Enhancing Spectral Resolution of Dipolar-Encoded HETCOR Spectra at 900 MHz

Riqiang Fu (NHMFL); Daniel J. Hibbard (Pacific Lutheran University); Myriam Cotten (Hamilton College)

Introduction

High resolution HETCOR (heteronuclear correlation) spectroscopy has recently been developed to obtain orientational restraints (i.e., both the anisotropic $^1$H and $^{15}$N chemical shifts and their $^1$H-$^{15}$N dipole-dipole couplings) from membrane proteins in a lamellar phase lipid environment. The HETCOR resonances of an ideal $\alpha$-helical structure form characteristic wheels. Similar to PISA wheels (polarity index slant angle) the size and position of these wheels in the spectra can be used to uniquely determine the helical tilt with respect to the bilayer normal without resonance assignments. In this report, we use a multiple $^{15}$N-backbone labeled amidated piscidin 1 (p1) peptide oriented in hydrated 3:1 DMPC/DMPG bilayers to illustrate the spectral resolution enhancement of the HETCOR spectra at high fields.

Experimental

The $^1$H-$^{15}$N HETCOR experiments were performed on an ultra-wide bore superconducting 21.1 T magnet with a Bruker Avance 900 NMR console. High resolution $^1$H chemical shifts were evolved in the $t_1$ dimension with high power $^1$H homonuclear decoupling, followed by a short isotropic mixing 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.

Results and Discussion

Fig. 1 shows the 2D $^1$H-$^{15}$N correlation spectra of a 10-site $^{15}$N labeled p1-NH$_2$ peptide oriented in 3:1 DMPC/DMPG bilayers. Out of ten resonances, nine are identified in the HETCOR spectrum (Fig. 1A). In the de-HETCOR spectrum (Fig. 1B), the resonances are split into two distinct groups due to the dipolar couplings. For the upper group, ten resonances are observed, implying that the overlapped resonances at ~52 ppm in the $^{15}$N chemical shift region of the HETCOR spectrum can be resolved in the de-HETCOR spectrum. Fig. 2 shows the $^1$H and $^{15}$N slices taken from the de-HETCOR spectra at different static field strengths for the $^{15}$N resonance at 37.8 ppm. Clearly, by going from the 600 to 900 MHz, the spectral resolution in both the $^1$H and $^{15}$N dimensions improves by ~150%. Therefore, with improved spectral resolution at higher magnetic fields, the 2D de-HETCOR spectra, allowing for simultaneous extraction of anisotropic $^1$H and $^{15}$N chemical shifts as well as their dipolar couplings, would become valuable in the structural studies of multiply or uniformly labeled membrane bound proteins and peptides.

Acknowledgements

This work is supported in part by the NHMFL and by the NIH R01 AI23007. MC is grateful for support from the National Science Foundation (CHE-0748916), Research Corporation, the Dreyfus Foundation, and the Undergraduate Research Summer program at Pacific Lutheran University.

References