Mtb Cell Division Membrane Protein "CrgA" Structure Studied by ssNMR Spectroscopy

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Introduction

Solid state NMR (ssNMR) spectroscopy is a powerful tool to study membrane protein structure in membrane environment at atomic resolution. Here, we present the structural studies of *M. tuberculosis* cell division membrane protein CrgA with two transmembrane helices by ssNMR spectroscopy. By a combined approach of Oriented State (OS) and Magic Angle Spinning (MAS) experiments the 3D structure of the CrgA transmembrane domains in POPC:POPG lipid bilayers were determined.

Experimental

Purified $^{15}N/^{13}C$ uniform labeled and amino acid specific labeled CrgA were reconstituted in POPC-POPG liposomes (4:1 molar ratio). Two types of solid state NMR samples were prepared. Glass plate supported OS samples (Protein to Lipid molar ratio 1:80) were used for collecting several 2D PISEMA spectra. MAS samples (Protein to Lipid molar ratio 1:30) were used to collect 2D DARR and 3D NCACX, NCOCX and CAN(CO)CX experiments. ssNMR structural restraints obtained from these experiments were used directly to calculate CrgA membrane protein structure by Xplor-NIH followed by a refinement in POPC/POPG lipid bilayer by restrained molecular dynamics simulation.

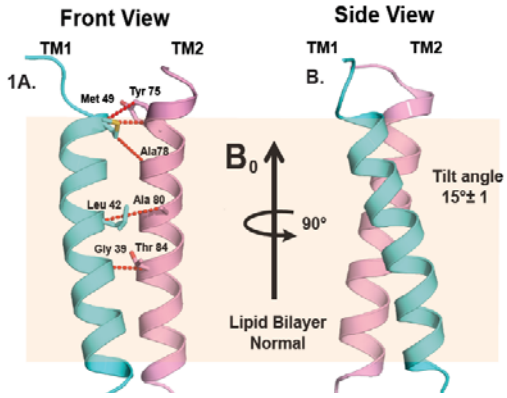

Figure 1: Front and side view of CrgA TMD showing the interhelical distances and tilt angle.

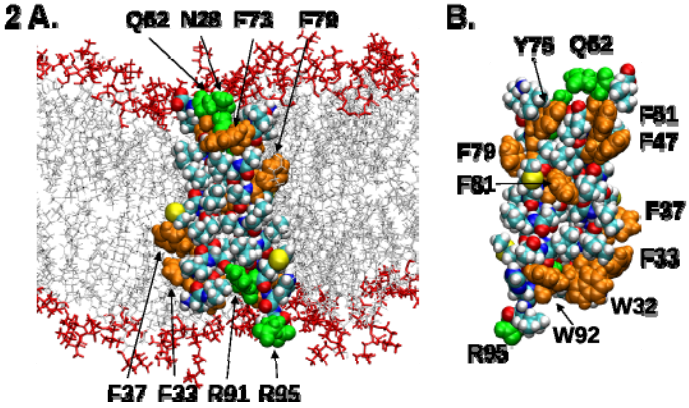

Figure 2: positions of aromatic residues (orange) and polar charged residues near membrane interface anchoring CrgA to the membrane.

Results and Conclusions

2D PISEMA spectra generated anisotropic ^{15}N chemical shift (ppm) and dipolar coupling (kHz) restraints for each residue in the CrgA TMD. An initial NMR structure of the two TM helices of CrgA with a tilt angle of 16°±1 with respect to the bilayer normal was achieved using these PISEMA restraints (Figure 1). Rotation angles for each helix were also defined. A sparse set of distance restraints obtained from MAS experiments were used for helix-helix packing. Combining both ssNMR restraints an initial CrgA-TMD structure was calculated with an average backbone pairwise r.m.s.d of 0.38Å. The refined structure is consistent with many native-like membrane protein structural features (Figure 2). Most of the hydrophobic bulky side chains (Leu, Ile, Val, Met) were membrane facing with the Tyr, Trp residues oriented towards the interfacial regions of the lipid bilayer anchoring the TM helices to the membrane environment. In addition, the Arg, Asn side chains also contributed to this anchoring and the positions of Ser, Thr polar residues were buried in the helix-helix interface. Another important aspect of the structure is, instead of Gly residues Met residue (Met 41) is involved in the formation of a kink in the middle of the TM1 helix. Also Gly residues in the TMD are facing towards the lipid bilayer instead of the helix helix interface, suggesting that CrgA membrane protein is involve in protein protein interaction through the TM domain.

Acknowledgements

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References

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