Structural Characterization of the N-Terminus of CrgA Using a Transient NOE TOBSY Pulse Sequence with Gd^{3+} Chelated Lipids for PRE Effect

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Introduction

The structure of the CrgA transmembrane (TM) domain has been characterized [1]. In addition to the TM domain, the extramembrane domain plays a crucial role for a function of TM proteins. Thus, another approach is required to study the extramembrane domain due to the different environment in terms of dynamics. In this project, Gd^{3+} chelated lipid is used for Paramagnetic Relaxation Enhancement (PRE) effect, since Gd^{3+} is known as a strong PRE reagent. 16:0 PE-DTPA (Gd^{3+}) is added to a proteoliposome sample in order to observe the changes of signals from the N-terminus in the extramembrane domain. However, rapid ^{1}H T_1 relaxation suppresses all crosspeaks in the previous TOBSY (total through-bond correlation spectroscopy) type experiment. It is found that a new TOBSY pulse sequence using transient NOE [3] solves this problem.

Experimental

Magic angle spinning solid state NMR is exploited with ^{13}C uniformly labeled CrgA in synthetic lipid bilayers. Two types of 2D ^{13}C-^{13}C correlation TOBSY experiments (INEPT and transient NOE) are utilized to describe the dynamic N-terminus in CrgA. Also, spectra from preteoliposome samples both with and without Gd^{3+} chelated lipids are compared to observe PRE effects.

Results and Discussion

Gd^{3+} is bound to a lipid head group, and therefore, amino acid residues which are located close to the head groups are more susceptible to the PRE effect. A new TOBSY using transient NOE avoids broad lines due to rapid H T_1 relaxation. Thus, it prevents the loss of crosspeaks as seen in the **Fig. 1** and **2**, and therefore it will enable further titration experiment to examine the PRE effect depending on the concentration of Gd^{3+} lipids in samples.

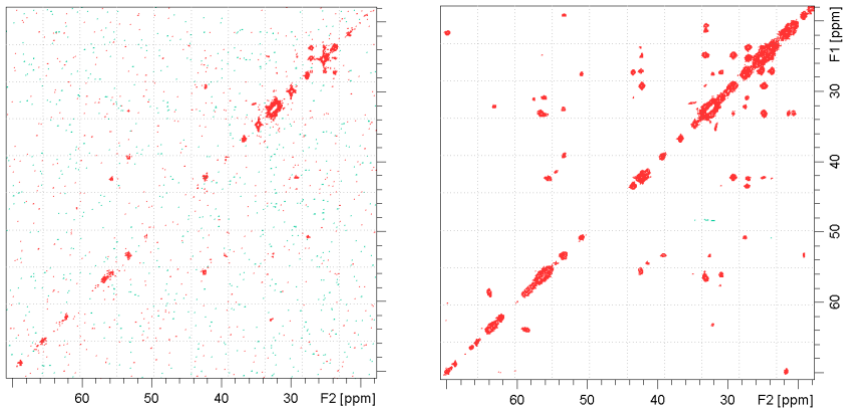

Fig. 1 & 2 1. A 2D ^{13}C-^{13}C INEPT TOBSY Spectrum and **2.** A 2D ^{13}C-^{13}C Transient NOE TOBSY Spectrum. Both spectra are from the same sample, ^{13}C uniformly labeled CrgA with additional 0.1% Gd^{3+} chelated lipids. The sample is spinning at 12kHz and at 300K in a 800 MHz magnet at NHMFL.

Conclusions

The new transient NOE pulse sequence solves the problem caused by rapid H T_1 relaxation when using the PRE effect. This method is expected to help characterize the location of the side chains of certain residues in the N-terminus through a series of titration experiments by increasing Gd^{3+} chelated lipids in the samples.

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References

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