THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

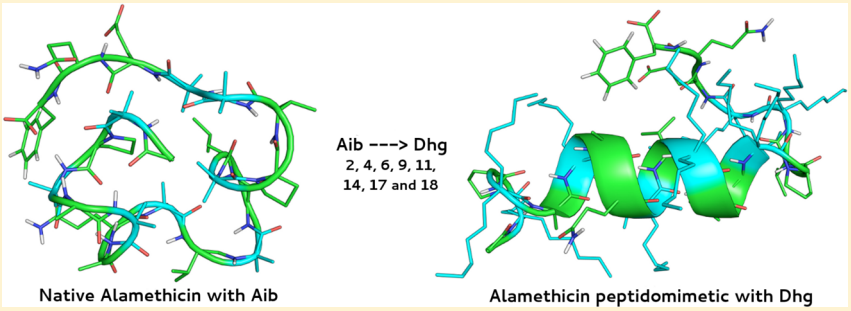
pubs.acs.org/JPCB

Conformational and Thermodynamic Properties of Non-Canonical α,α-Dialkyl Glycines in the Peptaibol Alamethicin: Molecular Dynamics Studies

Tarsila G. Castro and Nuno M. Micaêlo*

Departamento de Química, Escola de Ciências, Universidade do Minho, Largo do Paço, Braga 4704-553, Portugal

Supporting Information



ABSTRACT: In this work, we investigate the structure, dynamic and thermodynamic properties of noncanonical disubstituted amino acids (α,α-dialkyl glycines), also known as non-natural amino acids, in the peptaibol Alamethicin. The amino acids under study are Aib (α-amino isobutyric acid or α-methyl alanine), Deg (α,α-diethyl glycine), Dpg (α,α-dipropyl glycine), Dibg (α,α-di-isobutyl glycine), Dhg (α,α-dihexyl glycine), DΦg (α,α-diphenyl glycine), Db_2g (α,α-dibenzyl glycine), Ac_6c (α,α-cyclohexyl glycine), and Dmg (α,α-dihydroxymethyl glycine). It is hypothesized that these amino acids are able to induce well-defined secondary structure in peptidomimetics. To test this hypothesis, new peptidomimetics of Alamethicin were constructed by replacing the native Aib positions of Alamethicin by one or more new α,α-dialkyl glycines. Dhg and Ac_6c demonstrated the capacity to induce well-defined α-helical structures. Dhg and Ac_6c also promote the thermodynamic stabilization of these peptides in a POPC model membrane and are better alternatives to the Aib in Alamethicin. These noncanonical amino acids also improved secondary structure properties, revealing preorganization in water and maintenance of α helical structure in POPC. We show that it is possible to optimize the helicity and thermodynamic properties of native Alamethicin, and we suggest that these amino acids could be incorporated in other peptides with similar structural effect.

INTRODUCTION

α,α-Dialkyl glycines are noncanonical amino acids where the Cα is substituted with two alkyl side chains. This substitution can be symmetrical or not. It is proposed that the double substitution at the Cα of α,α-dialkyl glycines induces a more constrained conformation of the φ and ψ main-chain dihedral angle pair. Consequently, these amino acids should explore a more restrictive range of dihedral angles of the Ramachandran space corresponding to the helical secondary structure conformation, observed for the natural amino acids encoded by DNA.[1] Such structural arrangement, combined with the steric hindrance caused by the presence of the second alkyl group attached to Cα, leads to the formation of constrained peptides. These properties can be very important to evaluate the foldamer potential—capability to induce always the same secondary structure, independent of the amino acids sequence or the solvent used—of these amino acids in the modeling of peptides with a particular secondary structure.[2]

In fact, noncanonical amino acids already have a relevant role on the conformation and design of peptidomimetics with biomedical applications.[3−6] It is also shown that the incorporation of noncanonical amino acids is capable to induce specific types of secondary structure in peptides with a significant increase on the bioavailability and stability in physiological conditions.[7−12] This type of amino acids may occur naturally in some peptides but are not encoded by DNA. Known natural examples of this class of amino acids are Aib (α-amino isobutyric acid) and IVA (isovaline or isovaleric acid).[2] Aib occurs naturally in peptaibols with antibiotic activity such as Alamethicin, Zervamicin, and Antiamoebin I.[13,14] In these three peptides, this amino acid is responsible for the formation of α-helical structures. This type of arrangement is essential for their insertion into lipid bilayers of cell membranes and formation of barrel stave type channels.[15−17] In a recent modeling study done by us, we also reported the α-helical preorganization of a small peptide Peptaibolin, as a result of the incorporation of this class of amino acids.[18]

Received: June 6, 2014
Revised: July 29, 2014
Published: August 5, 2014

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

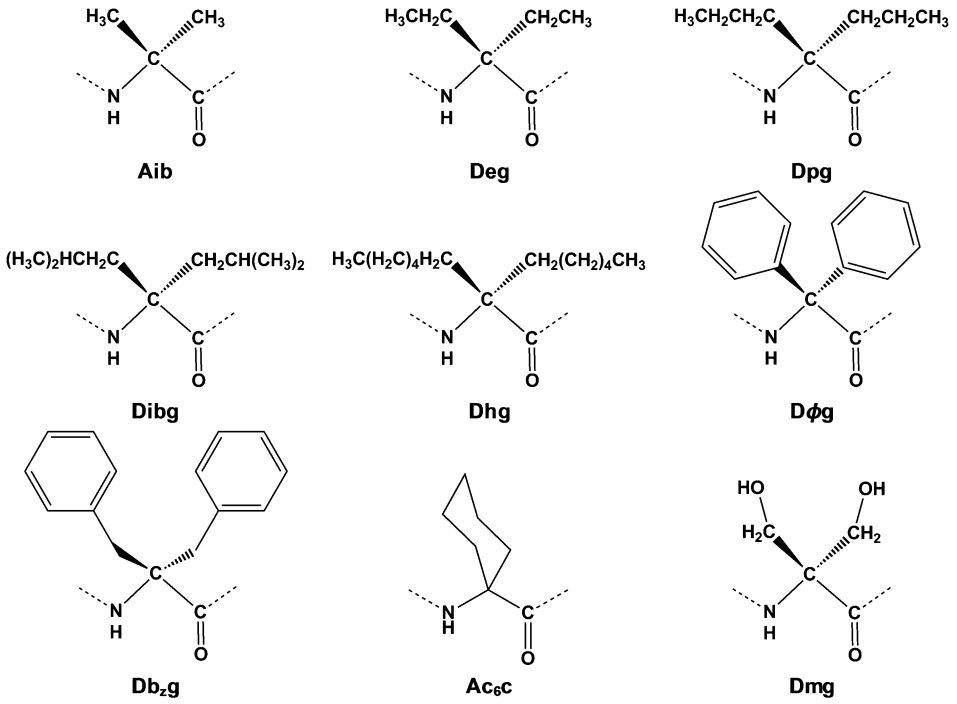


Figure 1. Two-dimensional structure of the α,α-dialkyl glycines studied in this work: α-amino isobutyric acid (Aib), α,α-diethyl glycine (Deg), α,α-dipropyl glycine (Dpg), α,α-di-isobutyl glycine (Dibg), α,α-dihexyl glycine (Dhg), α,α-diphenyl glycine (DΦg), α,α-dibenzyl glycine (Db$_z$g), α,α-cyclohexyl glycine (Ac$_6$c), and α,α-dihydroxymethyl glycine (Dmg).

In this work, we evaluate the structural and thermodynamic effects of replacing Aib by symmetric α,α-dialkyl glycines in Alamethicin, a peptaibol with well-known conformational structure and function, in order to establish if the noncanonical amino acids increase the helicity of novel Alamethicin peptides compared to the native structure. The new α,α-dialkyl glycines studied in this paper are α,α-diethyl glycine (Deg), α,α-dipropyl glycine (Dpg), α,α-di-isobutyl glycine (Dibg), α,α-dihexyl glycine (Dhg), α,α-diphenyl glycine (DΦg), α,α-dibenzyl glycine (Db$_z$g), α,α-cyclohexyl glycine (Ac$_6$c), and α,α-dihydroxymethyl glycine (Dmg). These α,α-dialkyl glycines form a heterogeneous group of amino acids that enable the design of peptidomimetics with different degrees of amphipathicity and structural behavior. This series of peptides includes five nonpolar aliphatic amino acids of different size and volume (Aib, Deg, Dpg, Dibg, Dhg), one cyclic amino acid (Ac$_6$c), two amino acids with aromatic side chains (DΦg and Db$_z$g), and one polar aliphatic amino acid (Dmg).

