^{17}O STUDY OF ION CHANNELS AT 21.2 T 105MM ULTRA-WIDE BORE SUPERCONDUCTING NMR MAGNET

Jun Hu (FSU, Chemistry), Eduard Y. Chekmenev (NHMFL, CIMAR/NMR), Zhehong Gan (NHMFL, CIMAR/NMR), William W. Brey (NHMFL, CIMAR/NMR), Saikat Saha (NHMFL, CIMAR/NMR), Kiran K. Shetty (NHMFL, CIMAR/NMR), Peter L. Gor'kov (NHMFL, CIMAR/NMR), Timothy A. Cross (FSU, Chemistry, NHMFL, CIMAR/NMR)

Introduction

Recent advances in development of high field magnets and NMR instrumentation tremendously aided to sensitivity and spectral resolution and made ^{17}O spectroscopy a rapidly growing area in the past 10 years. Oxygen was always an extremely attractive nucleus for scientists since it participates in a variety of interactions, which control both structure and function of biomolecules. Carbonyl sites of proteins are of special interest since they directly participate in interactions, which (i) define protein primary and secondary structure, (ii) play a crucial role in intermolecular contacts, but more importantly (iii) power the biological function. ^{17}O NMR holds the key to a variety of biologically important structural and functional information. Ion cannels are an important class of membrane proteins that are difficult to be studied by X-ray crystallography and solution NMR methods, we report here a preliminary study of ion channel gramicidin A (gA) by ^{17}O solid-state NMR.

Experimental

We inserted ^{17}O label (54% enrichment) in gA in Leu-10 position, which participates in ion binding. This single site ^{17}O labeled gA was inserted in hydrated DMPC bilayers with 1:8 molar peptide-to-lipid molar ratio. ^{1}H decoupled ^{17}O spectra were then acquired at 21.2 Tesla with in-house built static double-resonance probe.

Results and Discussion

Three ^{17}O spectra of gA (Fig.1) show the sufficient sensitivity and, therefore, feasibility of studying ion channel by solid-state ^{17}O NMR spectroscopy. The spectrum of lyophilized or dry gA (no water signal observed) shows the static spectral shape typical for carbonyl sites in peptides, while the other two spectra are dramatically affected in membrane environment by dynamics effects of gA molecules. Moreover, the presence of K^+ ion (2.4 M concentration) significantly, >50 ppm, shifts the chemical shielding tensor components of the ^{17}O site. This observation is in agreement with our results in model peptide systems.

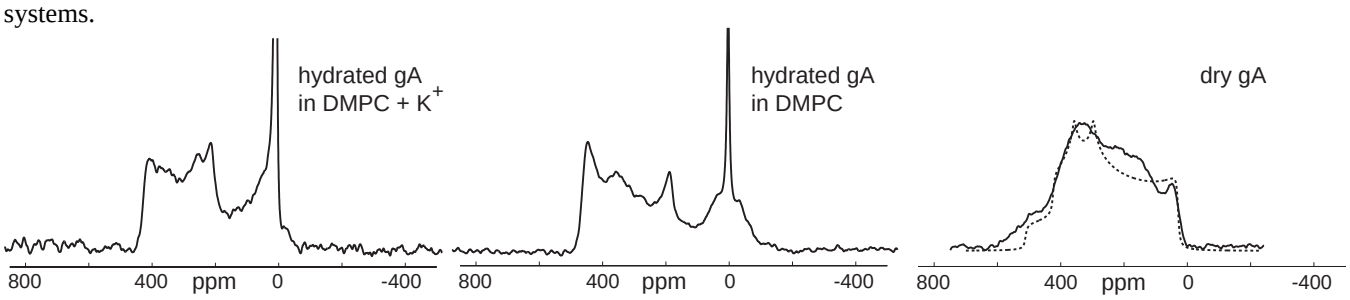

Figure 1. ^{1}H decoupled ^{17}O spectra and simulation (dashed line) gA in various environments obtained at 21.2 T. The chemical shifts are referenced to H_2O (ext.) at 0 ppm. Chemical shielding tensor (δ_{11}=510 ppm, δ_{22}=370 ppm, δ_{33}=20 ppm) and quadrupole coupling (χ=8.1 MHz, η=0.300) NMR parameters for dry gA are in qualitative agreement with those reported for stationary amides.

Conclusions

NMR sensitivity enhancement by high fields makes ^{17}O solid-state NMR spectroscopy a potential method for studying ion channels. We plan more experiments at 21.2 T to obtain the detailed information about ion channels in membrane environment.