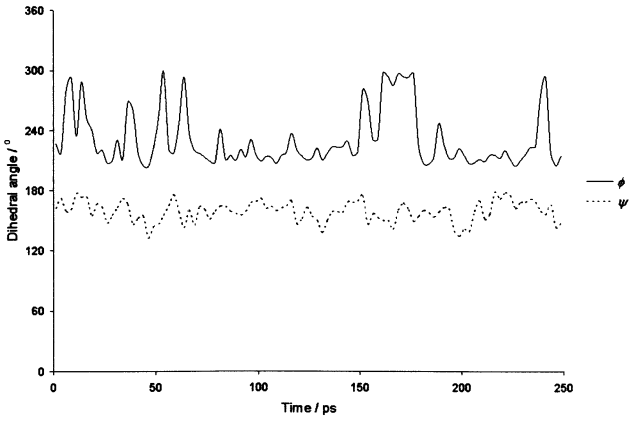

Figure 15. Behavior of the ϕ and ψ dihedral angles of the *O*-methyltyrosine amino acid, during the molecular dynamics simulation of the Gly−TyrCH_3–Gly peptide. The presented data was averaged in 50 fs windows.

while C(α)−C(α)dialkylglycines with very short side chains and monosubstituted residues have the opposite effect (they possess greater flexibility). As a result, the insertion of the first type of amino acids in the active region is important to confer specificity to a mimetic peptide, since they are capable of retaining a more restricted conformation. On the other hand, the insertion of the latter type residue is important to retain the flexibility of nonspecific regions, which will allow the accommodation of the peptomimetic molecule in the receptor.

Acknowledgment. Financial support from Fundação para a Ciência e Tecnologia, project Sapiens 35380/99 is gratefully acknowledged.

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