Structural Characterization of CwsA in a Lipid Bilayer Environment

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Introduction

CwsA is a small membrane protein from *Mycobacterium tuberculosis* (TB) that functions in bacterial cell division. The depletion or overproduction of CwsA has been shown to cause defects in cell wall synthesis [1]. Furthermore, CwsA has been shown to interact with two other TB division related proteins: CrgA and Wag31 [2]. In order to reveal the mechanism behind this cellular division process, it is crucial to understand the atomic structure of CwsA in a native like environment. To achieve this goal, static solid-state NMR was used to study the tilt of CwsA with respect to lipid bilayer normal using oriented samples. In addition, MAS solid-state NMR was applied to probe the interaction between CwsA and CrgA.

Experimental

CwsA and CrgA proteins were overexpressed in *E.coli* and purified with nickel affinity chromatography. For alignment study, amino acid selective-^{15}N labeled CwsA was incorporated into POPC liposomes, followed by a rehydration process on glass slides. For interaction study, uniform-^{13}C labeled CwsA with and without CrgA was incorporated into POPC liposomes at a protein to lipid molar ratio of 1/30. All NMR data were acquired at NHMFL NMR/MRI facility, specifically, 2D SAMPI4 spectra were acquired on 720 MHz magnet, and 2D SAMMY spectra were acquired on 600 MHz and 900 MHz magnets. 2D DARR and INEPT MAS spectra were acquired on 600 MHz and 800 MHz magnets. 2D HSQC solution spectra were acquired on 800 MHz solution magnet.

Results and Discussion

Fig.1 shows 2D SAMMY spectra of ^{15}N-Ile (Grey), Ala (Red), and Phe (Blue)-labeled CwsA with superimposed PISA wheels at various tilt angles. The main resonances from aligned Transmembrane (TM) helix portion (175 ppm to 225 ppm) for all three sets of data lie within 24° to 26° PISA wheels suggesting a small tilt angle (~25°) for the CwsA TM helix CwsA with respect to bilayer normal. The limited sample alignment could be due to the presence of a large water soluble domain. Fig.2 shows an overlay of 2D INEPT MAS spectra of uniform-^{13}C labeled CwsA with and without CrgA (Unlableled). As shown, the loss of cross peaks upon the addition of CrgA suggests the binding of CrgA affects the dynamics of the extracellular domains of CwsA, which are the regions that can be probed by INEPT type experiments. There is predicted intrinsically disordered region (IDP) present in the extracellular domains of CwsA, it is possible that the binding of CrgA induces folding of CwsA IDP.

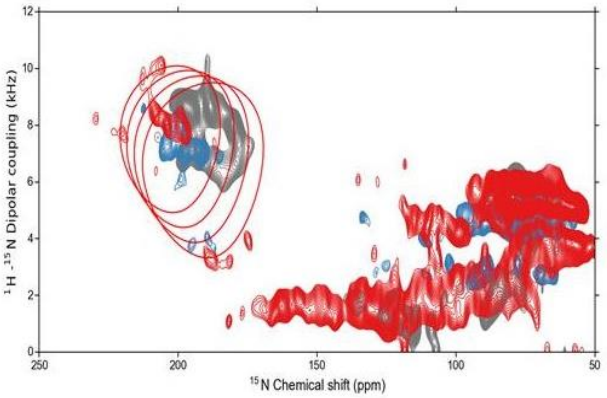

Fig.1 2D SAMMY spectra of ^{15}N-Ile (Grey), Ala (Red), and Phe (Blue)-labeled CwsA with superimposed PISA wheels at various tilt angles (22°, 24°, 26°, and 28°).

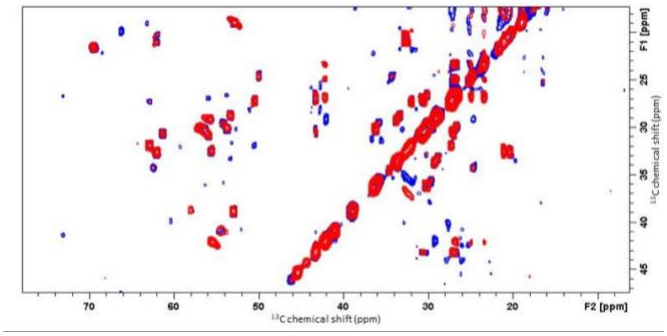

Fig. 2 2D INEPT spectra of uniform-^{13}C labeled CwsA with (Red) and without (Blue) CrgA.

Acknowledgements

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References

[1] Plocinski, P., *et al.*, Tuberculosis, **93**, S21–S27 (2013).
[2] Plocinski, P., *et al.*, J. Bacteriol., **194**, 6398–6409 (2012).