The incorporation of Aib in peptides has been extensively investigated in the past decades due to its ability to induce α-helix conformation,[19−22] and it has been observed that Aib stabilizes a type II β-turn in small peptides (2−4 residues) and 3_{10}-helix in peptides with 4−6 residues.[23,24] Deg and Dpg induce both fully extended C_5 conformations and helical conformations in crystal structures.[23,24] Dibg has also been synthesized previously,[25] but there are few results about its structure. It is expected that, in the same way as the others amino acids with two or more carbons in each side chain (Deg, Dpg, Db$_z$g and DΦg), Dibg also prefers an extended conformation of C_5 type.[26]

Experimental and theoretical investigations indicate that the noncanonical amino acids DΦg and Db$_z$g induce C_5 and C_7 backbone conformations.[26−29] Previous results about cyclic amino acids such as Ac$_6$c (Ac$_n$c, $n > 3$) suggest that the C$\alpha\leftrightarrow$Cα cyclization constrains the main chain dihedrals, even more than the constraint resulting of the double substitution at the Cα, as in Aib.[30,31]

Our case study, Alamethicin, is a peptaibol with known antimicrobial activity isolated from the fungus *Trichoderma viride*, and its structure was studied by X-ray diffraction by Fox and Richards.[32] It consists of a sequence with 19 residues (Ac-Aib-L-Pro-Aib-L-Ala-Aib-L-Ala-L-Gln-Aib-L-Val-Aib-Gly-L-Leu-Aib-L-Pro-L-Val-Aib-Aib-L-Glu-L-Gln-Phe), including eight Aib residues at positions 1, 3, 5, 8, 10, 13, 16, and 17.[33] This peptide can be an alternative to conventional antibiotics,[34,35] affecting the membrane permeability and leading to cell death due to osmotic shock and leakage of intracellular material.[16,36,37]

Modeling studies done by Tieleman and co-workers employing molecular dynamics simulations, investigated the structural and dynamic properties of Alamethicin in water, methanol, and the phosphatidylcholine bilayer membrane.[38,39] The authors have found substantial loss of structure in aqueous environment, especially at the C-terminal segment of the peptide. Furthermore, the formation of channels was investigated in three studies from Tieleman and co-workers that evaluate the most stable Alamethicin bundles consisting of 4, 5, 6, 7, or 8 helices.[39−41] It was observed in the bilayer and methanol that Alamethicin underwent partial loss of structure about its central Gly-X-X-Pro sequence motif.

Alamethicin has been the most investigated Peptaibol. Before the contributions of Tieleman and co-workers, MD simulations had been employed to analyze this Peptaibol in other solvent environments.[42,43] Modeling studies in methanol and chloroform suggest that the Alamethicin structure is mostly α-helical, but it can present some residues organized in the 3_{10}-helix form, from the tenth residue.[36,43−46] Also, the C-terminal loses its initial helical structure, presenting more flexibility than the N-terminal and the central part of the peptide.[36,38,39,43−46]

Alamethicin channels were predominantly investigated by theoretical approaches.[47,48] Fox and Richards[32] and other

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

researchers[49−53] suggest the formation of a barrel-stave channel with Alamethicin monomers using experimental techniques. Further insights were also obtained through molecular dynamics methods in different membrane models (POPC, DMPC, DOPC, DMPC/DHPC).[40,54−57]

MATERIALS AND METHODS

Noncanonical Amino Acid FF Parameters. The three-dimensional structure of the new noncanonical amino acids (Figure 1) were designed with the program Pymol.[58] The GROMOS topologies (bonded and nonbonded parameters) for each amino acid were transferred from the corresponding natural amino acids parametrized with the GROMOS 54a7 force field (FF).[59,60] (Supporting Information) provides the bonded and nonbonded parameters of each noncanonical amino acid under study using the FF GROMOS 54a7 syntax (Table 1S).

System Preparation. The X-ray structure of Alamethicin used in this study is available in the Protein Data Bank,[61] with the code 1AMT.[32] We created nine Alamethicin peptidomimetics by replacing all eight Aib residues by one of the new α,α-dialkyl glycines (Figure 1) and Ala. These peptidomimetics were named by the acronym of the new α,α-dialkyl glycine that was inserted.

The new Alamethicin peptidomimetics were modeled in water with the simple point charge (SPC) water model,[62] ethanol, and POPC membranes. These three solvents allow the evaluation of the peptidomimetics structure in solvents of different polarities and molecular environments. In water, Alamethicin and its mimetics were simulated in a dodecahedral box considering a hydration layer of at least 1 nm between the peptide and the walls, in all three directions. Thus, the systems have about 3300−3500 water molecules. In ethanol, the systems were modeled in a cubic box, with dimensions of 7 × 7 × 7 (nm) and containing approximately 3300 molecules of ethanol. In both media, the systems were neutralized with the addition of two Na^+ ions.

Peptide simulations in membrane were done using a POPC membrane composed of 128 phospholipids, previously equilibrated with water.[63] Each peptide (Alamethicin and peptidomimetics) was manually inserted in a transmembrane orientation into the equilibrated POPC membrane. It was necessary to remove three phospholipids of each monolayer to minimize collisions with the peptides. This procedure yielded 10 different peptide POPC systems; system 1: the native Alamethicin; system 2: the Alamethicin analog carrying Ala in the native Aib positions; systems 3 to 10: eight Alamethicin mimetics resulting from the insertion of the eight noncanonical α,α-dialkyl glycines.

Molecular Dynamics Simulations. All simulations were performed using the GROMACS 4.0.5 version.[64,65] For the treatment of long-range interactions, we used the Reaction Field method, with a cutoff of 1.4 nm and dielectric constant of 54 for SPC water model[62,66] and 24.3 for ethanol.[67−69] The van der Waals interactions were also truncated with a twin-range cutoff of 0.8 and 1.4 nm. The algorithm LINCS[70,71] was used to constrain the chemical bonds of the peptides and the algorithm SETTLE[72] in the case of water. The pressure and temperature Berendsen algorithms were used to control the temperature and pressure at 300 K and 1 atm, respectively.[73] In water and ethanol, we used a coupling constant of $\tau_T = 0.1$ ps and $\tau_P = 0.5$ ps, respectively, and in POPC these parameters were $\tau_T = 0.2$ ps and $\tau_P = 1.0$ ps.

In all systems (peptide in water, ethanol, and POPC membranes), three steps of energy minimization were performed. In the first two steps of energy minimization, position restraints (with force constant of 1000 $kJ{\cdot}mol^{-1}{\cdot}nm^{-2}$) were applied to all heavy atoms of the peptide and afterward on the main chain. In the third step of energy minimization, no position restraints were applied. Two molecular dynamics simulations of 100 ps were done with position restraints (force constant of 1000 $kJ{\cdot}mol^{-1}{\cdot}nm^{-2}$) on the heavy atoms and afterward on the main chain. The systems were equilibrated and sampled using 100 ns molecular dynamics simulations with an integration interval of 2 fs. To ensure a better sampling of the conformational states of these peptides in water and ethanol, five replicates of each system were done. Conformations were recorded every 1 ps.

Free Energy Calculations. To evaluate the relative free energy cost ($\Delta\Delta G^{1-2}$, see Figure 2) of replacing each Aib position of Alamethicin inserted on a POPC membrane, by a new noncanonical amino acid, we performed free energy perturbation (FEP) experiments using the Thermodynamic Integration (TI) technique.[74−76] This $\Delta\Delta G^{1-2}$ will measure and allow the comparison of the relative thermodynamic stability of each new noncanonical amino acid at every Aib position and indicate its contribution for the peptide stability. Negative $\Delta\Delta G^{1-2}$ indicates that it is thermodynamically favorable to replace Aib by a given noncanonical amino acid; a positive value indicates the opposite. In this approach, 21 intermediate Hamiltonian states separating the initial and final state were simulated using a coupling parameter λ. The relative free energy was given by the integration of the Hamiltonian derivative relative to the coupling parameter (λ) that connects the initial and final states. The trapezoidal rule was employed for this integration.

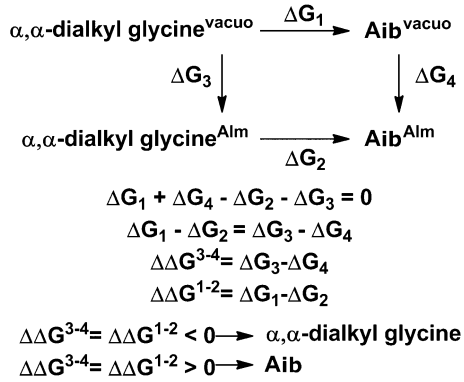


Figure 2. Thermodynamic cycle and equations used to determine the relative free energy of insertion ($\Delta\Delta G^{3-4}$) of a new α,α-dialkyl glycine relative to every Aib position in the native Alamethicin. ΔG_1 and ΔG_2 are the free energy resulting from the conversion of an α,α-dialkyl glycine into Aib in vacuo and in the Alamethicin peptide, respectively. ΔG_3 and ΔG_4 correspond to the free energy of incorporating an α,α-dialkyl glycine or Aib, respectively, into the Alamethicin peptide.

