The High Resolution Structural Characterization and Detection of Fast Motions in Antimicrobial Piscidins Provide Insight about their Mechanism of Action

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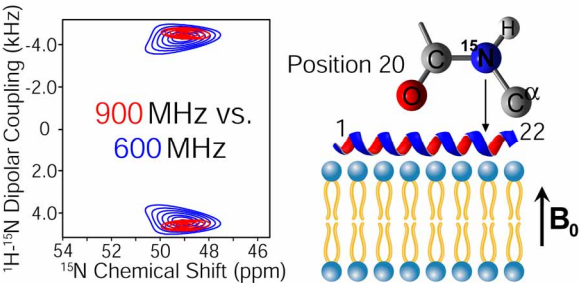

Figure 1. ^{15}N-^{1}H PISEMA spectrum of ^{15}N-Leu$_{20}$ amidated piscidin 3 in hydrated 3:1 DMPC/DMPG at pH 6, and T = 40ºC, 900 (red) and 600 MHz (blue).

Amidated and non-amidated piscidins 1 and 3, amphipathic cationic antimicrobial peptides from fish[1,2], were studied to characterize structural and dynamic similarities and differences between these peptides and better understand the structural motifs involved in functional activity and diversity among amidated and non-amidated isoforms. Site-specific high-resolution solid-state NMR orientational and distance restraints were obtained from ^{15}N- and ^{13}C/^{15}N-labeled amidated and non-amidated piscidins 1 and 3 in the presence of hydrated oriented lipid bilayers. REDOR (Rotational Echo DOuble Resonance)[3] distance measurements results indicate α-helicity while PISEMA (Polarization Inversion Exchange at the Magic Angle)[4,5] data, as exemplified in Figure 1, show that the peptides adopt an orientation parallel to the membrane surface. In this orientation at the water-bilayer interface, peptide-lipid interactions appear to be enhanced for these amphipathic cationic antimicrobial peptides. Figure 1 also indicates that enhanced resolution is obtained at 900 MHz, which points at a significant advantage of ultra high field magnetic field NMR for the structural determination of multiple-labeled peptides and proteins. To investigate peptide dynamics, ^{15}N solid-state NMR data was obtained from oriented samples positioned so that the bilayer normal was perpendicular to B_0 ("90° orientation"). As shown in Figure 2 for piscidin 1, the results indicate that the peptides experience fast, large amplitude backbone motions around an axis parallel to the bilayer normal above the lipid phase transition temperature. Under the conditions tested here, amidation does not affect the structural and dynamic parameters studied to this day. In light of similarities between piscidins 1 and 3, we hypothesize that their topology and fast dynamics work synchronically to define their mechanism of action.

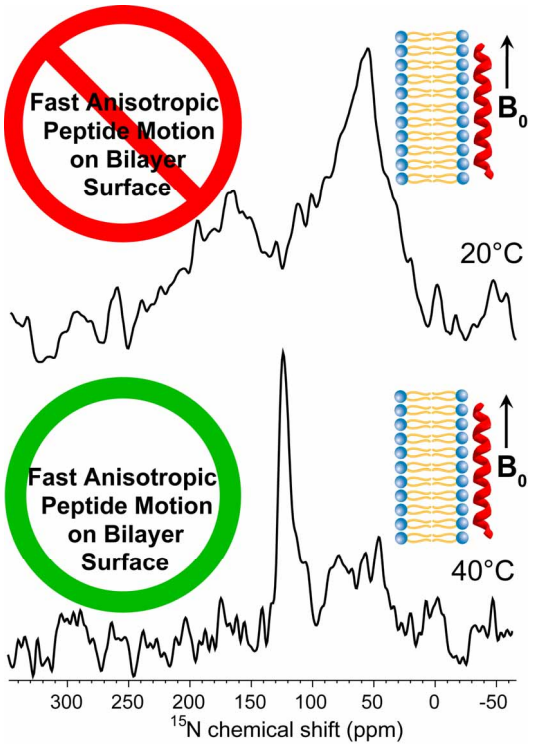

Figure 2. Proton decoupled ^{15}N spectrum of ^{15}N-Val$_{12}$ piscidin 1 in oriented 3:1 DMPC/DMPG lipid bilayers at 20 and 40 ºC. The sample was oriented so that the bilayer normal was perpendicular to the static magnetic field, B_0 ("90° orientation"). The spectra were referenced to saturated aqueous solution of $^{15}NH_4NO_3$.

References

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