Influence of Carboxyamidation on the Activity of Mastoparan-AF Peptides: a ^{1}H NMR Structural Study

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Abstract: *Antimicrobial peptides display different types of post-translational modification, such as amidation in the C-terminal region, which can modify their activity significantly. The eumenine mastoparan-AF peptide, (EMP-AF-NH_2, INLLKIAKGIIKSL-NH_2), and its analogue containing a carboxylate C-terminal (EMP-AF-OH), characterized by a reduced antimicrobial activity, were investigated using Circular Dichroism spectroscopy (CD) and ^{1}H NMR. CD measurements suggest that both peptides are random in water and undergo a helical conformational transition in the presence of 30% TFE or in the presence of SDS micelles. The helical content is higher in the carboxyamidated peptide. In order to understand the reason for their different activity, the three dimensional structures of the two peptides were determined in 30%TFE:70%H_2O solvent mixture by means of 2D ^{1}H-NMR. The experiments were carried out on a Varian Inova 500AS spectrometer, operating at 11.7T. The NMR data show that the carboxyamidated peptide displays a larger number of side chain NOEs, suggesting a higher structural organization of this peptide with respect to EMP-AF-OH. The calculated NMR-derived 3D models for the two peptides show, for EMP-AF-NH_2, the presence of a helical segment spanning residues Leu3-Leu14. In the case of EMP-AF-OH, the helix extends only through residue Leu3-Lys8. It appears therefore that the presence of a negative charge at the peptide C-terminus may interfere with the α-helix macro-dipole partially destabilizing the peptide secondary structure. Studies are in progress to describe in more details the possible correlation between the reduced activity and the structure instability of the carboxy form of the peptide.*

Antimicrobial peptides display different types of post-translational modifications, such as amidation at the C-terminus, which can significantly modify their structure. . In fact, studies by Konno et al.[1] have shown that the eumenine mastoparan-AF (EMP-AF-NH_2, INLLKIAKGIIKSL-NH_2) has a greater biological activity than that of its analogue containing a carboxylate C-terminal (EMP-AF-OH). On the basis of this evidence, we undertook a comparative study of the structure-function relationship of these two peptides by using Circular Dichroism (CD) and Nuclear Magnetic Resonance (^{1}H NMR) spectroscopies

CD spectroscopy (Jasco 810 spectropolarimeter), ^{1}H NMR (Varian Inova 500AS spectrometer operating at 11.7T) and molecular modeling have been used to study the structure of the two peptides in 30%TFE:70%H_2O solvent mixture. The peptide

resonance peaks were assigned by means of standard methods (DQF-COSY, TOCSY and NOESY). Data were processed using the nmrPIPE/nmrVIEW[2] software. To obtain the distance constraints, cross-peaks volumes were estimated from the 300 ms NOESY spectra. The 3D structure of EMP-AF-NH_2 and EMP-AF-OH were computed using simulated annealing methods in the DYANA[3] refine module.

CD spectra (Figure 1), show that both peptides change from an unordered state in water to a helical conformation either in a 30%TFE:70% H_2O mixture either in the presence of 8 mM SDS micelles. Nonetheless, EMP-AF-NH_2 shows a higher helicity than EMP-AF-OH. Figure 2 reports NOE patterns and $^3J_{NH-\alpha}$ coupling constants for both peptides.

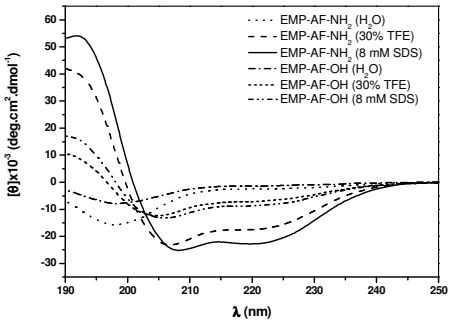

Figure 1. CD spectra in the far-UV region of EMP-AF-NH_2 and EMP-AF-OH, pH 4.5 at 20 °C in water, 30% TFE aqueous solution and 8 mM SDS.