The TI experiments of Alamethicin in membrane, comprised the alchemical mutation of each eight new α,α-dialkyl glycines and, in separate FEP calculations, into native Aib residue (ΔG_2, see Figures 1 and 2) . The same alchemical transformation of each α,α-dialkyl glycine into Aib residue was also made in vacuo, to complete the necessary thermodynamic cycle (ΔG_1, see Figures 1 and 2). We choose to use vacuo in order to obtain a relative free energy solely correlated to the mutation of Aib

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

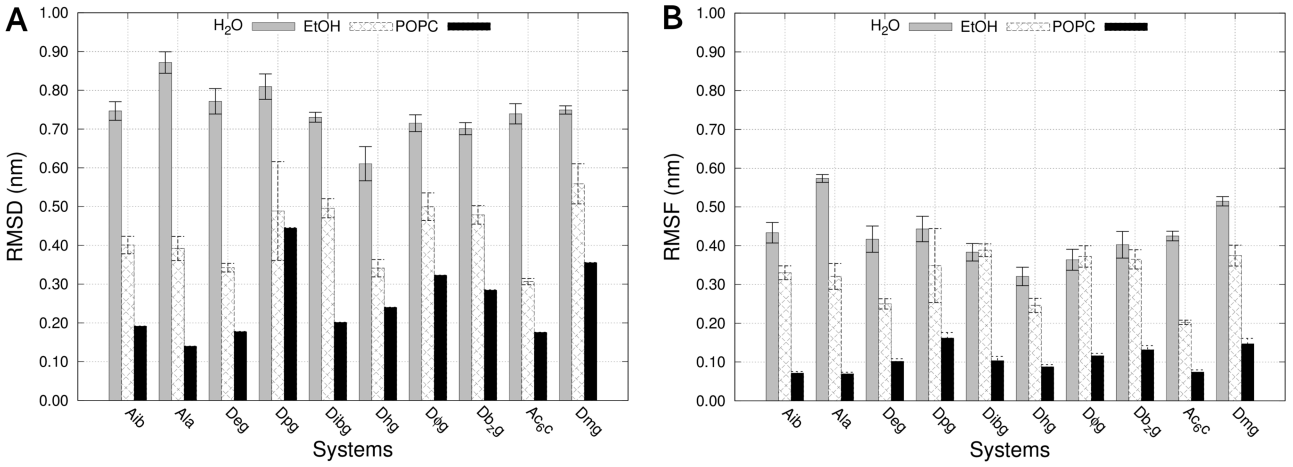


Figure 3. (A) Cα RMSD and (B) Cα RMSF averages for Alamethicin and peptidomimetics, in water, ethanol, and POPC. Fitting of Cα relative to the experimental X-ray structure of Alamethicin in α-helix. Average values obtained from five replicate simulations (in water and ethanol), including standard deviation error bars.

into each noncanonical amino acid without any solvent effect. For the purpose of this evaluation, this approach is sufficient. With these experiments, it was possible to evaluate the free energy of insertion of each α,α-dialkyl glycine in every Alamethicin position in a membrane environment. In total, with this protocol, it was possible to study the thermodynamic properties of 72 Alamethicin mimetics inserted on the membrane. The coupling parameter λ was varied from 0 to 1, with incremental steps of 0.05 λ for each simulation, resulting in 21 simulations for each of the 72 new systems (these systems comes from individual substitution of the 8 Aib positions for one of the 8 new α,α-dialkyl glycines or Ala, resulting in 9 residues for 8 possible positions = 72 different peptides). We used an integration interval of 2 fs and simulations of 10 ns sampling for each of the 21 λ points, resulting in a total sampling time of 210 ns.

Analysis. RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), SS (Secondary Structure Analysis), and Ramachandran plots analysis were performed over all the conformations from the 100 ns simulations.[65] All measurements are averaged over five replicates, and the corresponding standard deviation is presented. Average structures (central conformations) shown on figures are the ones that minimize the RMSD variance when used for fitting against all other conformations of the trajectory.

RESULTS AND DISCUSSION

The 2-dimensional structures of all α,α-dialkyl glycines investigated in this work are shown in Figure 1. Aib and Ala were used as reference residues. Deg, Dpg, Dibg, and Dhg are noncanonical amino acids with nonpolar, aliphatic side chains. DΦg and Db$_z$g are disubstituted amino acids with aromatic side chains, Ac$_6$c has a cyclic side chain and Dmg is the only noncanonical amino acid under study with aliphatic, polar side chain. Some noncanonical amino acids of this collection are similar to natural amino acids. That is the case for amino acids Aib, Dibg, Dmg, and Db$_z$g, which are similar to alanine, leucine, serine, and phenylalanine, respectively. These disubstituted amino acids lose their chirality because of the addition of the second symmetrical side chain.

Figure 3 shows the Cα RMSD and RMSF averages of each Alamethicin system in three different environments (water, ethanol, and POPC) relative to the experimental X-ray structure of Alamethicin in α-helix. In Figure 3A, the peptide with the highest RMSD relative to the native α-helix structure is the one substituted by Ala in all Alamethicin Aib positions. This result is a first indication that the Ala amino acid in Alamethicin is unable to promote preorganization in α-helix. Furthermore, the native form of Alamethicin loses a substantial part of its helical structure in water, as observed by Tieleman and co-workers.[38] On the other hand, in water, Dhg appears to promote a conformation more native-like relative to the reference structure in α-helix than Aib, indicating that this amino acid may induce α-helical conformations. The peptidomimetics containing the amino acids Dibg, DΦg, Db$_z$g, Ac$_6$c, and Dmg were shown to be poorly structured, similarly to native Alamethicin in water. Also, in all solvents, Alamethicin substituted by Dmg does not seem to have conformations similar to the native structure in α-helix.

All Alamethicin peptidomimetics structure (including Ala) are more native-like when solvated in ethanol (Figure 3A). This fact seems to correlate with the preference of these peptides to adopt α-helical structures in low polar environments. Ethanol was used here to evaluate the structure of the peptides in a media with an intermediate dielectric constant between the water and POPC membrane. We observe that Alamethicin substituted by Aib, Ala, Dpg, Dibg, DΦg, and Db$_z$g present high RMSD values in ethanol, and the ones carrying the residues Ac$_6$c, Dhg, and Deg have low deviation in ethanol. Note that Dhg induces low RMSD both in water and in ethanol.

In POPC (Figure 3A), it is observed that the less bulky residues, such as Aib, Ala, Deg and Ac$_6$c promote lower RMSD in comparison to the amino acids with large side chains. This behavior might be related to the fact that these small residues are well-arranged between phospholipids chains, and therefore, they do not suffer large structural rearrangement when inserted on the membrane. On the other hand, the peptide-containing residues with longer and bulky side chains must need to rearrange these amino acids between the phospholipid chains to minimize steric hindrance, causing some structural perturbation on the native helical conformation of the peptides.

The conformational sampling of the peptides under study was evaluated using a RMSF analysis. Figure 3B shows that, in water, most peptides bearing a noncanonical disubstituted

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

amino acid are more constrained than the peptide with Ala in all Aib positions. It is also apparent that the amino acid that exhibits the lowest RMSF in aqueous environment is Dhg. The other peptides have similar RMSF relative to the native Alamethicin. The RMSF behavior of the peptides with Dibg, DΦg, and Db_zg, in ethanol is very similar to what is observed in water. However, the general trend seems to be the reduction of the amplitude of the peptide RMSF in this medium.

In POPC, peptides containing residues of Aib, Ala, Dhg, and Ac_6c suffer the smallest structural fluctuations. Despite the large and bulky side chain of Dhg, this amino acid is capable to induce lower fluctuations when the peptide is inserted in the membrane, similarly to what is observed for the smallest amino acids discussed in this work, Ala and Aib. These facts suggest that peptides bearing Dhg are, in general, more conformationally restrained in different environments than most of the other residues under study.

The secondary structure analysis was used to determine and quantify the type of secondary structure conformations explored by these peptides and the number of residues that are involved in a particular type of secondary structure. Figure 4

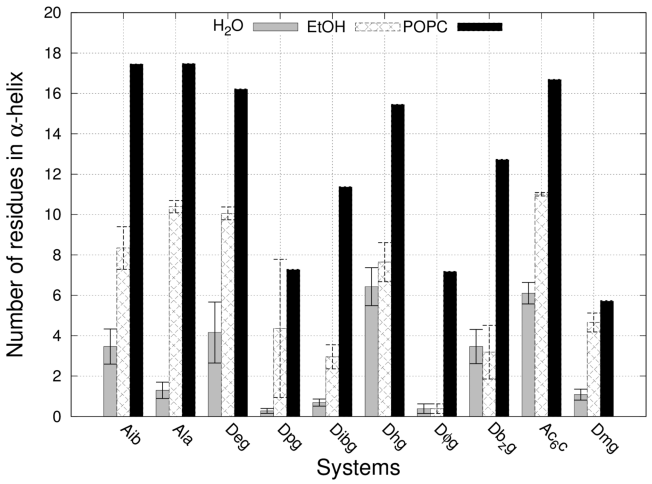


Figure 4. Average number of residues in α-helix of all peptides in water, ethanol, and POPC. Average values obtained from five replicate simulations (in water and ethanol), including standard deviation error bars.

shows the average number (over five replicates) of residues involved in α-helix throughout the 100 ns simulation, obtained for each peptide investigated in water, ethanol, and POPC.

