HIGH RESOLUTION HETERONUCLEAR CORRELATION SPECTROSCOPY IN SOLID-STATE NMR OF ALIGNED SAMPLES

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Introduction

*P*olarization *i*nversion *s*pin *e*xchange at the *m*agic *a*ngle (PISEMA)[1] has been widely used to obtain high-resolution orientational restraints (i.e., anisotropic ^{1}H-^{15}N dipole-dipole couplings and ^{15}N chemical shifts) from membrane proteins in a lamellar phase lipid environment. In this report, we demonstrate that an additional ^{1}H anisotropic chemical shift restraint from the peptide plane can be obtained by a novel high-resolution heteronuclear correlation spectroscopy, which provides valuable complementary information for structural analyses.

Experimental

The ^{1}H-^{15}N correlation experiments were performed on a Bruker Avance 600 NMR spectrometer at NHMFL. The *m*agic *s*andwich *h*igh *o*rder *t*runcation (MSHOT) homonuclear decoupling sequence [2] was used in the t_1 dimension. A short cross polarization contact time of 50 μs was used to ensure that the ^{15}N magnetization was transferred from its closest ^{1}H. The ^{1}H B_1 fields were 62 and 40 kHz during the MSHOT decoupling and cross polarization, respectively, while the ^{15}N B_1 field of 40 kHz was used for cross polarization and ^{15}N decoupling in the t_1 dimension. A total of 48 t_1 increments were used with a recycle delay of 4 s. For each increment, 512 scans were used for signal accumulation. A line-broadening of 1200 Hz was applied to the t_1 dimension before Fourier transform.

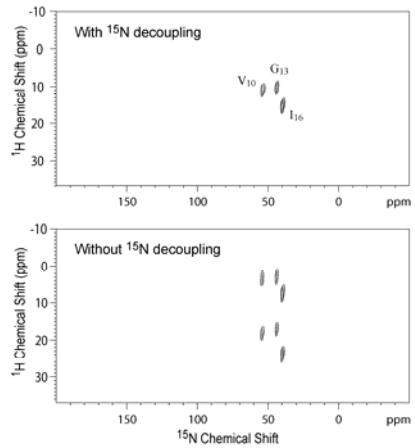

Fig. 1. ^{1}H-^{15}N correlation spectra of ^{15}N-$V_{10}G_{13}I_{16}$ p1-NH_2 oriented in hydrated 3:1 DMPC/DMPG at 1:20 peptide-to-lipid ratio, pH6, with (Top) and without (Bottom) ^{15}N decoupling during the ^{1}H chemical shift evolution. In the spectra, the scaling factor has been taken into account in the ^{1}H chemical shift dimension. The ^{15}N chemical shift was referenced to the ^{15}N resonance peak of saturated $^{15}NH_4NO_3$ solution at 0 ppm, while the ^{1}H chemical shift was referenced to the water signal at 4.7 ppm of the solution.

Results and Discussion

Piscidins 1 (p1) an Amphipathic Cationic Antimicrobial Peptides (ACAPs) from the mast cells of animals, believed to play a crucial and direct role in the fight against many aquatic bacterial infections[3,4], were structurally analyzed using solid state NMR. P1 was structurally characterized by PISEMA experiments[5,6] and simulations to be α-helical and oriented perpendicular to (~89º) the bilayer normal. Detailed characterization of the p1 structure is a necessary step in the search for relationships between structural motifs, interactions with biological membranes, potency, and mechanisms of action. Fig. 1 shows the ^{1}H-^{15}N correlation spectra of ^{15}N-$V_{10}G_{13}I_{16}$ p1-NH_2 oriented in hydrated bilayers. In addition to the ^{15}N chemical shifts and ^{15}N-^{1}H dipolar couplings, as obtained in the PISEMA experiments, the correlation spectra provide the ^{1}H chemical shift information, which may give new insights about the interactions of the peptide with the bilayer and aqueous environment.

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