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Modeling of Peptaibol Analogues Incorporating Nonpolar α,α-Dialkyl Glycines Shows Improved α-Helical Preorganization and Spontaneous Membrane Permeation

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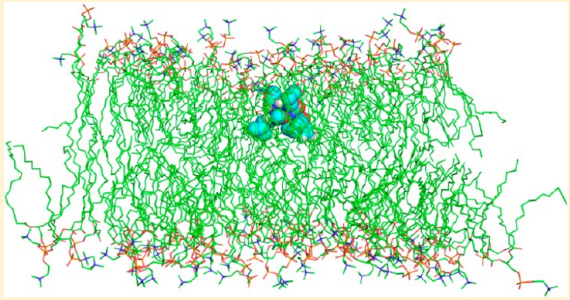
Supporting Information

ABSTRACT: In this study, we investigate the effect of nine noncanonical α,α-dialkyl glycines on the structure, dynamics, and membrane permeation properties of a small peptaibol, peptaibolin. The noncanonical amino acids under study are Aib (α-amino isobutyric acid), 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_zg (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 structures in peptidomimetics. To investigate this hypothesis, we designed new peptaibolin peptidomimetics by replacing the native Aib positions with a new α,α-dialkyl glycine. We show that Dhg and Ac_6c noncanonical amino acids are able to induce α-helix secondary structures of peptaibolin in water, which are not present in the native structure. We also demonstrate that the α,α-dialkyl glycines increase the membrane permeability of peptaibolin in 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) membranes. However, there is no apparent correlation between increased helicity and membrane permeability. In summary, we show that some α,α-dialkyl glycines under study induce the formation of α-helix secondary structures in peptaibolin and promote spontaneous membrane permeation. Our findings increase the knowledge of the membrane permeability and folding of peptides incorporating α,α-dialkyl glycines.



INTRODUCTION

Peptaibols are a family of membrane-active peptides biosynthesized by soil fungi.[1,2] The native sequence of these peptides incorporates the symmetric α,α-dialkyl glycine Aib (α-aminoisobutyric acid), a C-terminal alcohol having a length of 5 to 20 residues. Noncanonical amino acids Hyp (imino acid hydroxyproline) and Iva (isovaleric acid) are also very common in the peptaibol sequence.[1,3] This unique family of peptides has been investigated for the past four decades because of its antibacterial and antifungal properties and potential clinical applications. Furthermore, these peptides are very useful for investigating transmembrane ion transport through model lipid membranes, cells, and organelles.[4−7] In our study, we focus on peptaibolin, the smallest peptaibol reported.[1] Peptaibolin was isolated from two fungal strains, *Sepedonium sp.* HKI-0117 and *Sepedonium ampullosporum* HKI-0053, and characterized as a particular α-helical peptide. The structure is characterized by an N-terminus on an intramolecular three-center double hydrogen bond forming a type-III β-turn (C_{10}-ring structure) fused with an α-turn (C_{13}-ring structure). Acyl C═O0 (atom numbers in Figure 1) is the acceptor of two hydrogen bonds, with N3−H and N4−H being the donor groups. This structural motif develops into an additional α-turn (C═O1-H-N5), giving rise to an incipient α-helix. Also, C═O2-H-O was related as the fourth hydrogen bond involved in this unusual α-helix. However, less information regarding membrane interaction and permeability has been reported.[8,9]

Peptaibolin is a leucine-based peptide carrying two Aib residues in its sequence. Aib is an important example of a noncanonical amino acid (α,α-dialkyl glycine) that occurs naturally in some peptides but is not encoded by DNA.[10] The α,α-dialkyl glycines are disubstituted amino acids at the Cα, and it is proposed that the double substitution induces a constrained conformation of the ψ and φ main-chain dihedrals.[11] Also, the steric hindrance caused by the second alkyl group attached to Cα contributes to the constrained peptide.[10] The incorporation of noncanonical amino acids has been extensively used in the design of peptidomimetics with biomedical applications.[12−14] In fact, it is shown that these amino acids are capable of inducing specific types of secondary structure that are correlated with improved peptide function. Furthermore, the insertion of these noncanonical amino acids increases the enzymatic resistance under physiological conditions.[15−19]

In this work, we investigate the conformational and membrane permeation properties of peptaibolin incorporating eight noncanonical amino acids in the native Aib positions. The