It was noted earlier that Ac_6c and Dhg are more capable of inducing Alamethicin conformations closer to the native X-ray structure in α-helix than peptides with Aib or Ala (Figure 3A). This fact is confirmed by our secondary structure analysis. We show in Figure 4 that, in water, the peptides with higher number of amino acids in α-helix are those containing Ac_6c and Dhg.

In water, the analogue containing Ala presents an average of less than two residues in α-helix. The analogues containing Deg and Db_zg have an average of four residues in α-helix, whereas the analogues containing Dpg, Dibg, DΦg, and Dmg have a residual number of amino acids in α-helix.

In POPC, most of the peptides show a high number of residues in α-helix. This observation agrees with the results of Tieleman and co-workers about the capability of the native Alamethicin in maintaining or reorganizing the conformational structure when near or inserted in a membrane.[38] Only the amino acids Dpg, DΦg, and Dmg have lower tendency to induce this type of secondary structure in this medium. Ethanol behaves as a medium with intermediate properties between water and POPC, because there is a considerable increase in the number of residues in α-helix, relative to those seen in water.

To illustrate the previous analyses, Figure 5 shows the central structures (see Material and Methods) of all peptides in water. This analysis provides the most representative structure of the ensemble of conformations sampled during the simulation. It is clear that the most of them have lost their initial helical structure, except for the case of Dhg (Figure 5F).

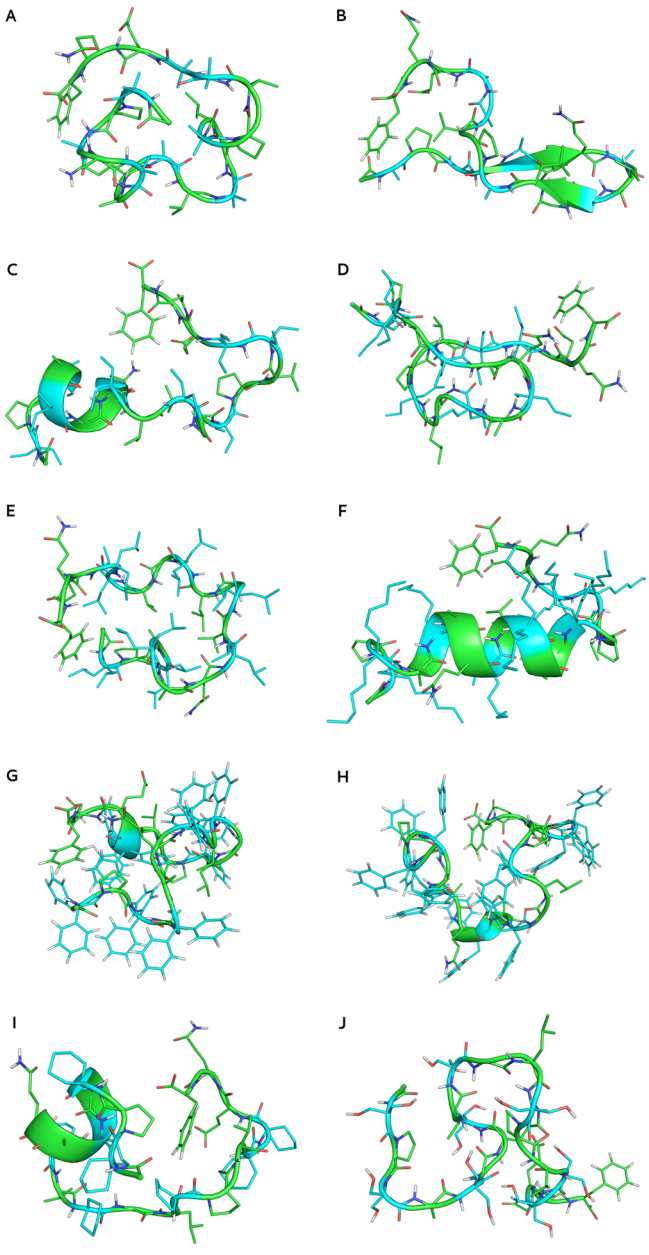


Figure 5. Central structures of one replicate of Alamethicin and analogues in water: (A) Aib, (B) Ala, (C) Deg, (D) Dpg, (E) Dibg, (F) Dhg, (G) DΦg, (H) Db_zg, (I) Ac_6c, and (J) Dmg. The coloring of the atoms follows the convention: green for carbon, blue for nitrogen, red for oxygen, white for hydrogen, and cyan to highlight amino acid of interest. The water molecules were omitted for better visualization, and peptides show the cartoon that defines its secondary structure.

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

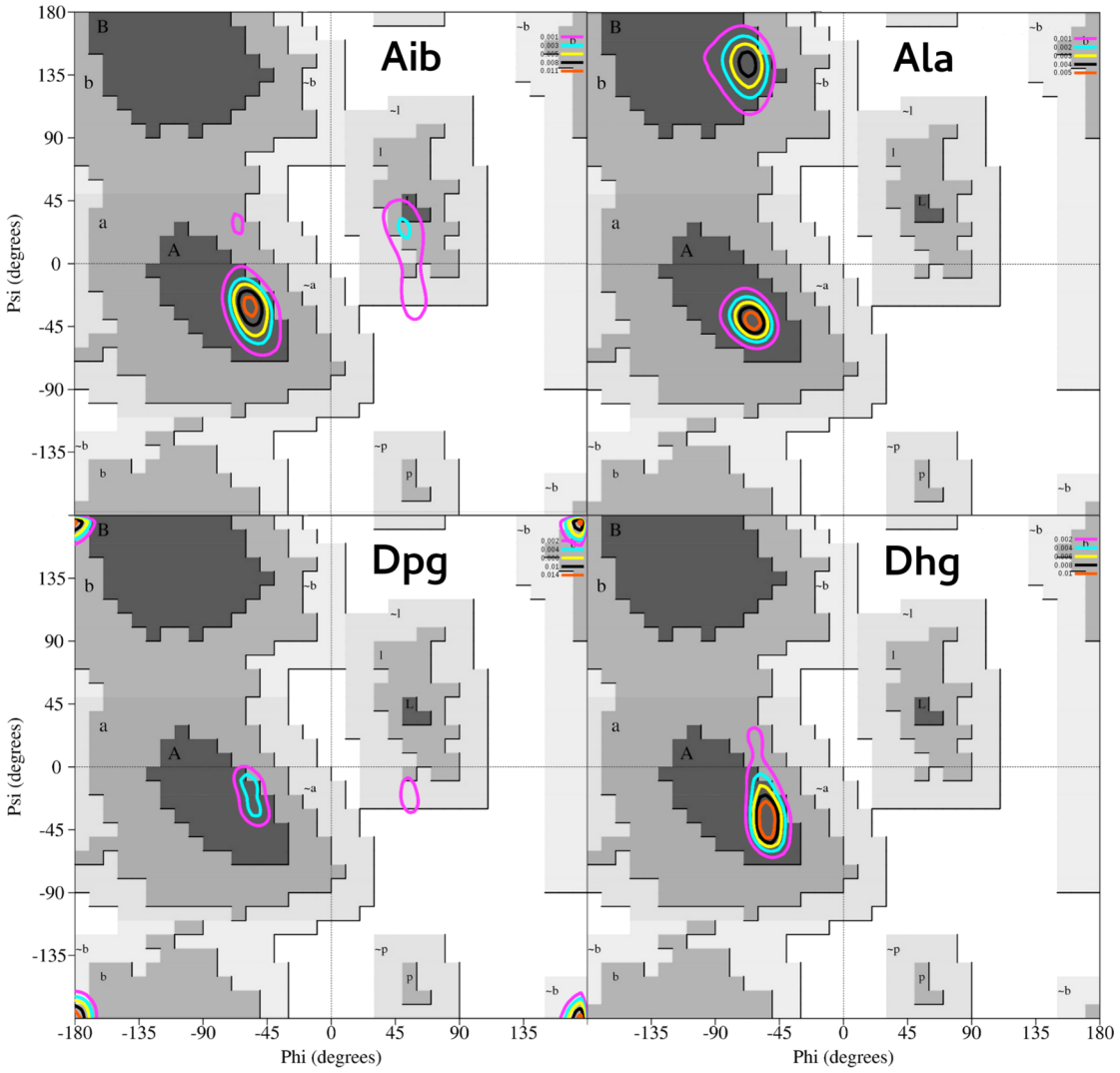


Figure 6. Probability density contours of φ and ψ pairs for the amino acids Aib, Ala, Dpg, and Dhg in water. These contours are superimposed on the Ramachandran diagram in which region (A) corresponds to typical dihedrals of right α-helix, (B) corresponds to β-sheets space, and (L) to left α-helix region.

