Dipolar-Encoded HETCOR Spectroscopy in NMR of Aligned Samples

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Introduction

PISEMA (*P*olarization *i*nversion *s*pin *e*xchange at the *m*agic *a*ngle) [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 particular, the resonance patterns in the PISEMA spectra of uniformly labeled membrane proteins either in mechanically aligned on glass plates or magnetically aligned bicelle form characteristic PISA wheels (*p*olarity *i*ndex *s*lant *a*ngle)[2], from which the helix axis tilt with respect to the alignment axis can be directly extracted. In this report, we demonstrate that our recently developed high resolution HETCOR (*het*eronuclear *cor*relation) spectroscopy[3] also shows characteristic resonance patterns for aligned membrane proteins. These patterns are sensitive not only to the helix axis tilt but also the amide ^{1}H chemical shifts, which may provide valuable information about the interactions of the helix with the bilayers and its aqueous environment.

Experimental

The ^{1}H-^{15}N HETCOR experiments were performed on a Bruker Avance 600 NMR spectrometer. High resolution ^{1}H chemical shifts were evolved in the t_1 dimension with high power ^{1}H homonuclear decoupling, followed by a short cross polarization contact time to ensure that the ^{15}N magnetization observed in the t_2 dimension was transferred from its closest ^{1}H. With the presence of ^{15}N decoupling in the t_1 dimension, only the ^{1}H chemical shifts are evolved, resulting in typical ^{1}H-^{15}N HETCOR spectra. On the other hand, with the absence of ^{15}N decoupling in the t_1 dimension, the ^{1}H chemical shifts are encoded by the ^{1}H-^{15}N dipolar interaction, leading to the dipolar-encoded HETCOR (or de-HETCOR) spectra.

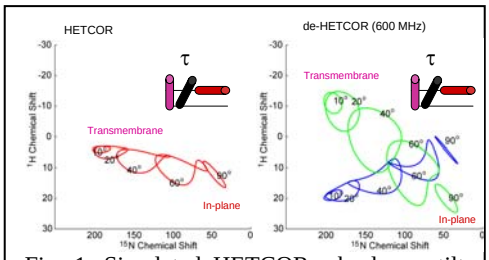

Fig. 1. Simulated HETCOR wheels vs. tilt angle τ. The curves were simulated with a MATLAB program using the ^{15}N and ^{1}H chemical shift tensors in an ideal α-helical structure.

Results and Discussion

Fig. 1 shows the simulated HETCOR wheels as a function of tilt angle with a MATLAB program using the ^{15}N and ^{1}H chemical shift tensors [4] in an ideal α-helical structure. Clearly, the size and position of those wheels strongly depends on the helix axis tilt. For de-HETCOR, there exist two asymmetric wheels for each tilt angle. It has been found in our simulations that the shapes of the two wheels are very sensitive to the amide ^{1}H chemical shifts, which are greatly influenced by the lipids environment. Fig. 2 shows the ^{1}H-^{15}N correlation spectra of multiply ^{15}N labeled Piscidins 1, p1, (an amphipathic cationic antimicrobial peptide from the mast cells of animals) oriented in hydrated 3:1 DMPC/DMPG at 1:20 peptide-to-lipid ratio. 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.

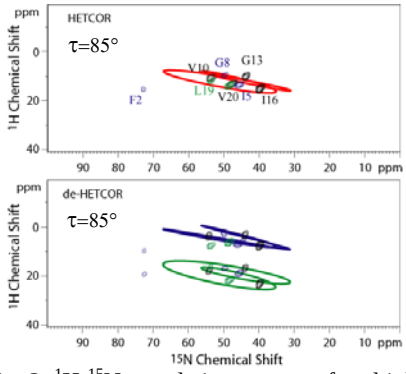

Fig. 2. ^{1}H-^{15}N correlation spectra of multiply ^{15}N labeled p1-NH_2 oriented in hydrated 3:1 DMPC /DMPG at 1:20 peptide-to-lipid ratio, pH6. In the spectra, the scaling factor has been taken into account in the ^{1}H chemical shift dimension. The ^{15}N chemical shift was referenced to the ^{15}N 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. The curves were simulated with a MATLAB program using the ^{15}N and ^{1}H chemical shift tensors in an ideal α-helical structure.

Acknowledgements

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References

[1] Wu, C.H., *et .al.*, J. Magn. Reson. Ser. **A 109**, 270 (1994).
[2] Wang, J., et. al., J. Magn. Reson. **144**, 162 (2000);
Marassi, F.M. and Opella, S.J., J. Magn. Reson. **144**, 150 (2000).
[3] Fu, R., et. al., J. Magn. Reson. **188**, 41 (2007).
[4] Wu, C.H., *et. al.*, J. Am. Chem. Soc. **117**, 6148 (1995).