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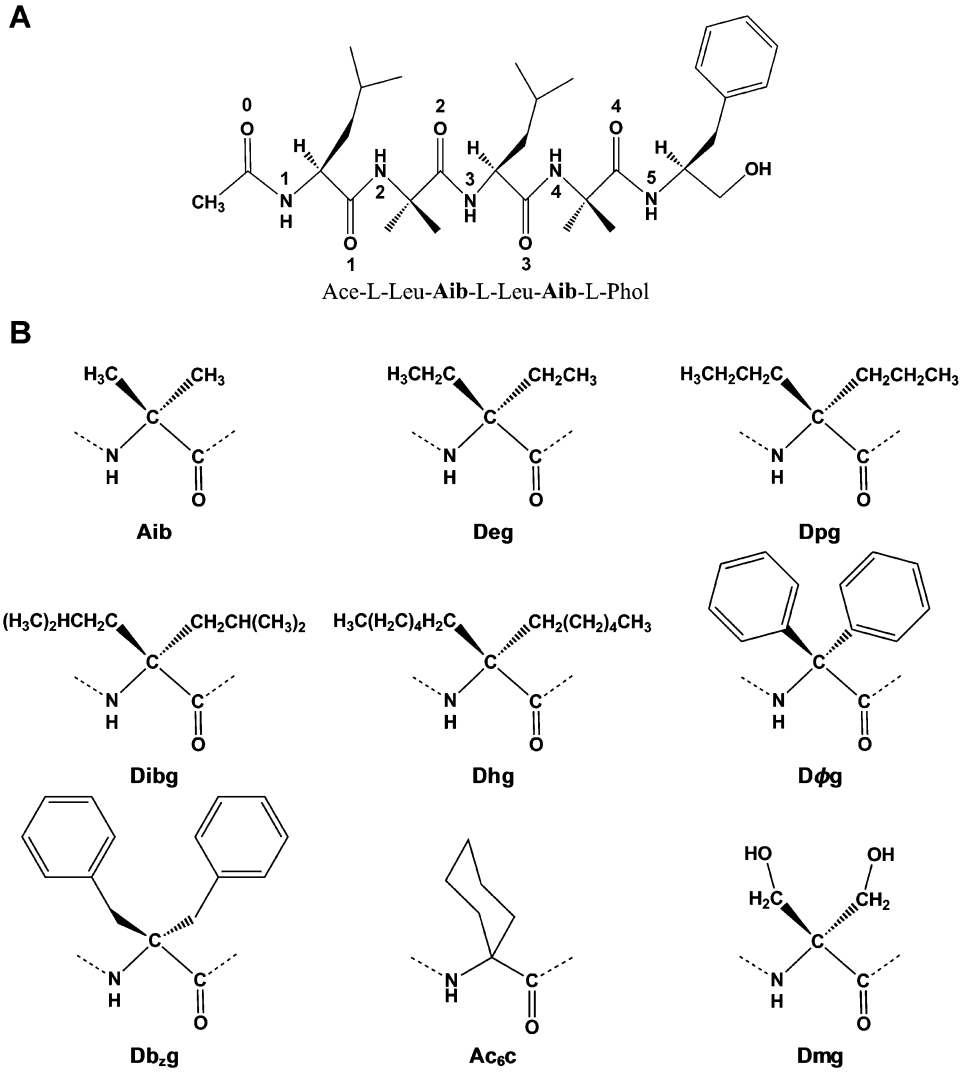

Figure 1. Two-dimensional structure of (A) peptaibolin and (B) α,α-dialkyl glycines under study: α-amino isobutyric acid (Aib), α,α-diethyl glycine (Deg), α,α-dipropyl glycine (Dpg), α,α-di-isobutyl glycine (Dibg), α,α-dihexyl glycine (Dhg), α,α-diphenyl glycine (DΦg), α,α-dibenzyl glycine (Db$_z$g),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

α,α-dialkyl glycines studied in this model peptide 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). This series includes five nonpolar aliphatic amino acids of different sizes and volumes (Aib, Deg, Dpg, Dibg, and 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 smallest residues Deg and Dpg are known to induce both fully extended C_5 conformations and helical conformations in crystal structures.[20] Dibg has already been synthesized,[21] but there are few reports concerning its structure. Experimental and theoretical investigations indicate that DΦg and Db$_z$g induce C_5 and C_7 backbone conformations.[22−25] Previous results for cyclic amino acids such as Ac$_6$c (Ac$_n$c, $n > 3$) suggest that C$\alpha \leftrightarrow$ Cα cyclization constrains the main-chain dihedrals even more than double substitution at the Cα in Aib.[26,27] However, it is reported that the Ac$_6$c residue has folding properties similar to those of Aib. Our studies investigate the structural properties of all of these noncanonical amino acids using peptaibolin and determine which amino acids have a greater tendency to induce α-helical secondary structures (SS) in this peptide. Furthermore, we also investigate the membrane (1-palmitoyl-2-oleoylphosphatidylcholine, POPC) permeability of peptaibolin incorporating each noncanonical amino acid.

MATERIALS AND METHODS

Noncanonical Amino Acid Force Field Parameters. The 3D structure of the α,α-dialkyl glycines was designed with the program PyMOL.[28] The GROMOS topologies (bonded and nonbonded parameters) for each noncanonical amino acid were based on the corresponding amino acid parametrized with the GROMOS 54a7 force field (FF).[29,30] The topologies for each α,α-dialkyl glycine under study are shown in the Supporting Information.

System Preparation. The peptaibolin experimental structure, characterized by Crisma and co-workers,[8] is not available in a database but was kindly provided by these researchers for analysis and comparison. We designed a peptaibolin α-helix structure with PyMOL and nine peptide analogues by replacing the native Aib positions with a α,α-dialkyl glycine and ALA. Aib and ALA were used as control