The conformations observed in Figure 5 confirm the previous observation made on the RMSD analysis and number of residues in the α-helix (Figures 3A and 4). It is visible clear that the Dhg residue induces α-helical conformations of Alamethicin in water (Figure 5F), and the native peptide has lost its helical conformation (Figure 5A). Experiments conducted by Tieleman et al. demonstrate that the loss of structure in water for the native Alamethicin (Figure 3A) can be explained by the hydrophobic effect. The authors also demonstrate that the peptide reorganizes into a α-helical conformation at the membrane water interface.[38] A similar behavior for the new peptidomimetics is expected, and in fact, the implemented analysis has shown that a great part of these analogues are unstructured in water but maintain a helical conformation when inserted in the membrane. It is interesting to note that the analogue containing Ala (Figure 5B) completely loses the initial structure in α helix and promotes the formation of an antiparallel β-sheet during the simulation in water.

The conformations of analogues containing Dpg, Dibg, DΦg, and Dmg (Figure 5D, E, G, and J, respectively) suggest the formation of random coil structures. It is clear that the peptide containing Dhg (Figure 5F) has most of its residues in α-helix (approximately half of the residues of this peptide), and only the amine and carboxyl terminal are unstructured. This α-helix preorganization suggests that the insertion of the peptide in the membrane will be thermodynamically less costly compared to a unstructured peptide. In principle, some peptides (for example, Ala, Deg, or Dibg) will require a higher reorganization cost to adopt an α-helix conformation in POPC (see Figure 4). The peptide containing the cyclic amino acid Ac_6c (Figure 5I) has also a α-helix region at its N-terminal. It is, therefore, an amino acid less bulky than the Dhg residue that also promotes the formation of α-helical conformations.

Within the context of structural analysis, we investigated the dihedral angle pairs Psi (ψ) and Phi (φ) of the Ramachandran space for each α,α-dialkyl inserted in Alamethicin.[77] This type of analysis is essential to understand the backbone degrees of freedom and secondary structure of each amino acid compared to the natural amino acids. Disubstituted amino acids have two symmetric and sometimes bulky side chains constraining the amino acid structure around the Cα. Therefore, the dihedral conformational space of these disubstituted amino acids might adopt conformations that lie outside of the classical

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

Ramachandran plot regions of canonical amino acids. Figure 6 shows the probability density of the φ and ψ pairs of four amino acids of interest obtained from our simulation of Alamethicin in water, and they are Ala, Aib, Dpg, and Dhg. The distribution was calculated from the ψ and φ angles recorded from the eight Alamethicin Aib positions replaced by Ala and the α,α-dialkyl glycines. A total of 4 000 000 points were used to calculate the probability densities shown in Figure 6 (5 replicate simulations × 8 residue positions × 100 000 conformations). The Ramachandran diagrams for the others amino acids discussed in this work are shown in the Supporting Information (Figure 1S).

Figure 6 shows that the highest density of φ and ψ dihedral pairs obtained for Aib (Figure 6 − upper left) inserted in Alamethicin are in regions corresponding, as expected, to left and right α-helices, with higher preference for the right α-helix region. Aib is a symmetrical amino acid, where the carbon alpha has no chirality, and consequently, it is neither an L- or D-amino acid. This fact has clear consequences on the propensity to sample both left and right regions of the Ramachandran plot. Another important observation is that the probability density observed for Aib, when compared to those obtained by Ala (Figure 6 − upper right), also confirms that the Aib is more suitable for constraining the peptide structure in α-helix. Note that, as expected, Ala explores dihedral pairs in the region of β sheets. We show that the double-methylation at Cα on Aib eliminates completely the conformations in the β-sheet Ramachandran space.

The Dhg residue (Figure 6 − lower right) has φ and ψ pairs only in the right α-helix region, suggesting that it is not possible to establish a correlation between the lack of chirality with the propensity to induce both left and/or right α-helices. The new amino acid Dpg (Figure 6 − lower left) has dihedral angles scattered at 180°, indicating the possibility of the arrangement in extended conformation as previously suggested by Valle and co-workers for amino acids with two or more carbons in the branched side chain.[23,24,26] The results presented by the Aib, Dpg, and Dhg residues clearly indicate that disubstituted amino acids constitute a diverse class of new residues with great conformational variability that are not exclusively in α-helix conformations.

So far, the structural and dynamics findings presented above, suggested that some new noncanonical amino acids, such as Dhg and Ac_6c, are able to induce peptides to adopt helical secondary structures compared to Aib in native Alamethicin. However, from a thermodynamic point of view, it is important to evaluate the relative free energy cost of replacing each Aib for a new α,α-dialkyl glycine. This aspect is relevant, taking into account the function of Alamethicin in the insertion and disruption of cell membranes. We evaluated the relative free energy cost of replacing each Aib position by a new α,α-dialkyl glycine in Alamethicin inserted in a membrane environment. This was accomplished using the thermodynamic cycle shown in Figure 2 (see Material and Methods).

In this thermodynamic cycle, ΔG_1 corresponds to the free energy associated with the transformation of an α,α-dialkyl glycine to Aib in vacuo, whereas ΔG_2 refers to the free energy of the transformation of an α,α-dialkyl glycine to Aib insert in Alamethicin. In this thermodynamic cycle, we do not want to evaluate any solvent effect, and for this reason, we chose to close the thermodynamic cycle with an alchemical transformation in vacuo. In this thermodynamic cycle, if $\Delta\Delta G^{3-4} < 0$, it is thermodynamically favorable to replace Aib by a new α,α-dialkyl glycine in this position, and if $\Delta\Delta G^{1-2} > 0$, it is preferable to maintain the native Aib. The relative free energy values of insertion of a new α,α-dialkyl glycine at each position previously occupied by Aib are show in Figure 7.

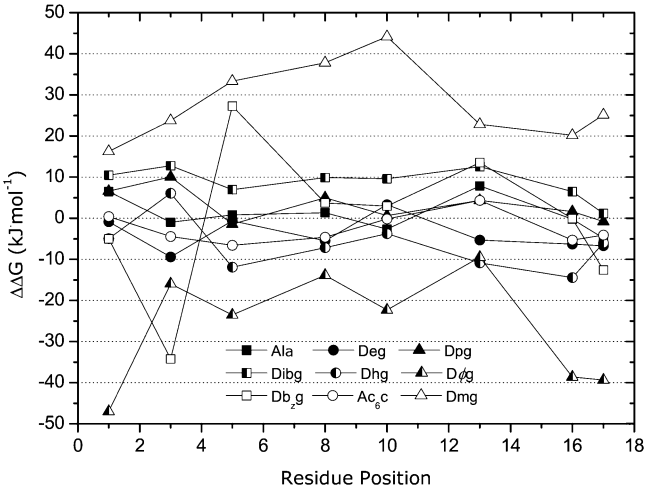


Figure 7. Relative free energy of insertion ($\Delta\Delta G^{3-4}$) of the new non-natural amino acids in Alamethicin, in each position previously occupied by Aib (1, 3, 5, 8, 10, 13, 16, and 17), in the POPC membrane. Negative free energy values indicate the preference toward the noncanonical amino acid.

In Figure 7, it is observed that for all positions of interest (1, 3, 5, 8, 10, 13, 16, and 17), we found alternative residues that are thermodynamically more favorable than Aib. DΦg seems to be one of the best amino acids to replace the native positions belonging to Aib in six out of the eight positions. It is also important to note that in the position 10, the amino acids Dhg, Ala, Dpg, Db$_z$g, and Deg lead to similar relative free energies. This thermodynamic data can also be used to design a novel Alamethicin peptidomimetics with improved thermodynamic stability in membrane environments.

Taking into account only the thermodynamic data, the best options for replacing Aib at the eight positions are DΦg, Db$_z$g, DΦg, DΦg, Dhg, DΦg, and DΦg. However, to suggest a novel peptidomimetic for Alamethicin, we may also take into account the structural and dynamics properties previously observed for each new α,α-dialkyl glycine. The previous analysis indicates that the choice of DΦg at positions 1, 5, 8, 10, 16, and 17 might not be the best option because it did not promote good α-helical preorganization of Alamethicin in water and in the POPC membrane.

