Gramicidin Ion Binding and Conductance: New Insights from 17O Solid State NMR at 35T SCH Magnet

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Introduction

Gramicidin A (gA) forms a dimeric ion channel with a pore lined by the backbone carbonyl oxygens (1). It has been shown that 17O NMR is very sensitive to cation binding to gA nevertheless it suffers from low sensitivity and poor resolution. We have performed 17O NMR of gA using the SCH Magnet at 35.2T and report here a dramatic gain in spectral sensitivity from the high field and better resolution from improved alignment. These improvements reveal a split into two ^{17}O peaks that have never been observed before.

Experimental

gA 17O Leu10 in DMPC bilayers aligned sample was prepared as described previously (2). Spectra were acquired at the SCH magnet at the NHMFL in Tallahassee. The SCH was set at 35.2T (19.39 kA), 203 MHz 17O frequency. A NMR probe optimized for static samples developed for the SCH magnet by the RF group in the NMR facilities of the NHMFL was used. The spectra was acquired with a Hahn echo pulse sequence with a 90° solid pulse of 1.5 μs and 20 μs echo time in the absence and presence of low power 1H decoupling. Spectra of the same sample were also acquired at a 19.4T superconducting magnet in the NMR facilities of the Maglab. Acquisition conditions were similar to the ones described for the SCH magnet.

Results and Discussion

Spectrum of 17O of gA 17O-Leu10 at 1.5 GHz (1H frequency) showed an S/N increase by an order of magnitude over 19.4T field (Fig. 1). Also, line narrowing due to the increase in field revealed for the first time 2 peaks with linewidths of about 5 ppm as compared to a broad 33 ppm wide resonance for Leu10 site acquired for the same sample at 19T. Moreover, the presence of the two peaks persists in the presence of the binding ions Li^+, K^+ and Ba^{2+}. These two peaks could possibly be correlated to different monomer geometry and/or differences in water hydrogen bonding.

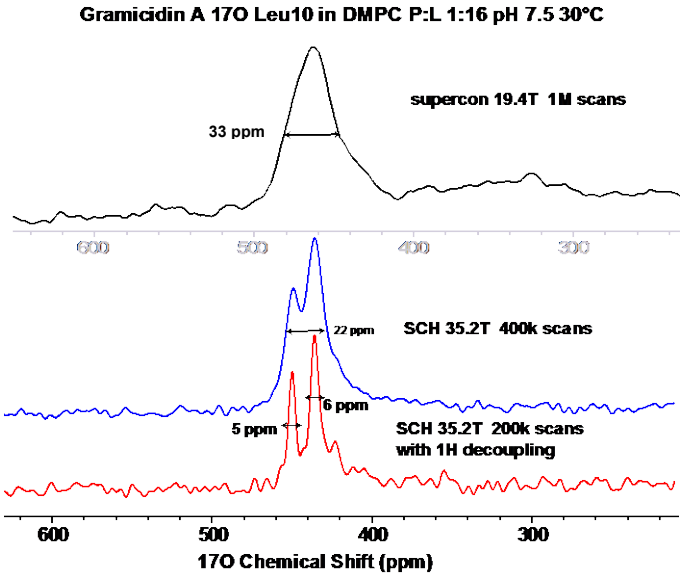

Fig.1 Comparison of Gramicidin A 17OLeu10 in DMPC spectra acquired in 19.4T superconducting magnet (black line) and in the 35.2T SCH magnet (blue and red lines. Line narrowing of Leu10 carbonyl oxygen peak reveals the spectra is composed of 2 peaks. Use of weak (30 kHz) 1H decoupling reveals line widths (~5ppm or 1kHz) are dominated by spin-spin relaxation (T_2~360 µs).

Conclusions

Early 17O of gA 17O-Leu10 results using the SCH NMR spectrometer at 1.5 GHz (1H frequency) shows dramatic increase in sensitivity and resolution that greatly surpasses what we can accomplish with superconducting magnetic fields up to 20T. Commonly, protein NMR studies are based on 1H, 13C and 15N nuclei. Oxygen, on the other hand, is involved in many different processes, as ion conductance in gA and potassium channels. This dramatic increase on sensitivity and resolution could open a new front on the study of proteins by NMR.

Acknowledgements

The 1500 MHz NMR console was funded by NSF DMR-1039938. This work was performed at the NHMFL, supported by NSF DMR-1157490 and the State of Florida. This work was also supported by NIH P41GM122698.

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