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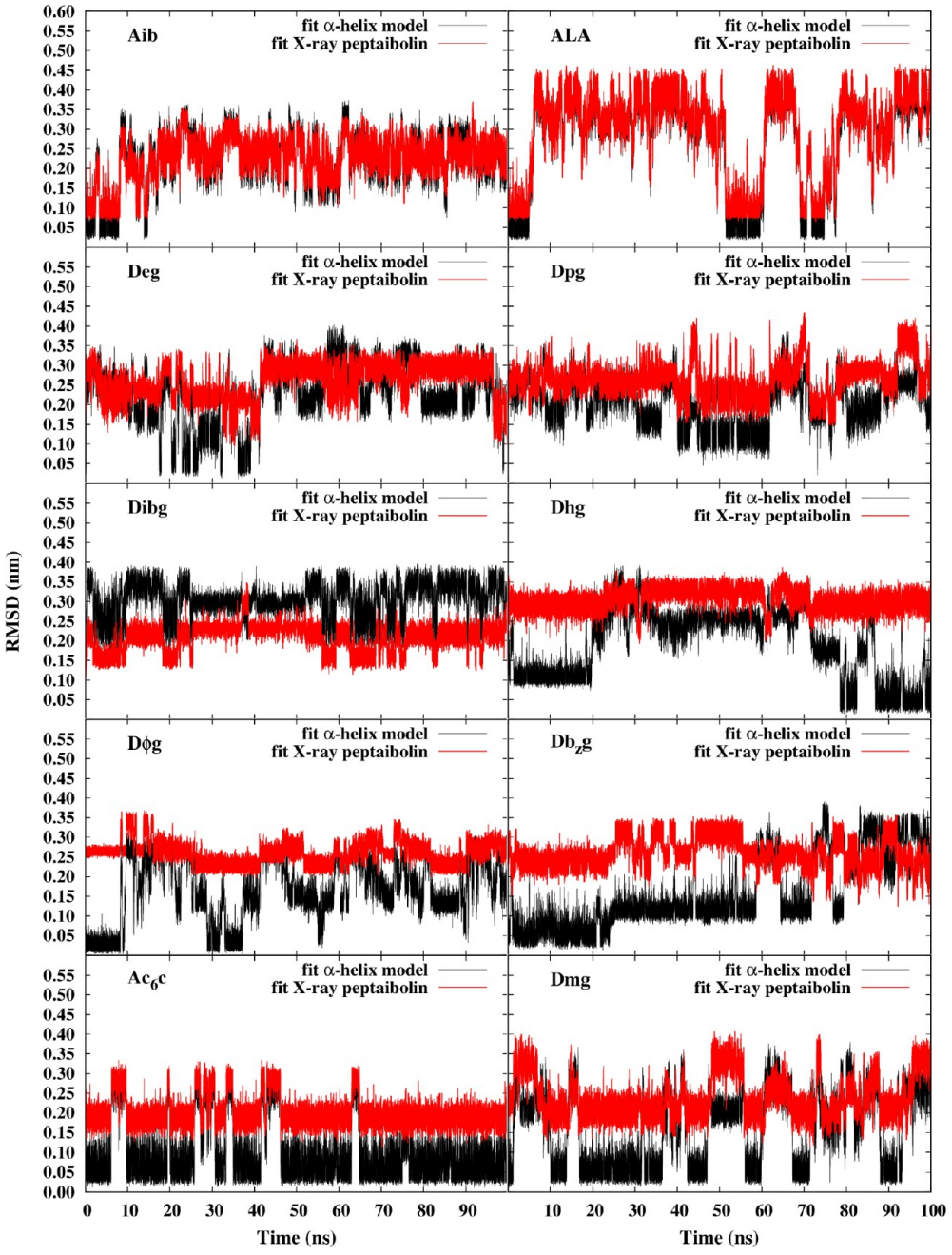

Figure 2. Cα rmsd of peptaibolin and analogues fitting the starting α-helix model vs Cα rmsd of peptaibolin and analogues fitting the X-ray structure of peptaibolin.

amino acids to compare with the properties of the new α,α-dialkyl glycines. These peptidomimetics were named with the three letter code for each α,α-dialkyl glycine that was inserted into the peptide (Figure 1).

Peptaibolin and its analogues were modeled in water, with the simple point charge (SPC) water model,[31] using dodecahedral boxes with a layer of at least 1 nm between the peptides and the walls in all three directions. The systems have about 800−1200 water molecules.

In-membrane simulations were done using a POPC membrane composed of 128 phospholipids, previously equilibrated with water.[32] Each peptide (minimized structure) was manually placed on the surface of the membrane with three different orientations: N-terminus close to the polar heads of the phospholipids, C-terminus near the polar heads, and peptide parallel to the membrane (Figure 6). It was necessary to remove a small number of water molecules to create a cavity for peptide insertion in the aqueous phase of the membrane system. This procedure yielded 30 different peptide−POPC systems (10 peptides with 3 orientations).

Molecular Dynamics Simulations. All simulations were performed using GROMACS, version 4.0.5.[33] The reaction field method, with a cutoff of 1.4 nm and a dielectric constant of 54 for water,[31,34] was used for the treatment of long-range interactions. The van der Waals interactions were also truncated with twin-range cutoffs of 0.8 and 1.4 nm. The LINCS algorithm[35] was used to constrain the chemical bonds of the peptides and SETTLE algorithm[36] in the case of water. The systems were simulated in the isothermal−isobaric ensemble. The temperature (300 K) and pressure (1 atm)

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were controlled using the Berendsen algorithms[37] with coupling constants of τ_T = 0.1 ps and τ_P = 0.5 ps, respectively. In POPC, these parameters were τ_T = 0.2 ps and τ_P = 0.5 ps.

For the peptides in water, three steps of energy minimization were performed. In the first two steps, position restraints were applied to all heavy atoms of the peptides and afterward on the main chain, with a force constant of 1000 kJ·mol^{-1}·nm^{-2}. In the third step of energy minimization, no position restraints were applied. In the peptide POCP systems, one step of energy minimization was done without position restraints.

In water, molecular dynamics (MD) simulations of 100 ns length were done. In peptide−POPC systems, 150 ns of MD simulations was used for each peptide orientation. For both systems, conformations were recorded every 1 ps. It is important to note that no force was applied to peptides to initiate the process of insertion into the membrane.

Analysis. For the peptides in water, the rmsd (root-mean-square deviation) fitted against a conformation modeled with an ideal α-helical secondary structure, Ramachandran plots, and central structure analysis was obtained over all of the conformations from the 100 ns simulations. The central structure of the peptides is obtained from an rmsd matrix that calculates the conformation that minimizes the rmsd variance against all of the conformations of a trajectory, indicating the most representative structure of the simulation. Quantitatively, we calculate a folding free energy using the rmsd analysis, assuming the conformations under the 0.15 nm cutoff to be folding states. For our isothermal−isobaric ensemble, it is possible to estimate the folding free energy as $\Delta G = -RT \ln(N_{\text{folded conformations}}/N_{\text{unfolded conformations}})$. We also calculate a conformational entropy using the quasi-harmonic approximation[38] and the percentage of frames presenting zero, one, two, or three hydrogen bonds of a typical α-helix ($i \rightarrow i + 4$) secondary structure via a hydrogen bond analysis.