We can suggest that the amino acid sequence to replace all the Aib positions that combine the best thermodynamic and α-helical propensity in Alamethicin are Dhg, Deg, Dhg, Dhg, Aib, Dhg, Dhg, and Deg, replacing positions 1, 3, 5, 8, 10, 13, 16, and 17 in Alamethicin. This suggestion of amino acids reflects the best combination of structural characteristics and thermodynamic properties for Alamethicin. In this regard, this new peptide was modeled and evaluated under the same simulation conditions (in water and POPC). A summary of the structural properties of this peptide is compared with some of the previous Alamethicin peptidomimetics in Figure 8. Figure 8A shows that the structure of the new peptide is more native-like than Alamethicin with Aib, Deg, or Ac_6c and it clearly adopts conformations with α-helix organization (Figure 8C). It is also interesting to note that the RMSD of this new peptide

dx.doi.org/10.1021/jp505400q | J. Phys. Chem. B 2014, 118, 9861−9870

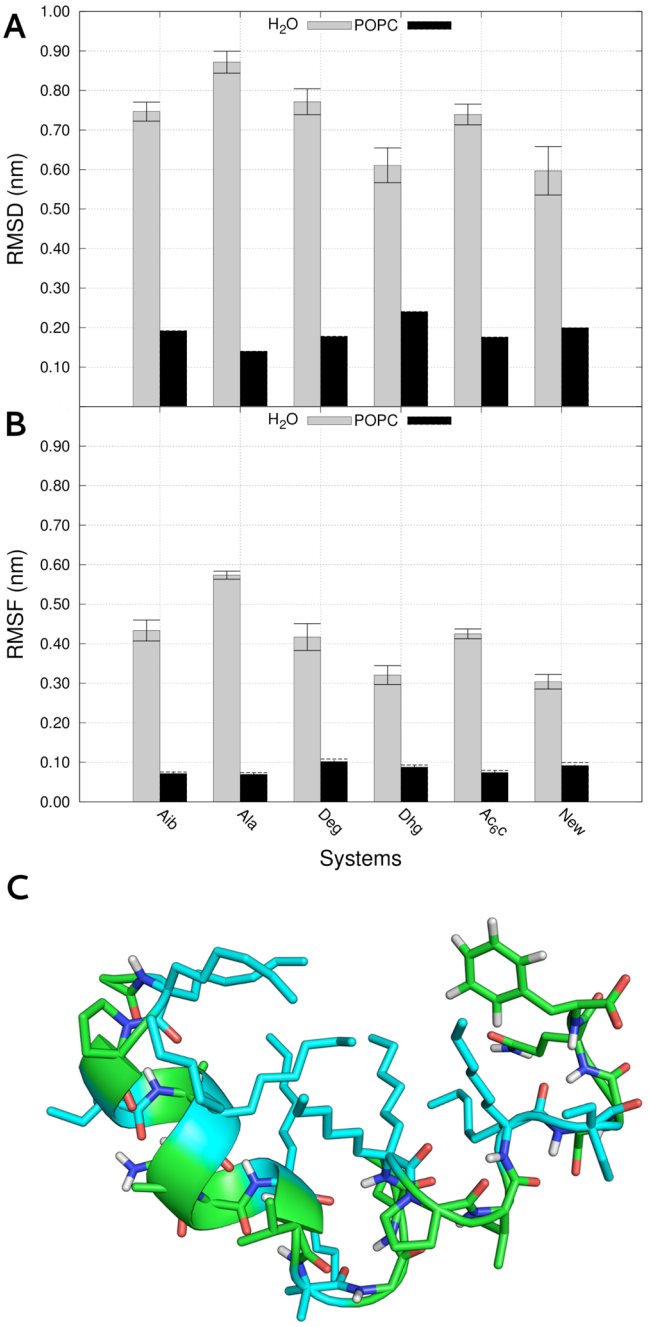


Figure 8. (A) Cα RMSD and (B) Cα RMSF averages for Alamethicin, peptidomimetics with Ala, Deg, Dhg, and Ac_6c and for the new Alamethicin peptide (New), in water and POPC. Fitting of Cα relative to the experimental X-ray structure of Alamethicin in α-helix. Average values obtained from five replicate simulations (in water), including standard deviation error bars. (C) Central structure of one replicate of the new Alamethicin analogue.

and the Alamethicin substituted by Dhg are statistically equivalent. This means that the expected conformational constraining imposed by the Dhg residue was observed on this new peptidomimetic. In POPC (Figure 8A), it is apparent that the structural deviation of the new peptide is equivalent to the other Alamethicin analogues. A similar observation can be made for the dynamic properties compared to Alamethicin with Aib, Deg, Dhg, and Ac_6c residues (Figure 8B). The chosen amino seems to favor the stabilization of the peptide in α helix, otherwise, we would have found higher values of RMSD and RMSF, as well as a less folded central structure. We cannot tell yet if there are any positive, negative or cancelation of both correlations effects between the chosen residues. However, the low RMSD and RMSF seem to indicate the absence of negative correlations.

CONCLUSIONS

The modeling studies of the α,α-dialkyl glycines using Alamethicin as a model peptaibol provided significant results about the structure and function of the new noncanonical amino acids here proposed. In water, Dhg, DΦg, and Db_zg impose more constrained and helical structures than Aib. The lack of chirality around Cα and bulky side chains of these amino acids must be responsible for this effect. In ethanol, Deg, Ac_6c, and Dhg are the amino acids that induce higher peptide helicity. In POPC, Ala, Deg, and Ac_6c rendered analogues with structural behavior similar to native Alamethicin. In this environment, it was noted that smaller amino acids are well-arranged between phospholipids chains, and therefore, the peptides do not suffer large structural rearrangement.

The analyses implemented indicate that Dhg and Ac_6c are the ones more capable of inducing α-helix conformations in Alamethicin, in all solvents under study. Moreover, they seem to improve the thermodynamic stabilization of the peptides in the membrane. This result is consistent with our prior study in Peptaibolin[18] where these residues were the most capable to induce helical conformations. This indicates that these residues seem to have a foldamer profile; however, further experiments using different peptides are required to propose a definitive conclusion about their foldamer role.

Ramachandran analysis demonstrated that the disubstituted amino acids do not only induce α-helix conformations. Dpg and Db_zg may prefer an extended conformation, and this fact agrees with previous results.[23,24,26] The α,α-dialkyl glycines show to have different propensities to induce secondary structures, particularly right and left α-helix, β-sheets, and planar structures. These residues seem to constitute a class of amino acids with great conformational variability, not restricted to α-helical conformations. The foldamer potential of this class of amino acids needs to be further evaluated in future studies using other peptides. This is necessary to evaluate if other peptide context affects the conformational properties observed in this study. The relative free energy of replacing Aib by a new α,α-dialkyl glycine suggests that there are better alternatives to Aib in almost all positions previously occupied by Aib, except at position 11. We proposed a new analogue of Alamethicin that combines the best structural, dynamic, and thermodynamics properties. The modeling of Alamethicin analogues by inserting new α,α-dialkyl glycines suggests that it is possible to optimize the characteristics of native Alamethicin and obtain novel peptides that may have improved antibiotic activity.

ASSOCIATED CONTENT

Supporting Information

All parametrizations for the new amino acids discussed in this article and Ramachandran plots are available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: micaelo@itqb.unl.pt. Tel.: +351 253 604 384.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was in part financially supported by FCT (SFRH/BD/79195/2011), (PEst-C/QUI/UI0686/2011), (PTDC/QUI-BIQ/118389/2010), and (FCOMP-01-0124-FEDER-022716). The authors thank the access to the Minho University GRIUM cluster and for contract research grant C2008-UMINHO-CQ-03.

■ REFERENCES

(1) Fu, Y. W.; Hammarstrom, L. G. J.; Miller, T. J.; Fronczek, F. R.; McLaughlin, M. L.; Hammer, R. P. Sterically hindered C-alpha,C-alpha-disubstituted alpha-amino acids: Synthesis from alpha-nitro-acetate and incorporation into peptides. *J. Org. Chem.* **2001**, *66*, 7118−7124.

(2) Mendel, D.; Ellman, J.; Schultz, P. G. Protein-biosynthesis with conformationally restricted amino-acids. *J. Am. Chem. Soc.* **1993**, *115*, 4359−4360.

(3) Gentilucci, L.; Tolomelli, A.; Squassabia, F. Peptides and peptidomimetics in medicine, surgery and biotechnology. *Curr. Med. Chem.* **2006**, *13*, 2449−2466.

(4) Giannis, A. Peptidomimetics for receptor ligand discovery, development, and medical perspectives. *Angew. Chem., Int. Ed.* **1993**, *32*, 1244−1267.

(5) Grauer, A.; Konig, B. Peptidomimetics—A Versatile Route to Biologically Active Compounds. *Eur. J. Org. Chem.* **2009**, 5099−5111.

