Structure Determination of LspA, an *M. Tuberculosis* Transmembrane Protein

Mooney, V.L. (NHMFL); Cross, T.A. (Florida State University, Chemistry and Biochemistry/NHMFL)

Introduction

The pathogen *Mycobacterium tuberculosis* (*Mtb*) causes the highly infectious disease tuberculosis, which is estimated by the World Health Organization to have infected 8.6 million people and caused 1.3 million deaths worldwide in 2012 (*1, 2*). Many possible *Mtb* drug targets are under investigation. Among them is the type II signal peptidase, LspA, which cleaves signal peptides from prolipoproteins' N-termini, thus converting them into their mature lipoprotein form. As these mature lipoproteins are necessary for *Mtb's* cell growth and virulence (*3, 4*), the structure and function of the LspA protein are of utmost importance. Using the TMHMM program, this protein is predicted to have four transmembrane helices. Our aim in this study is to use solid-state NMR techniques to determine the tilt and rotation of each helix and their relative orientations.

Experimental

After expressing *Mtb* LspA in E. coli, the membrane was detergent solubilized, the LspA was isolated using Ni-NTA affinity chromatography, and the LspA was then incorporated into proteoliposomes containing a 4:1 mole ratio of POPC:POPG. The 600 MHz and 720 MHz magnets at the National High Magnetic Field Laboratory were used to conduct solid-state magic angle spinning (MAS) and oriented sample (OS) experiments, respectively. The facility's 400 MHz magnet was also used to check the lipid alignment of the oriented samples. Various labeling strategies were used when preparing the MAS samples for which 2D DARR data were collected. The OS NMR samples were specifically labeled with ^{15}N.

Results and Discussion

The DARR ^{13}C-^{13}C intra-residue correlations seen using short mixing times are being used to identify amino acid types and to assign chemical shift resonances. A few inter-residue interactions, particularly those between helical residues, have been identified using a 500 ms mixing time (Figure 1). This same technique can be used to identify interhelical ^{13}C-^{13}C correlations; however, prior knowledge of helix orientations would aid in determining labeling strategies.

In order to determine the tilt and orientation of the transmembrane helices, SAMPI4 spectra were collected of LspA. Signals between 200 ppm and 250 ppm suggest that at least one helix has a tilt of 5°-10° (Figure 2). However, spectra with enhanced signal-to-noise will be needed to determine this conclusively. In addition, all of the SAMPI4 spectra collected thus far have a significant amount of signal around 100 ppm. While some of this may be due to unaligned protein, it is also possible that one or both of the large periplasmic loops contains an amphipathic helix. This possibility is supported by the distribution of non-polar amino acids in these loops.

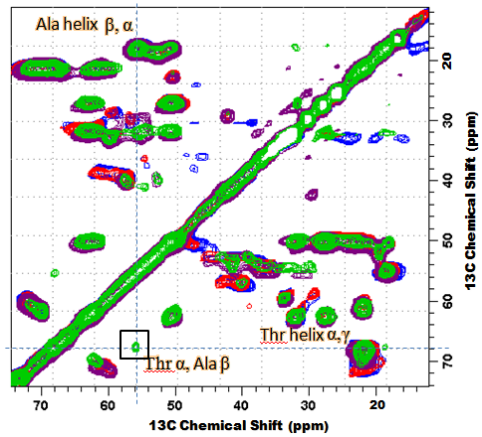

Fig.1 DARR spectra of LspA with reverse labeled V,I,L,G,H, and R. The mixing times used were 2 ms (blue), 8 ms (red), 50 ms (purple), and 500 ms (green).

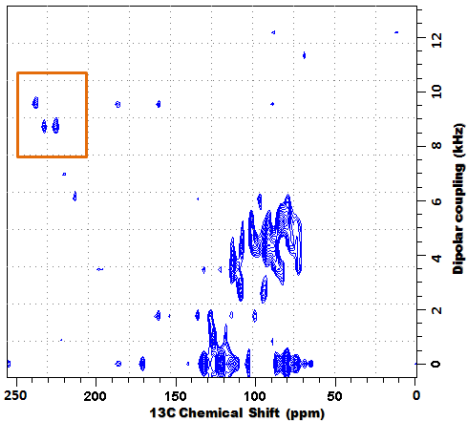

Fig.2 SAMPI4 spectrum of 15N-I labeled LspA. The signals indicated by the orange box are in the region of helices with tilts between ~5° and 10°.

Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by NSF Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy. This work was also funded by NIH/NIAID grants R01AI1073891 and P01AI074805.

References

[1] *Reported Tuberculosis in the United States, 2012*. Atlanta, GA: U.S. Department of Health and Human Services, CDC. October 2013.
[2] "Global Tuberculosis Report 2013," (World Health Organization, 2013).
[3] Kovacs-Simon, A., *et al.*, Infection and Immunity, **79**, 548-561 (2011).
[4] Sander, P., *et al.,* Molecular Microbiology, **52**, 1543-1552 (2004).