For the peptide−POPC systems, we calculate the distance traveled by each peptide into the membrane, that is, the distance traveled along the z axis from the starting point in the aqueous phase into the interior of the membrane. In this analysis, we considered that the origin of this reference (z = 0) is the plane that passes through the middle of the membrane bilayer.

RESULTS AND DISCUSSION

α-Helical Preorganization in Water. Figure 1 shows the 2D structure and sequence (Figure 1A) of the native peptaibolin. Labels in Figure 1A highlight the oxygen and nitrogen atoms expected to form a typical α-helix ($i \rightarrow i + 4$). The α,α-dialkyl glycines that were inserted into the peptaibolin native Aib positions are also shown in Figure 1B.

We calculate two types of peptide Cα rmsd's (Figure 2) for all conformations for each simulation. The first one fits the peptide Cα against the initial structure modeled as an α-helix, and the other one fits the peptide Cα against the experimental structure provided by Crisma and co-workers.[8] The former identifies which noncanonical amino acids promote α-helix conformations, and the latter identifies the preference to induce a more nativelike conformation, similar to the X-ray structure.

Figure 2 shows the time evolution of the Cα rmsd of peptaibolin and its mimetics carrying ALA or a new α,α-dialkyl glycine residue. Every panel has the rmsd fit against a modeled α-helix (black traces) superimposed on the rmsd fit against the X-ray structure (red traces) for each peptide under investigation. For the black traces, the fitting of the conformations was done against the initial structure modeled in the α-helix, thus lower rmsd values (<0.15 nm) are an indication that the peptide structure is close to an ideal α-helix. In this case, the rmsd analysis reveals that the α,α-dialkyl glycines have a different propensity to maintain the peptide structure close to a typical α-helix conformation. Peptaibolin carrying Dpg and Dibg seem to populate conformations

Table 1. Folding Free Energy (ΔG) of Peptaibolin and Analogues in Water Using an rmsd Criterion of 1.5 nm to Define Folded and Unfolded States (Materials and Methods)

peptides	ΔG (kJ/mol)
peptaibolin (Aib)	5.17
ALA	3.56
Deg	4.65
Dpg	3.56
Dibg	17.79
Dhg	1.56
DΦg	0.82
Db_zg	−1.56
Ac_6c	−4.08
Dmg	0.58

Table 2. Percentage of Conformations with Zero, One, Two, or Three Hydrogen Bonds Involved in a Typical $i \rightarrow i + 4$ α-Helix[a]

peptides	0	1	2	3
peptaibolin (Aib)	81.42	12.24	5.49	0.85
ALA	78.42	9.37	10.77	1.44
Deg	82.38	13.73	3.67	0.23
Dpg	72.20	27.62	0.18	0.00
Dibg	100.00	0.00	0.00	0.00
Dhg	81.84	8.62	9.19	0.34
DΦg	77.73	13.25	9.02	0.00
Db_zg	76.88	12.60	10.30	0.22
Ac_6c	39.19	30.71	25.34	4.76
Dmg	61.82	25.13	12.54	0.52

[a]See also Figure 1.

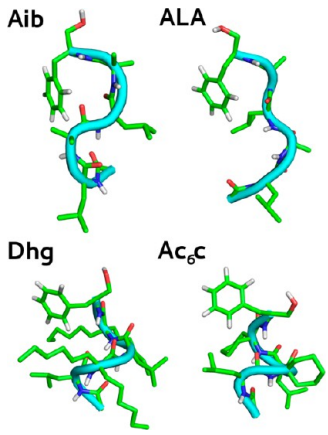

Figure 3. Central structures of peptaibolin and peptidomimetics carrying ALA, Dhg, and Ac_6c from the last 25 ns in water. The coloring of the atoms follows the convention: green for carbon, blue for nitrogen, red for oxygen, white for hydrogen, and cyan to highlight the cartoon that defines the SS. The water molecules were omitted for better visualization.