(6) Vagner, J.; Qu, H. C.; Hruby, V. J. Peptidomimetics, a synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292−296.

(7) Ballet, S.; Feytens, D.; Buysse, K.; Chung, N. N.; Lemieux, C.; Tumati, S.; Keresztes, A.; Van Duppen, J.; Lai, J.; Varga, E.; Porreca, F.; Schiller, P. W.; Broeck, J. V.; Tourwe, D. Design of Novel Neurokinin 1 Receptor Antagonists Based on Conformationally Constrained Aromatic Amino Acids and Discovery of a Potent Chimeric Opioid Agonist-Neurokinin 1 Receptor Antagonist. *J. Med. Chem.* **2011**, *54*, 2467−2476.

(8) Feytens, D.; Cescato, R.; Reubi, J. C.; Tourwe, D. New sst(4/5)-selective somatostatin peptidomimetics based on a constrained tryptophan scaffold. *J. Med. Chem.* **2007**, *50*, 3397−3401.

(9) Mallareddy, J. R.; Borics, A.; Keresztes, A.; Kover, K. E.; Tourwe, D.; Toth, G. Design, Synthesis, Pharmacological Evaluation, and Structure−Activity Study of Novel Endomorphin Analogues with Multiple Structural Modifications. *J. Med. Chem.* **2011**, *54*, 1462−1472.

(10) Oh, J. E.; Lee, K. H. Synthesis of novel unnatural amino acid as a building block and its incorporation into an antimicrobial peptide. *Bioorgan. Med. Chem.* **1999**, *7*, 2985−2990.

(11) Ressurreicao, A. S. M.; Bordessa, A.; Civera, M.; Belvisi, L.; Gennari, C.; Piarulli, U. Synthesis and conformational studies of peptidomimetics containing a new bifunctional diketopiperazine scaffold acting as a beta-hairpin inducer. *J. Org. Chem.* **2008**, *73*, 652−660.

(12) Whitby, L. R.; Ando, Y.; Setola, V.; Vogt, P. K.; Roth, B. L.; Boger, D. L. Design, Synthesis, and Validation of a beta-Turn Mimetic Library Targeting Protein−Protein and Peptide−Receptor Interactions. *J. Am. Chem. Soc.* **2011**, *133*, 10184−10194.

(13) Chugh, J. K.; Wallace, B. A. Peptaibols: models for ion channels. *Biochem. Soc. Trans.* **2001**, *29*, 565−570.

(14) Whitmore, L.; Wallace, B. A. The Peptaibol Database: a database for sequences and structures of naturally occurring peptaibols. *Nucleic Acids Res.* **2004**, *32*, D593−D594.

(15) Marr, A. K.; Gooderham, W. J.; Hancock, R. E. W. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Curr. Opin. Pharmacol.* **2006**, *6*, 468−472.

(16) Shai, Y. Mode of action of membrane active antimicrobial peptides. *Biopolymers* **2002**, *66*, 236−248.

(17) Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature* **2002**, *415*, 389−395.

(18) Castro, T. G.; Micaêlo, N. M. Modeling of Peptaibol Analogues Incorporating Nonpolar α,α-Dialkyl Glycines Shows Improved α-Helical Preorganization and Spontaneous Membrane Permeation. *J. Phys. Chem. B* **2014**, *118*, 649−658.

(19) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. Factors Governing Helical Preference of Peptides Containing Multiple alpha,alpha-Dialkyl Amino-Acids. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 487−491.

(20) Improta, R.; Barone, V.; Kudin, K. N.; Scuseria, G. E. Structure and Conformational Behavior of Biopolymers by Density Functional Calculations Employing Periodic Boundary Conditions. I. The Case of Polyglycine, Polyalanine, and Poly-α-aminoisobutyric Acid in Vacuo. *J. Am. Chem. Soc.* **2001**, *123*, 3311−3322.

(21) Karle, I. L.; Balaram, P. Structural Characteristics of Alpha-Helical Peptide Molecules Containing Aib Residues. *Biochemistry* **1990**, *29*, 6747−6756.

(22) Marshall, G. R.; Bosshard, H. E. Angiotensin II. Studies on the biologically active conformation. *Circ. Res.* **1972**, *31* (Suppl 2), 143−50.

(23) Schweitzer-Stenner, R.; Gonzales, W.; Bourne, G. T.; Feng, J. A.; Marshall, G. R. Conformational manifold of alpha-aminoisobutyric acid (Aib) containing alanine-based tripeptides in aqueous solution explored by vibrational spectroscopy, electronic circular dichroism spectroscopy, and molecular dynamics simulations. *J. Am. Chem. Soc.* **2007**, *129*, 13095−13109.

(24) Rodrigues, L. M.; Fonseca, J. I.; Maia, H. L. S. Synthesis and conformational investigation of tetrapeptide analogues of the fragment B23-B26 of insulin. *Tetrahedron* **2004**, *60*, 8929−8936.

(25) Fu, Y.; Etienne, M. A.; Hammer, R. P. Facile Synthesis of α,α-Diisobutylglycine and Anchoring Its Derivatives onto PAL-PEG-PS Resin. *J. Org. Chem.* **2003**, *68*, 9854−9857.

(26) Valle, G.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Lelj, F.; Barone, V.; Fraternali, F.; Hardy, P. M.; Langrangoldsmith, A.; Maia, H. L. S. Structural versatility of peptides from C-alpha,alpha disubstituted glycines − preferred conformational of the C-alpha,alpha-dibenzylglycine residue. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1481−1487.

(27) Casanovas, J.; Nussinov, R.; Alemàn, C. Intrinsic Conformational Preferences of Cα,α-Dibenzylglycine. *J. Org. Chem.* **2008**, *73*, 4205−4211.

(28) Casanovas, J.; Zanuy, D.; Nussinov, R.; Alemán, C. Intrinsic Conformational Characteristics of α,α-Diphenylglycine. *J. Org. Chem.* **2007**, *72*, 2174−2181.

(29) Pavone, V.; Lombardi, A.; Saviano, M.; De Simone, G.; Nastri, F.; Maglio, O.; Omote, Y.; Yamanaka, Y.; Yamada, T. Conformational behavior of C-alpha,C-alpha-diphenyl glycine: Extended conformation in tripeptides containing consecutive D phi G residues. *Biopolymers* **2000**, *53*, 161−168.

(30) Rodriguez-Ropero, F.; Zanuy, D.; Casanovas, J.; Nussinov, R.; Aleman, C. Application of 1-aminocyclohexane carboxylic acid to protein nanostructure computer design. *J. Chem. Inf. Model* **2008**, *48*, 333−343.

(31) Alemán, C. Conformational Properties of α-Amino Acids Disubstituted at the α-Carbon. *J. Phys. Chem. B* **1997**, *101*, 5046−5050.

(32) Fox, R. O.; Richards, F. M. A voltage-gated ion channel model inferred from the crystal-structure of Alamethicin at 1.5-A resolution. *Nature* **1982**, *300*, 325−330.

(33) Pandey, R. C.; Cook, J. C.; Rinehart, K. L. High-resolution and field desorption mass spectrometry studies and revised structures of Alamethicin I and Alamethicin II. *J. Am. Chem. Soc.* **1977**, *99*, 8469−8483.

(34) Bechinger, B. The structure, dynamics and orientation of antimicrobial peptides in membranes by multidimensional solid-state NMR spectroscopy. *Biochim. Biophys. Acta, Biomembr.* **1999**, *1462*, 157−183.

(35) Bechinger, B.; Salnikov, E. S. The membrane interactions of antimicrobial peptides revealed by solid-state NMR spectroscopy. *Chem. Phys. Lipids* **2012**, *165*, 282−301.

