Conformation of the N-terminus of CrgA from *M. Tuberculosis* with lipids chelating Gd^{3+} to the head group for observing Paramagnetic Relaxation Enhancements by solid state NMR

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Solid state NMR is one of the most powerful techniques to study small membrane proteins in lipid bilayers. However, it is difficult to selectively observe the extramembrane domain using typical NMR experiments that work well for studying the transmembrane (TM) domain. It is known that TOBSY (Through Bond Correlation Spectroscopy) pulse sequence allows for establishing ^{13}C-^{13}C correlations between covalently bonded carbon sites and thus can be utilized to characterize the dynamic N-terminus in the cytoplasmic domain. To preserve the native interaction between the N-terminus and its lipid environment, full length CrgA in a liquid crystalline preparation of POPC/POPG bilayers is prepared. Additionally, the bilayer is doped with 0.1% to 2% of 16:0 PE-DTPA (Gd^{3+}) such that residues close to the lipid head group are more susceptible to the PRE effect. Here, we use two different methods to polarize the ^{13}C signals for the TOBSY experiments. In the INEPT TOBSY experiment, most cross peaks are greatly suppressed in the presence of Gd^{3+} cation due to rapid ^{1}H relaxation. On the other hand, in the NOE TOBSY experiments, all cross peaks are uniformly enhanced compared to the INEPT experiments, owing to the fact that the ^{13}C signals are polarized through cross relaxation effect. By comparing two sets of experiments, it is observed that the positively charged residues are more susceptible to the PRE effect in the INEPT experiments compared to the negatively charged residues. This suggests that charge-charge interaction between positively charged residues and lipid head groups play an important role in the conformation of the intrinsically disordered region (IDR) in the N-terminus. It is concluded that 9 residues form a tether to the membrane surface by the electrostatic interactions.