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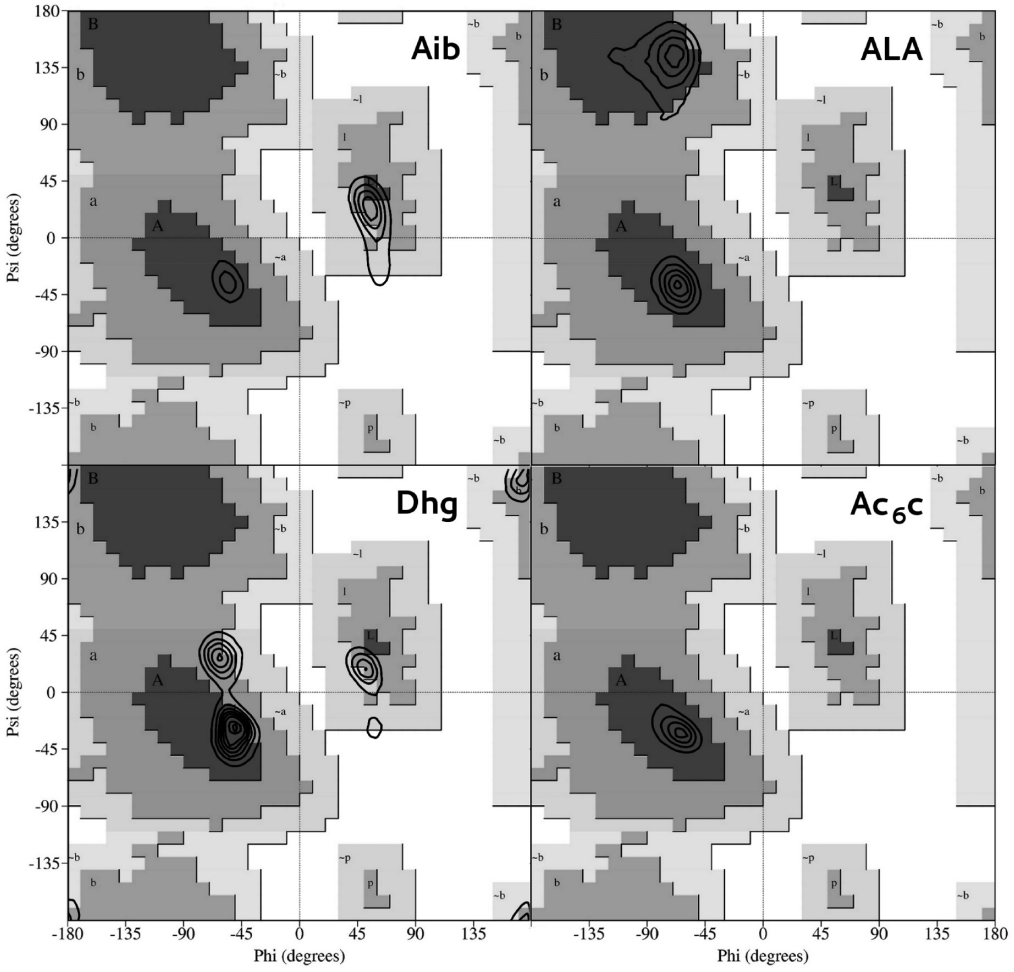

Figure 4. Probability density contours of φ and ψ pairs for amino acids AIB, ALA, Dhg, and Ac_6c in water. These contours are superimposed on the Ramachandran diagram in which region A corresponds to typical dihedrals of the right α-helix, B corresponds to the β-sheet space, and L corresponds to the left α-helix region. The contours levels used were 0.002−0.012 spaced by 0.002 for peptaibolin, 0.001−0.006 spaced by 0.001 for ALA, 0.001−0.009 spaced by 0.001 for Dhg, and 0.005−0.025 spaced by 0.005 for Ac_6c.

Table 3. Peptide Conformational Entropy (S) in Water, Estimated with the Quasi-Harmonic Approximation Method (Materials and Methods)

peptides	S (kJ/mol/K)
peptaibolin (Aib)	1.67
ALA	1.73
Deg	1.88
Dpg	2.10
Dibg	1.88
Dhg	2.78
DΦg	2.26
Db_zg	2.35
Ac_6c	1.30
Dmg	1.92

dissimilar to the initial structure. However, Deg, Dhg, Dϕg, Db_zg, and Dmg peptides have some conformations close to the α-helix structure at several time intervals during the simulation, indicating that these substitutions promote the formation of this type of secondary structure (SS). The peptide with Ac_6c is the one that remains close to an α-helix secondary structure during most of the 100 ns simulation time. Ac_6c is the only Cα cyclized amino acid of this series. Previous results suggest that the Cα ↔ Cα cyclization constrains the main-chain dihedrals even more than the constraint resulting from the double substitution at Cα, as in Aib.[26,27]

The red traces show the Cα rmsd fit to the peptaibolin X-ray structure (Figure 2). We highlight the peptide-containing Dibg residue; it is the only one that presents conformations more similar to the native peptaibolin X-ray structure compared to an ideal α-helix. For the peptides more similar to sample α-helical structures, like the ones containing Dhg, DΦg, Db_zg, Ac_6c, and Dmg, we see a shift in the rmsd's (fit against the native structure) to higher values. This fact is an indication that these α,α-dialkyl glycines tend to induce structures closer to typical α-helical conformations than to the native peptaibolin X-ray structure.

We estimate a folding free energy (ΔG) for the modeled peptaibolin and analogues (Table 1) using the rmsd data fitted against an ideal α-helical peptaibolin reference structure.

Table 1 demonstrates quantitatively the same trend observed on the black rmsd traces shown in Figure 2: the peptides carrying Ac_6c and Db_zg demonstrate the preference to induce α-helical folded states of peptaibolin; DΦg and Dmg also have a reasonable number of folding states, resulting in a folding ΔG close to zero. The peptide carrying Dibg has the highest free energy, indicating that this residue induces a significant deviation from the initial α-helix structure.

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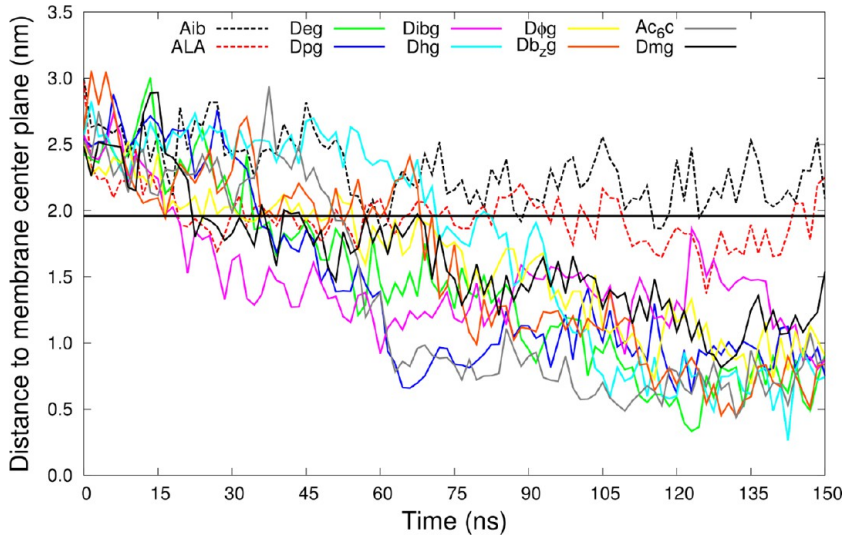

Figure 5. Center of mass position of each amino acid along the *z*-axis component of the simulation box. Peptides migrate spontaneously from the aqueous environment toward the center of the membrane during the 150 ns simulation time. The line at 1.96 nm corresponds to the water/membrane interface.