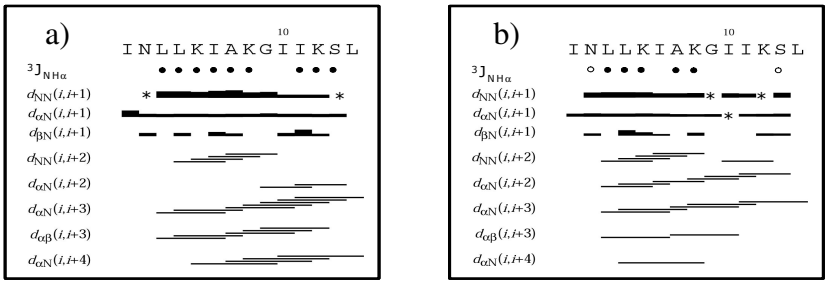

Figure 2. Summary of the sequential and medium-range NOE connectivity for (a) EMP-AF-NH_2 and (b) EMP-AF-OH in 30%TFE:70%H_2O, pH 4.5 at 20°C. The intensities of the observed NOEs are represented by the thickness of lines and were classified as strong, medium and weak, corresponding to upper bound constraints of 2.5, 3.5 and 5 Å, respectively. The stars indicate potential NOE connectivity that could not be obtained due to resonance overlap. $^3J_{NH-\alpha}$ < 6.0 Hz are indicated by filled circle and $^3J_{NH-\alpha}$ > 7.0 Hz by open circle.

The greater density of $d_{\alpha N}(i,i+3)$, $d_{\alpha N}(i,i+4)$ and $d_{\alpha\beta}(i,i+3)$ NOEs in the carboxyamidated peptide and the $^3J_{NH-\alpha}$ of less than 6.0 Hz confirm the higher helicity observed in the CD spectra. In particular, NMR data not only indicate that the α-helix extends over a larger number of residues in EMP-AF-NH_2 in comparison with EMP-AF-OH (L3 toL14 and L3 to K8, respectively). They also suggest that

the helical secondary structure is quite stable in the amidated peptide.

Figure 3 displays the superposition of the 20 final models of EMP-AF-NH_2 and EMP-AF-OH (RMSD 0.10 ± 0.02 and 0.47 ± 0.11, respectively) in the region 3-14. An amphipatic helical fold encompassing residues 3-14 is better defined for the carboxyamidated peptide. The amphipatic nature of the helix is evidenced by the distribution of charged residues with respect to the hydrophobic ones. Interestingly, EMP-AF-NH_2, besides displaying a larger number of side chain NOEs, presents a structural backbone NOEs involving CH_α of residues I11, K12 and S13 with the NH_2 terminal.

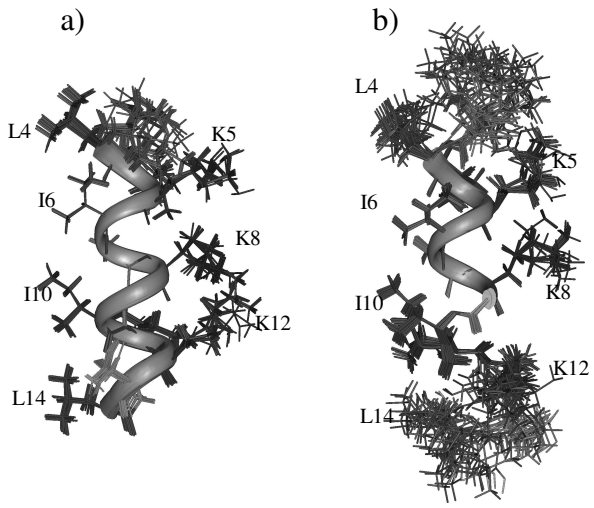

Figure 3. Superposition of final 20 calculated structures of (a) EMP-AF-NH_2 and (b) EMP-AF-OH in 30 % TFE at 20°C based on the minimum pair wise RMSD of the peptide backbone spanning residues 3 to 14. The C-terminus is shown at the bottom.

It appears therefore that the presence of a negative charge at the peptide C-terminus of EMPAF-OH may interfere with the α-helix macro-dipole, partially destabilizing the peptide secondary structure. Studies are in progress to verify the possible correlation between the reduced activity and structural features of the carboxy form of EMP-AF.

Acknowledgements

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