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High Resolution Heteronuclear Correlation NMR Spectroscopy of an Antimicrobial Peptide in Aligned Lipid Bilayers: Peptide−Water Interactions at the Water−Bilayer Interface

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Since the earliest demonstration[1] that high-resolution structural constraints could be obtained by solid-state NMR (ssNMR) of proteins in anisotropic environments, high-resolution structural NMR studies of membrane-bound proteins and peptides oriented in "native-like" hydrated lamellar-phase lipid environments have intensified.[2] Information from these studies feed a larger body of knowledge aimed at improving our understanding of structure−function relationships in biomolecules that perform vital functions at cell membranes. With improved resolution in recently developed Separated-Local-Field (SLF) experiments,[3] the correlation between the orientation-dependent, anisotropic ^{1}H−^{15}N heteronuclear Dipolar Couplings (DCs) and ^{15}N Chemical Shifts (CSs) has become an effective way[4,5] to characterize the helical tilt, τ (i.e., the angle between the peptide helical axis and the bilayer normal), of uniformly labeled aligned proteins and peptides. However, until recently,[6−8] the CSs of amide protons received little attention in the structural and topological studies of aligned proteins or peptides, although they may yield complementary information, such as hydrogen bonding partnership and geometry in peptide planes.[8,9] With this regard, we demonstrate in this communication the resolution improvement of ^{1}H−^{15}N Heteronuclear Correlation (HETCOR)[7] NMR spectroscopy at high static magnetic field, B_0, and its advantages in characterizing both the topology and solvent interactions of $^{15}N_\alpha$-labeled piscidin aligned in hydrated lipid bilayers.

Piscidin, an Amphipathic Cationic Antimicrobial Peptide (ACAP) from fish, is believed to play a fundamental and direct role in the fight against many aquatic bacterial infections.[10,11] The recent surge in bacterial resistance to the most potent conventional antibiotics has motivated active research on ACAPs since they evidence little, if any, resistance effects.[12] The actual mode of action of most ACAPs remains undetermined[13] even though some theoretical models, which involve interactions with cell membranes,[14−17] exist.[18−22] A better understanding of structure−function relationships in ACAPs, as needed to design improved therapeutics,[20,23,24] may be obtained by analyzing the high-resolution structures of their membrane-bound states. Our prior high-resolution ssNMR studies[25,26] have indicated that piscidin isoforms 1 and 3 (p1 and p3), which differ significantly in their antimicrobial and hemolytic effects, adopt an α-helical structure and lie almost in the plane of the bilayer, where they experience fast motion about the bilayer normal. Since piscidins are well adapted to partition at the water−bilayer interface,[25−27] and piscidin−bilayer interactions may have biological implications, they represent a suitable ground to test ^{1}H−^{15}N HETCOR to not only obtain high-resolution structures of peptides and proteins but also gain insight into their interactions with bilayers and the aqueous environment.

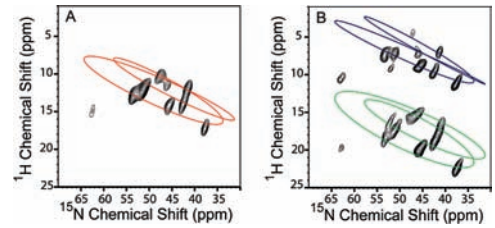

Figure 1. Anisotropic 2D ^{1}H−^{15}N correlation spectra of a 10-site ^{15}N-labeled p1 peptide oriented in lipid bilayers as recorded at 21.1 T. (A) HETCOR. (B) dipolar encoded-HETCOR with DCs. The curves (red, green, purple) were simulated using the ^{15}N and ^{1}H CS tensors of an ideal α-helix that has a tilt of 86° with respect to B_0 and the bilayer normal.

Figure 1A shows the 2D HETCOR spectrum of amidated ^{15}N−$I_5F_6G_8I_9V_{10}V_{12}G_{13}I_{16}L_{19}V_{20}$ p1 oriented in lipid bilayers. As described previously,[26] this peptide sequence FFHHIFRGIVHVGK-TIHRLVTG was chemically synthesized and a hydrated sample aligned on glass plates was prepared by mixing dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol in a 3:1 molar ratio and adding p1 at a 1:20 peptide/lipid molar ratio. At 21.1 T, nine resonances are identified in Figure 1A with the ^{15}N CSs spreading from 37 to 63 ppm, in contrast to values of ∼180 ppm for transmembrane peptides.[2] As shown in the Supporting Information, the ^{1}H−^{15}N HETCOR resonances of an ideal α-helix form characteristic patterns, from which τ values can be uniquely determined without resonance assignments. The simulated "red" curve in Figure 1A indicates that τ for p1 is 86° ± 4° with respect to B_0 and the bilayer normal, which is consistent with prior general observations on singly labeled p1.[25,26] While the ^{15}N resonance at 63 ppm does not fit the simulation in Figure 1A, presumably due to variations in the local chemical environment of p1, its ^{1}H−^{15}N DC follows the trend of the other residues, implying that the whole helix adopts a relatively constant orientation.

When the ^{1}H CSs are encoded by the ^{1}H−^{15}N DCs, the dipolar-encoded HETCOR (de-HETCOR) spectrum of the same sample (c.f. Figure 1B) displays two distinct groups of resonances split by the DCs. Unlike the SLF spectra[4,5] where the DCs result in two identical wheels mirrored along the zero frequency in the ^{1}H−^{15}N DC dimension, the de-HECTOR spectrum shows two asymmetric resonance patterns. For the upper group (i.e., in the low ^{1}H CS range) in Figure 1B, 10 resonances are clearly identified, implying that the resonances at the ^{15}N CS of ∼52 ppm, which were overlapped in the HETCOR spectrum, are now resolved in the de-HETCOR spectrum. The curves simulated for $\tau = 86° \pm 4°$ fit the experimental data very well, confirming again that the peptide helical axis is almost perpendicular to the bilayer normal.