Hydrogen bond analysis was employed to check if the peptides have the intramolecular interactions expect to form an α-helix (in Figure 1, C═O0-H-N4, C═O1-H-N5, and C═O2-H-O). Table 2 shows, for each peptide, the percentage of conformations sampled over the entire 100 ns simulation with zero, one, two, or three hydrogen bonds.

The analysis of the helicity based on the backbone hydrogen bonds agrees with the previous analysis. Residue Ac$_6$c is the most capable of sample conformations with an α-helix structure; the percentage of frames with one, two, or three $i \rightarrow i + 4$ hydrogen bonds corresponds to 61% of the sampled conformations. The peptides carrying Dmg, Db$_z$g, and DΦg also present a high percentage of conformations with $i \rightarrow i + 4$ hydrogen bonds.

Figure 3 shows the central structures obtained for the last 25 ns of simulation in water for peptaibolin and the peptidomimetics with ALA, Dhg, and Ac$_6$c. (Supporting Information Figure S1 shows the central structure of peptaibolin, ALA, and Dhg at other time intervals.)

The central structures shown in Figure 3 clearly reveal that the peptides carrying Dhg and Ac$_6$c have an α-helical conformation whereas Aib and ALA promote peptide unfolding. Dhg is the bulkiest aliphatic noncanonical amino acid and Ac$_6$c is the only one cyclized at the Cα, suggesting that these characteristics seem to promote the folding of the peptide into helical SS. Ramachandran plots (Figure 4) help us to understand the individual geometrical properties of the main-chain dihedrals for each amino acid incorporated into peptaibolin. The distribution was calculated from the ψ and φ angles recorded from the two Aib positions of peptaibolin, replaced by ALA or by any α,α-dialkyl glycine. A total of 200 000 points were used to calculate the probability densities shown in Figure 4 (1 peptide × 2 residue positions × 100 000 conformations) and are displayed as probability density contours. We show the main-chain dihedral geometry sampled by Aib, ALA, Dhg, and Ac$_6$c. The Ramachandran diagrams for the other noncanonical amino acids discussed in this work are shown in the Supporting Information (Figure 2S).

Figure 4 shows, as expected, that ALA has greater conformational freedom than Aib. ALA sampled conformations corresponding to the β-sheet and right α-helix regions, whereas Aib explores conformations only of right and left helices. The double substitution at the Aib Cα eliminates the β-sheet conformations present in ALA, restraining the conformational space toward right- and left-helical SS. Dhg main-chain dihedrals show a distribution of angles in helical regions but can also explore extended conformations ($\varphi \approx \pm 180°$, $\psi \approx \pm 180°$). The Ac$_6$c Ramachandran plot clearly shows that the dihedral pairs are exclusively constrained in the right α-helix space. This fact is clear evidence of the greater tendency of the peptide to adopt an α-helix SS as observed from the previous rmsd data (Figure 2). Although we cannot establish a rule stating that residues that populate main-chain dihedral angles of the Ramachandran plot typical of α-helices will lead to peptides rich in α-helical SS, we consider that this fact can be an indication of this effect.

The conformational entropy (S) of peptaibolin and its mimetics were estimated using a quasi-harmonic approximation.[38]

The conformational entropy indicates that the peptidomimetic containing Ac$_6$c has the lowest conformation entropy among the peptides under study. This fact indicates that the peptide is highly constrained, leading to the conclusion that Ac$_6$c not only induces α-helix SS in peptaibolin but also imposes lower conformation freedom on this peptide. The peptides containing the residues with short side chains—Aib, ALA, Deg, and Dmg—have conformational entropy lower than for the peptides carrying large, bulky side chains such as Dpg, DΦg, and Db$_z$g with the exception of Dibg, which seems to be restrained in an unfolded state.

Spontaneous Insertion in POPC Membranes. Experimentally, peptaibolin shows activity against gram-positive bacteria, as reported by Hulsmann and co-workers, but to date, no mechanism of insertion into membranes has been reported.[1,9] Our membrane permeation study of peptaibolin and analogues brings new knowledge about the insertion mechanism into membranes and, most importantly, about the effect of each noncanonical amino acid on this process. Our modeling experiments were done on a POPC membrane previously equilibrated in water. We emphasize that no

