Characterize the Transmembrane Helical Structure of CwsA in a Lipid Bilayer Environment

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Introduction

CwsA is a novel small membrane protein from *Mycobacterium tuberculosis* (TB) that functions in bacterial cell division and cell wall synthesis. The depletion or overproduction of CwsA has been shown to cause bulged cell poles, formation of rounded cells, and defects in cell wall synthesis [1]. Furthermore, CwsA has been shown to interact with two other TB division related proteins: CrgA and Wag31 [2]. The important role of CwsA drives the interest to study its atomic structure. To achieve this goal, static solid-state NMR was used to study the transmembrane domain of CwsA in a lipid bilayer environment using oriented samples. Previous attempts on the alignment of full-length CwsA in a POPC lipid bilayer did not give satisfactory alignment. Since we are aiming to characterize the tilt and rotation of the transmembrane helix with respect to the bilayer normal, a carefully designed reduced version of CwsA is constructed (Denoted as $CwsA_{76\text{-}125}$). A significant improvement on the alignment was observed and presented in this report.

Experimental

$CwsA_{76\text{-}125}$ was cloned into pTBGST vector. The GST tagged protein was expressed with BL21(DE3)RP *E.coli* strain and purified with nickel affinity chromatography. The GST tag was cleaved with TEV protease. The Ile-^{15}N specific labeled $CwsA_{76\text{-}125}$ was reconstituted into POPC/POPG (4/1 mol/mol) at a protein to lipid molar ratio of 1/120. The proteoliposomes were later spread onto 44 glass slides and allowed to rehydrate in a hydration chamber. All NMR data were acquired in the NHMFL NMR/MRI facility, 2D SAMPI4 spectra were acquired on 600 MHz (#1) and 800 MHz (#2) magnets.

Results and Discussion

Fig.1 shows 2D SAMPI4 spectra of ^{15}N-Ile-labeled CwsA with superimposed PISA wheels at various tilt angles (29°, 30° and 31°). The chemical shift dimension is scaled from 0 ppm to 250 ppm for a full observation of all resonances. There are four Ile residues in CwsA and all of them present in the transmembrane domain. Ile residue thus serves as a good indicator for alignment efficiency. The contour level in Fig. 1 was lowered to noise level. As seen from the spectra, several resonances were observed within 170 ppm to 200 ppm and no observable power pattern. This indicates excellent alignment. The positions of resonances agree well with transmembrane projection, and the tilt was estimated to be 30 ± 1° based on the superimposed PISA wheels. To look into the noise level carefully, a slice through 8.5 kHz dipolar dimension was plotted in Fig. 2 (blue line in Fig.1), two well resolved peaks with good sensitivity at chemical shift 188 ppm and 199 ppm are shown, which gives confidence on the resonances observed in Fig. 1.

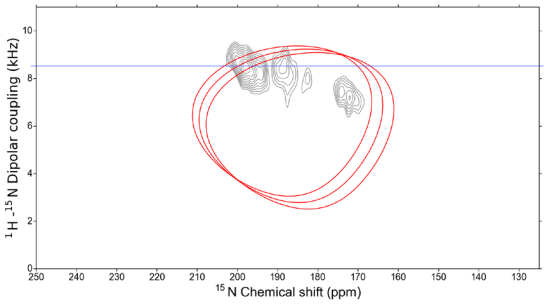

Fig.1 2D SAMPI4 spectrum of ^{15}N-Ile $CwsA_{76\text{-}125}$ with superimposed PISA wheels at various tilt angles (29°, 30°, and 31°).

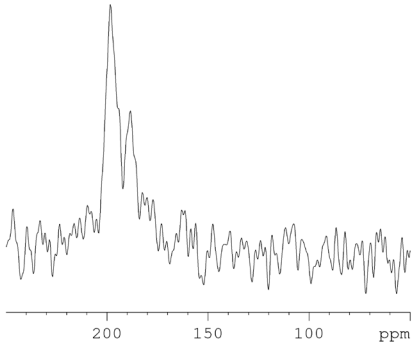

Fig.2 A slice through 8.5 kHz from 2D SAMPI4 spectrum of ^{15}N-Ile $CwsA_{76\text{-}125}$.

Conclusions

An optimized oriented sample preparation for $CwsA_{76\text{-}125}$ in POPC/POPG lipid bilayer was achieved. With this established method, preparation of other specific labeled samples will aid in the assignment and rotation determination of the transmembrane helix. A well-defined transmembrane helix will aid a full-length CwsA structural refinement in a lipid bilayer. This good alignment also opens potential approach to study CwsA and CrgA transmembrane interaction using oriented samples.

Acknowledgements

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References

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[2] Plocinski, P., *et al.*, J. Bacteriol., **194**, 6398–6409 (2012).