Figure 2 shows the ^{15}N and ^{1}H slices taken from the de-HETCOR spectra of the 10-site labeled sample at different B_0 strengths for

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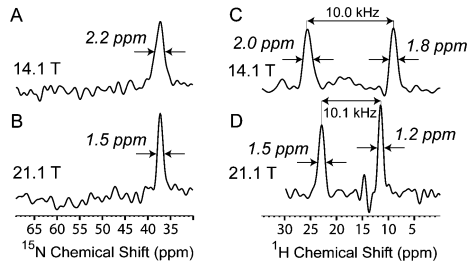

Figure 2. ^{15}N and ^{1}H slices taken from de-HETCOR p1 spectra recorded at two B_0 strengths for the ^{15}N CS resonance at 37.8 ppm. (A and C) ^{15}N and ^{1}H slices at 14.1 T; (B and D) ^{15}N and ^{1}H slices at 21.1 T. The splittings shown in (C) and (D) indicate the ^{1}H−^{15}N DC for this ^{15}N labeled site.

the ^{15}N resonance at 37.8 ppm. The ^{15}N line width (LW) at half-height was 2.2 ppm at 14.1 T and 1.5 ppm at 21.1 T. Similarly, the ^{1}H LW at half-height of the two split resonances decreased from 2.0 (left) and 1.8 ppm (right) to 1.5 (left) and 1.2 ppm (right) when B_0 increased from 14.1 to 21.1 T. That is, the improvement in spectral resolution at 21.1 T in both the ^{1}H and ^{15}N CS dimensions, as measured by the ratio of the LWs, reached ∼150%, commensurate with the increase in B_0, implying that the absolute LWs in unit of hertz remained almost the same even though B_0 increased. This result indicates that the uniform enhancement of resolution in both the ^{1}H and ^{15}N dimensions at 21.1 T is due to a combination of favorable shear alignment between glass plates and anisotropy of the diamagnetic susceptibility at higher B_0[28]. With simultaneously improved resolution of ^{1}H and ^{15}N CSs as well as ^{1}H−^{15}N DCs at higher B_0, de-HETCOR emerges as an advantageous technique for structural studies of multiply labeled aligned membrane-bound species that intrinsically yield crowded spectra.

An important aspect of HETCOR spectra is to provide information about the chemical environment of amide protons. Figure 3 shows the HETCOR spectrum of ^{15}N−K_{14} p1 oriented in lipid bilayers. The ^{15}N-oriented peak at 53.8 ppm correlates with two ^{1}H resonances at 10.7 and 4.1 ppm. As indicated by the red curve in Figure 1A, the resonance at 10.7 ppm originates from the backbone amide proton of K_{14}, while the resonance at 4.1 ppm appears to be the isotropic signal from bulk water protons. Such a resonance at ∼ 4 ppm is also present in the HETCOR spectrum of ^{15}N−H_4 p1 oriented in hydrated lipids (data not shown), but not observed in the spectrum of the ten-site labeled p1 that features ^{15}N labels on the hydrophobic side of the amphipathic helix. This suggests that the unique crosscorrelations in the 2D HETCOR spectra of ^{15}N−H_4 p1 and ^{15}N−K_{14} p1 may be due to the location of H_4 and K_{14} on the hydrophilic side of piscidin's amphipathic helix (c.f. Figure 3). The slice shown in Figure 3 indicates that the ^{1}H LWs for the resonances at 10.7 and 4.1 ppm appear to be broader (∼2.8 ppm) than the corresponding LWs of the hydrophobic sites

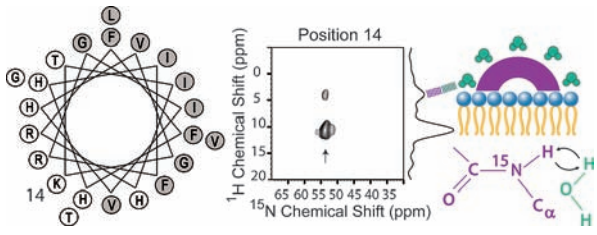

Figure 3. (Left) Helical wheel diagram of p1. The filled and open circles refer to hydrophobic and hydrophilic residues, respectively. (Center) HETCOR spectrum of ^{15}N−K_{14} p1 oriented, as recorded at 14.1 T, and the slice taken at 53.8 ppm along the ^{1}H CS dimension, as indicated by an arrow. (Right) Diagram showing p1 in the presence of lipids and the exchange between the K_{14} amide proton and protons from water.

(c.f. Figure 2), implying that protons from water molecules in the aqueous environment exchange with the amide proton at the K_{14} site at a slow rate.[29] To the best of our knowledge, this is the first direct evidence that piscidin bound to lipid bilayers interacts with the aqueous environment. This chemical information, which relates to the location of the peptide in the lipids, is useful to better understand the biological activity of piscidin, as the depth of insertion of membrane-active peptides is likely related to their ability to disrupt bilayers and induce cell death.[15−18] Therefore, with improved resolution at ultrahigh field and the ability to access ^{1}H CSs, the HETCOR technique featured here provides a new opportunity to characterize structure−function relationships of membrane-bound species, especially at the water−bilayer interface.

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Supporting Information Available: Materials and Methods; Simulated (de-)HETCOR resonance patterns; complete ref 26. This material is available free of charge via the Internet at http://pubs.acs.org.

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