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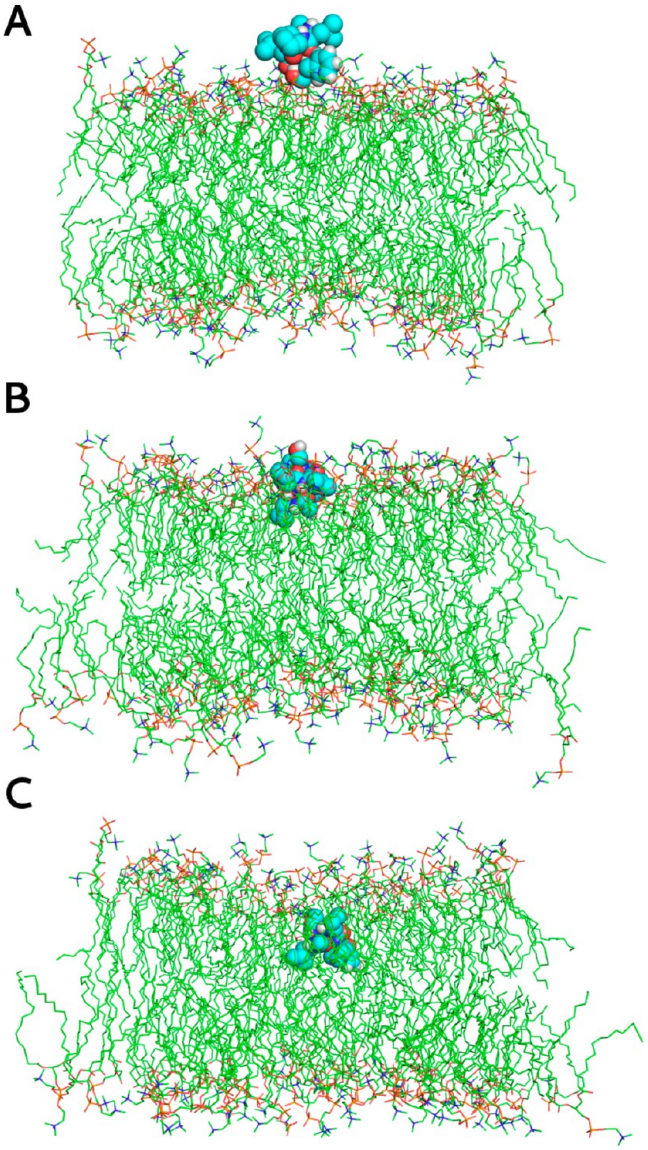

Figure 6. Snapshots of the spontaneous insertion of the Ac_6c analogue into a POPC membrane. Water molecules were omitted for better visualization. Snapshots: (A) t = 0, (B) t = 55 ns, and (C) t = 150 ns.

potential was applied to the peptides to promote membrane insertion. All peptides were manually placed on the surface of the membrane with three different orientations, close to the polar heads of the phospholipids.

Figure 5 shows the spontaneous insertion of the noncanonical peptidomimetics into a POPC membrane. This figure shows the distance traveled for each peptide (one peptide orientation only). We did not observe a specific tendency for one specific replicate. We observe that peptaibolin and the peptidomimetics incorporating ALA are preferentially located on the surface of the membrane. Our modeling experiment shows that, apparently, there is no spontaneous insertion of these two peptides in the POPC membrane. Although there is no experimental information regarding the membrane permeation properties of peptaibolin, we cannot conclude that this process will not occur in in vitro experiments or under physiological conditions. However, the peptidomimetics incorporating the new noncanonical amino acids permeate the POPC membrane in a spontaneous way. The peptide with Dmg, a polar α,α-dialkyl glycine, permeates the membrane and remains on the POPC water interface close to the polar heads of phospholipids. However, the peptide carrying the apolar noncanonical residues shows a greater tendency to enter the POPC membrane. This fact is somehow expected from the apolar characteristics of these residues. It must be emphasized that we cannot conclude that greater permeation will lead to improved antimicrobial activity without further experimental studies. Nevertheless, it is evident so far that peptaibolin incorporating Ac_6c was demonstrated to be able to permeate POPC membranes in a spontaneous way, in addition to his greater tendency to adopt a rigid α-helical SS. In light of the current models of action of these peptides, the helical form is considered to be the ideal conformation for the biological activity and the formation of barrel-stave-type channels;[39] consequently, we can assume that the preorganization in the α-helix of this peptide might help to reduce the structural rearrangement necessary to penetrate the membrane and formation of membrane channels. Figure 6 displays three frames corresponding to the spontaneous insertion of the peptide with Ac_6c into a POPC membrane at three simulation times: t = 0, 55, and 150 ns.

To investigate if the peptides that translocate into the membrane environment also adopt conformations close to an α-helical structure and if there is any correlation between the α-helical conformation and membrane permeation, we calculated the rmsd (fitted against the model α-helix conformation) and plotted this data as a function of the distance traveled toward the center of the POPC membrane (Figure 7). It is clear that peptaibolin and the peptide replaced with ALA remain on the surface of the membrane (Figure 7, top panels). However, α,α-dialkyl glycines Deg, Dpg, Dibg, Dhg, Db_2g, and Dmg promote the peptide insertion into the POPC membrane, as observed before. However, the conformations of these peptides that are found inside the membrane environment (d < 1.96 nm) have high rmsd values, indicating that they are quite dissimilar from the initial α-helix structure used for fitting. The peptides carrying DΦg and Ac_6c also permeate the POPC membrane and have low Cα rmsd values, indicating a more nativelike conformation similar to the α-helix. These results clearly indicate that the Ac_6c noncanonical residue promotes the folding of peptaibolin into helical SS in aqueous and membrane environments; however, we cannot establish a correlation between α-helical preorganization and membrane permeability. We have to clarify that current models of membrane disruption by these peptides, such as the barrel stave-type channels,[39] imply that more than one peptide monomer is necessary to disrupt the membrane. This suggests that these peptides could adopt α-helical structures in a cooperative way in the presence of more monomers. This aspect will be the subject of future studies.