(36) Bak, M.; Bywater, R. P.; Hohwy, M.; Thomsen, J. K.; Adelhorst, K.; Jakobsen, H. J.; Sørensen, O. W.; Nielsen, N. C. Conformation of Alamethicin in Oriented Phospholipid Bilayers Determined by 15N Solid-State Nuclear Magnetic Resonance. *Biophys. J.* **2001**, *81*, 1684−1698.
(37) Salnikov, E. S.; De Zotti, M.; Formaggio, F.; Li, X.; Toniolo, C.; O'Neil, J. D. J.; Raap, J.; Dzuba, S. A.; Bechinger, B. Alamethicin Topology in Phospholipid Membranes by Oriented Solid-state NMR and EPR Spectroscopies: a Comparison. *J. Phys. Chem. B* **2009**, *113*, 3034−3042.
(38) Tieleman, D. P.; Berendsen, H. J. C.; Sansom, M. S. P. Surface binding of alamethicin stabilizes its helical structure: Molecular dynamics simulations. *Biophys. J.* **1999**, *76*, 3186−3191.
(39) Tieleman, D. P.; Sansom, M. S. P.; Berendsen, H. J. C. Alamethicin helices in a bilayer and in solution: Molecular dynamics simulations. *Biophys. J.* **1999**, *76*, 40−49.
(40) Tieleman, D. P.; Hess, B.; Sansom, M. S. P. Analysis and evaluation of channel models: Simulations of alamethicin. *Biophys. J.* **2002**, *83*, 2393−2407.
(41) Tieleman, D. P.; Breed, J.; Berendsen, H. J. C.; Sansom, M. S. P. Alamethicin channels in a membrane: molecular dynamics simulations. *Faraday Discuss.* **1998**, *111*, 209−223.
(42) Leitgeb, B.; Szekeres, A.; Manczinger, L.; Vágvölgyi, C.; Kredics, L. The History of Alamethicin: A Review of the Most Extensively Studied Peptaibol. *Chem. Biodivers.* **2007**, *4*, 1027−1051.
(43) Fraternali, F. Restrained and unrestrained molecular dynamics simulations in the NVT ensemble of alamethicin. *Biopolymers* **1990**, *30*, 1083−1099.
(44) Biggin, P. C.; Breed, J.; Son, H. S.; Sansom, M. S. P. Simulation Studies of Alamethicin−Bilayer Interactions. *Biophys. J.* **1997**, *72*, 627−636.
(45) Gibbs, N.; Sessions, R. B.; Williams, P. B.; Dempsey, C. E. Helix bending in alamethicin: molecular dynamics simulations and amide hydrogen exchange in methanol. *Biophys. J.* **1997**, *72*, 2490−2495.
(46) Sessions, R. B.; Gibbs, N.; Dempsey, C. E. Hydrogen Bonding in Helical Polypeptides from Molecular Dynamics Simulations and Amide Hydrogen Exchange Analysis: Alamethicin and Melittin in Methanol. *Biophys. J.* **1998**, *74*, 138−152.
(47) Thogersen, L.; Schiott, B.; Vosegaard, T.; Nielsen, N. C.; Tajkhorshid, E. Peptide Aggregation and Pore Formation in a Lipid Bilayer: A Combined Coarse-Grained and All Atom Molecular Dynamics Study. *Biophys. J.* **2008**, *95*, 4337−4347.
(48) Mihajlovic, M.; Lazaridis, T. Antimicrobial peptides in toroidal and cylindrical pores. *Biochim. Biophys. Acta, Biomembr.* **2010**, *1798*, 1485−1493.
(49) Pan, J. J.; Tristram-Nagle, S.; Nagle, J. F. Alamethicin Aggregation in Lipid Membranes. *J. Membr. Biol.* **2009**, *231*, 11−27.
(50) Qian, S.; Wang, W. C.; Yang, L.; Huang, H. W. Structure of the alamethicin pore reconstructed by X-ray diffraction analysis. *Biophys. J.* **2008**, *94*, 3512−3522.
(51) Constantin, D.; Brotons, G.; Jarre, A.; Li, C.; Salditt, T. Interaction of alamethicin pores in DMPC bilayers. *Biophys. J.* **2007**, *92*, 3978−3987.
(52) He, K.; Ludtke, S. J.; Worcester, D. L.; Huang, H. W. Neutron scattering in the plane of membranes: Structure of alamethicin pores. *Biophys. J.* **1996**, *70*, 2659−2666.
(53) Hall, J. E.; Vodyanoy, I.; Balasubramanian, T. M.; Marshall, G. R. Alamethicin − A Rich Model for Channel Behavior. *Biophys. J.* **1984**, *45*, 233−247.
(54) Sansom, M. S. P.; Breed, J.; Berendsen, H. J. C.; Tieleman, P. Alamethicin channels − Simulation studies. *Biophys. J.* **1998**, *74*, A232−A232.
(55) Pan, J.; Tieleman, D. P.; Nagle, J. F.; Kučerka, N.; Tristram-Nagle, S. Alamethicin in lipid bilayers: Combined use of X-ray scattering and MD simulations. *Biochim. Biophys. Acta, Biomembr.* **2009**, *1788*, 1387−1397.
(56) Mihajlovic, M.; Lazaridis, T. Antimicrobial peptides bind more strongly to membrane pores. *Biochim. Biophys. Acta, Biomembr.* **2010**, *1798*, 1494−1502.
(57) Dittmer, J.; Thøgersen, L.; Underhaug, J.; Bertelsen, K.; Vosegaard, T.; Pedersen, J. M.; Schiøtt, B.; Tajkhorshid, E.; Skrydstrup, T.; Nielsen, N. C. Incorporation of Antimicrobial Peptides into Membranes: A Combined Liquid-State NMR and Molecular Dynamics Study of Alamethicin in DMPC/DHPC Bicelles. *J. Phys. Chem. B* **2009**, *113*, 6928−6937.
(58) *The PyMOL Molecular Graphics System*, version 1.3r1; Schrödinger, LLC: New York, 2010.
(59) Huang, W.; Lin, Z. X.; van Gunsteren, W. F. Validation of the GROMOS 54A7 Force Field with Respect to beta-Peptide Folding. *J. Chem. Theory Comput.* **2011**, *7*, 1237−1243.
(60) Schmid, N.; Eichenberger, A. P.; Choutko, A.; Riniker, S.; Winger, M.; Mark, A. E.; van Gunsteren, W. F. Definition and testing of the GROMOS force-field versions 54A7 and 54B7. *Eur. Biophys. J.* **2011**, *40*, 843−856.
(61) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. Protein Data Bank − Computer-based archival file for macromolecular structures. *J. Mol. Biol.* **1977**, *112*, 535−542.
(62) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. The missing term in effective pair potentials. *J. Phys. Chem.* **1987**, *91*, 6269−6271.
(63) Tieleman, D. P. Palmitoyl-linoleylphosphatidylcholine bilayer with 128 lipids. http://wcm.ucalgary.ca/tieleman/downloads.
(64) Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *J. Chem. Theory Comput.* **2008**, *4*, 435−447.
(65) Spoel, D. v. d.; Lindahl, E.; Hess, B.; Buuren, A. R. v.; Apol, E.; Meulenhoff, P. J.; Tieleman, P.; Sjibers, A. L. T. M.; Feenstra, K. A.; Drunen, R. v.; Berendsen, H. J. C. Gromacs user manual version 4.5. 2010. http://www.gromacs.org.
(66) Smith, P. E.; Vangunsteren, W. F. Consistent dielectric-properties of the simple point-charge and extended simple point-charge water models at 227 and 300K. *J. Chem. Phys.* **1994**, *100*, 3169−3174.
(67) Akerlof, G. Dielectric Constants of Some Organic Solvent−Water Mixture at Various Temperatures. *J. Am. Chem. Soc.* **1932**, *54*, 4125−4139.
(68) Dwyer, D. S. Molecular simulation of the effects of alcohols on peptide structure. *Biopolymers* **1999**, *49*, 635−645.
(69) Patel, S.; Brooks, C. L., Structure, thermodynamics, and liquid-vapor equilibrium of ethanol from molecular-dynamics simulations using nonadditive interactions. *J. Chem. Phys.* **2005**, *123*, -.
(70) Hess, B. P-LINCS: A parallel linear constraint solver for molecular simulation. *J. Chem. Theory. Comput.* **2008**, *4*, 116−122.
(71) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. LINCS: A linear constraint solver for molecular simulations. *J. Comput. Chem.* **1997**, *18*, 1463−1472.
(72) van der Spoel, D.; van Maaren, P. J.; Berendsen, H. J. C. A systematic study of water models for molecular simulation: Derivation of water models optimized for use with a reaction field. *J. Chem. Phys.* **1998**, *108*, 10220−10230.
(73) Berendsen, H. J. C.; Postma, J. P. M.; Vangunsteren, W. F.; Dinola, A.; Haak, J. R. Molecular-dynamics with coupling to an external bath. *J. Chem. Phys.* **1984**, *81*, 3684−3690.
(74) Kollman, P. Free Energy Calculations − Applications to Chemical and Biochemical Phenomena. *Chem. Rev.* **1993**, *93*, 2395−2417.
(75) Oostenbrink, C.; Van Gunsteren, W. F. Single-step perturbations to calculate free energy differences from unphysical reference states: Limits on size, flexibility, and character. *J. Comput. Chem.* **2003**, *24*, 1730−1739.
(76) Riniker, S.; Christ, C. D.; Hansen, N.; Mark, A. E.; Nair, P. C.; van Gunsteren, W. F., Comparison of enveloping distribution sampling and thermodynamic integration to calculate binding free energies of phenylethanolamine *N*-methyltransferase inhibitors. *J. Chem. Phys.* **2011**, *135*.
(77) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. Stereochemistry of polypeptide chain configurations. *J. Mol. Biol.* **1963**, *7*, 95−99.