Resolution and Sensitivity of Magic Angle Spinning Solid-State NMR Spectra of Membrane Proteins at Ultra High Magnetic Fields

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Introduction

Membrane proteins constitute approximately a third of all proteins. They are involved in many important physiological processes, and represent almost a half of known drug targets. The knowledge of structures of these proteins is a key to the understanding of their functions, yet the progress has been slow because of lack of reliable and generally applicable methods. Research in my group focuses on the development of magic angle spinning solid state NMR methodologies with particular emphasis on applications to large membrane proteins. We study alpha-helical proteins of both microbial and human origin, e.g., seven trans-membrane helical proteins (7TM) proteorhodopsin and *Anabaena* Sensory Rhodopsin, water channel aquaporin (AQP1), Adenosine A2a receptor, etc. In these relatively large proteins, both sensitivity and resolution are limiting factors, which hinder their structural and dynamic characterization, and the availability of state-of-the-art equipment such as high field magnets and highly sensitive NMR probes- are critical factors. The main objectives of this project were to evaluate the equipment available at the Magnet Lab, to compare it with the commercially available probes, and to identify potential avenues for improvement.

Experimental

One-dimensional ^{13}C- and ^{15}N- detected and two-dimensional ^{13}C-^{13}C, NCA, NCACX and NCOCX magic angle spinning solid state NMR experiments were performed on a 900 MHz NMR spectrometer using a triple resonance low-E field probe. We used 4.6 mg of uniformly ^{13}C,^{15}N labeled Anabaena Sensory Rhodopsin, a seven-helical photosensor, which gives spectra of excellent spectral sensitivity [1]. Resolution and sensitivity of these measurements were compared with those obtained using a commercial triple resonance Bruker E-free probe installed on a 800 MHz Bruker Avance III spectrometer at Guelph. Acquisition parameters were kept identical between the 900 MHz and 800 MHz experiments. Equal amounts of sample (~4.6mg) were used for both sets of measurements.

Results and Discussion

To assess and compare the sensitivities of both probes, well-resolved cross peaks were considered in the 2D NCA and ^{13}C-^{13}C correlation planes. We observed that the double cross-polarization transfer efficiency was higher on the 900 MHz probe: ~35% on the 800 MHz probe, and ~ 50% on the 900 probe. Average signal-to-noise ratios (SNR) for 15 well resolved peaks were approximately 8.2 on the 800 MHz probe, and 12.8 on the 900 MHz probe, indicating significant sensitivity improvement of approximately 55%. Likewise, ^{13}C-^{13}C correlation spectra collected with 30 ms of DARR mixing using the 900 MHz probe were ~30% more sensitive, with average SNRs of 12.9 ad 9.7 on the 900 MHz and 800 MHz probes, respectively.

Conclusions

While higher field is certainly a factor contributing to better signal-to-noise, this alone cannot explain the observed improvement in sensitivity. Our measurements indicate that the 900 MHz probe built at the NHFML shows better performance. Specifically, NCA transfer efficiency was much better, which is likely due to better radiofrequency field homogeneity. This identifies a potential avenue for improvement of commercial Bruker probes.

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References

[1] Shi, L., *et al.,* Angew. Chem. Int. Ed., **123**, 1338-1341 (2012).