Enhanced Sensitivity and Resolution for Orientational Restraints from Lipid Bilayer-Bound Gramicidin A

E. S. Mananga (NHMFL); R. Fu (NHMFL); M. Truong (FSU, Chemistry and Biochemistry); T. A. Cross (NHMFL, FSU, Chemistry and Biochemistry)

Introduction

Orientational restraints derived from solid-state NMR can be used to determine high resolution three-dimensional structures. Such an approach has already been used to define the structure of the ion channel, gramicidin A (gA), in lamellar phase lipids. 15 years ago, spectral sensitivity and resolution were minimal for achieving the first 3D structure in a lipid bilayer environment [1]. Today, we are using a new approach to study the gA backbone orientational constraints from PISEMA and HETCOR pulse sequences [2].

Experiment

CP and PISEMA experiments were carried out on Bruker Avance 400 and 600 NMR spectrometers. NHMFL low electrical field PISEMA probe with a rectangular solenoid coil (7.6 x 5.6 x 11 mm) for optimized filling factor to maximize sensitivity was used in all experiments. The SPINAL-64 decoupling sequence was used in all experiments during $^{15}$N detection. The chemical shifts (CS) of the resonances were referenced to $^1$H of water at 4.7 ppm and $^{15}$N of (NH$_4$)$_2$SO$_4$ at 0 ppm, respectively. Uniformly aligned samples containing isotopically labeled Gly$^2$-Ala$^3$ of gA in hydrated DMPC bilayers were prepared with 9 mg of peptide. In Fig. 1, the $^{15}$N CS spectra were obtained by using a conventional CP pulse sequence and 4.20 $\mu$s 90° pulse widths with just 400 acquisitions. Fig. 2 is the PISEMA spectrum obtained with a total of 48 $t_1$ increments and 160 acquisitions. Both experiments used a 6 s RD and a 1 ms contact time.

Results and Discussion

![Fig.1: CP Spectrum](image1.png)  
![Fig.2: PISEMA Spectrum](image2.png)  
![Fig.3: 1D slices taken at 113 (Top) and 198 ppm (Bottom)](image3.png)

The observed $^{15}$N CS is at 113 ppm for Gly$^2$ and 198 ppm for Ala$^3$ as reported previously [1]. PISEMA spectrum gives dipolar linewidths of 360 Hz. The measured $^{15}$N-$^1$H dipolar splitting was 12 kHz and 16 kHz respectively at Gly$^2$ and Ala$^3$ sites. We have observed an improvement in resolution and sensitivity comparatively to Ketchem.

Conclusions

This data demonstrated that gA offers an opportunity to refine the development of PISEMA experiments and spectral analysis through the excellent sensitivity and resolution afforded by state of the art spectra.

Acknowledgements

This work is supported by the NIH (T. A. C) and performed largely at the NHMFL.

References