CONCLUSIONS

Our findings on the insertion of α,α-dialkyl glycines in small peptaibols suggest that some noncanonical residues are more capable of inducing α-helical conformations and promoting spontaneous membrane permeation than the native Aib in peptaibolin. However, there is no correlation between the acquired helicity and membrane permeability because some of the peptides permeated the membrane without adopting α-helical conformations in either an aqueous or membrane environment. We demonstrate that Dhg and Ac_6c are able to maintain a structure closer to the reference one modeled in the

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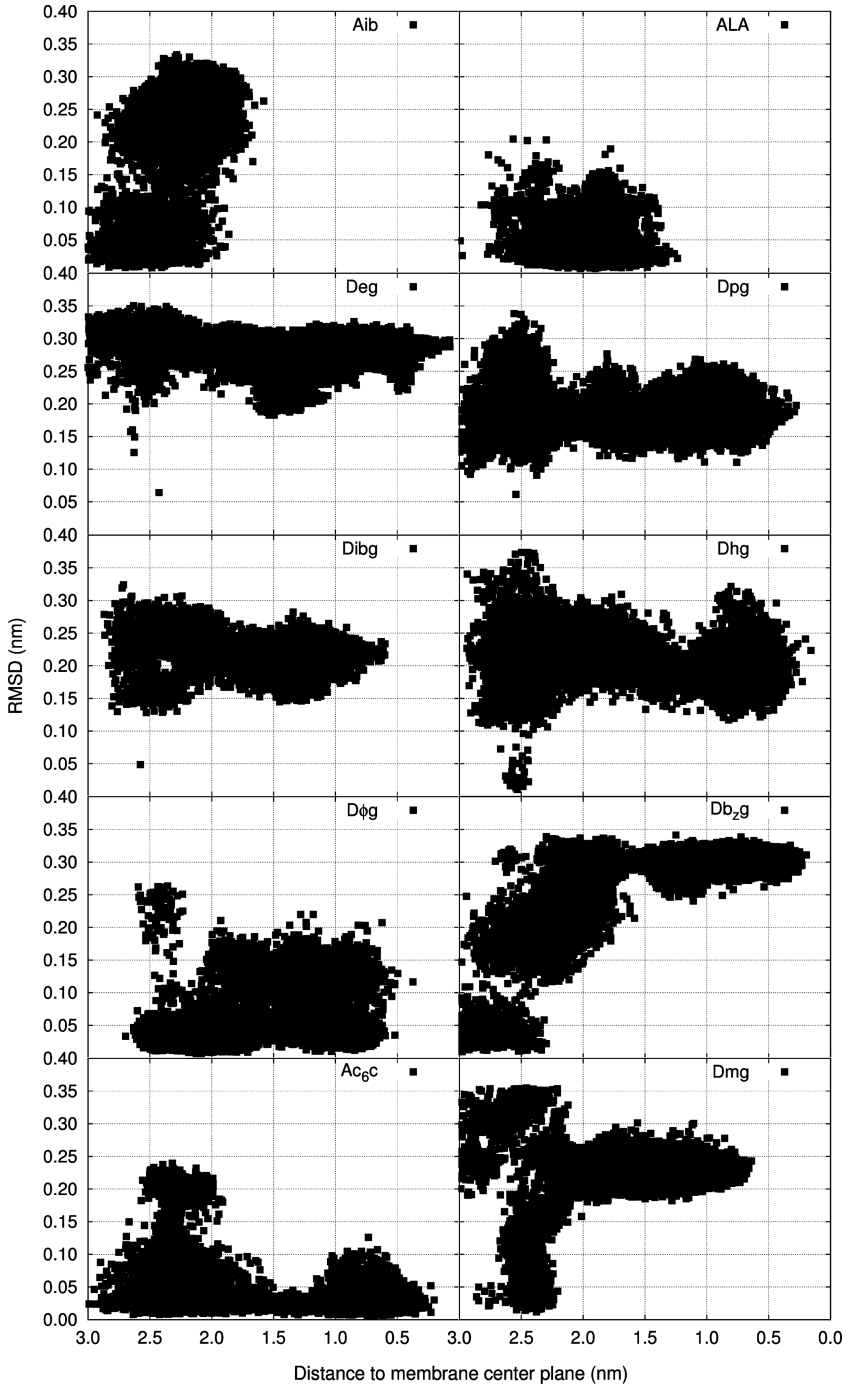

Figure 7. Cα rmsd of peptaibolin and analogues along the spontaneous insertion into the POPC membrane. Fitting of Cα relative to the minimized structure of each peptide.

α-helix. In fact, Ac$_6$c is the most efficient residue in constraining the conformations of the peptide close to the modeled structure in the α-helix. Furthermore, Ramachandran plots show that the dihedral pair of this residue explores only the right α-helix regions. These facts could be explained by the Cα cyclization of this residue that constrains its main-chain dihedrals. Furthermore, all nonpolar noncanonical amino acids promoted spontaneous membrane peptide permeation. This fact is somehow expected because of the apolar characteristics of these residues. Only peptaibolin and the corresponding peptidomimetic incorporating ALA did not permeate the membrane in our simulations; however, this result must be further validated experimentally. We emphasize that the peptide incorporating the Ac$_6$c residue also induces nativelike conformations inside the membrane environment. Maintaining the helicity of this peptide in the membrane environment could improve the antimicrobial properties of this peptide, but further experimental studies will be required to validate this hypothesis. If seems thus far that the Ac$_6$c noncanonical residue is a versatile residue that is capable of inducing helical conformations in short peptides, inducing lower conformational flexibility, and improving membrane permeability.

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We propose that peptaibolin peptidomimetics incorporating Ac_6c residues might improve their structure and antimicrobial function, and for this reason, further investigations of peptides incorporating Cα-cyclized residues will be presented in the near future.

■ ASSOCIATED CONTENT

Supporting Information

All parametrizations for the new amino acids discussed in this article. Central structures of peptaibolin and analogues with ALA and Dhg at different time intervals and Ramachandran plots for noncanonical amino acids Deg, Dpg, Dibg, DΦg, Db_2g, and Dmg. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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