Structural Characterization of FtsX by Solid-State NMR

C.A. Escobar (FSU, Chemistry & Biochemistry, NHMFL); T.A. Cross (FSU, Chemistry & Biochemistry, NHMFL)

Introduction

FtsX is a protein of 32 kDa with 4 transmembrane helices, which in complex with FtsE constitutes an ABC transporter. It has been demonstrated that it plays an important role during the final steps of cell division on *E. coli*[1] and experimental evidence suggests a similar role on *M. tuberculosis*[2], which makes this protein an interesting target for drug development against *M. tuberculosis*. In this study, ssNMR will be used to characterize the structure of FtsX from *M. tuberculosis* because it offers the possibility of study this and other membrane proteins in a membrane-like environment. In addition, due to the importance of the periplasmic loop for FtsX function, solution NMR experiments have been carried out for its characterization.

Experimental

Recombinant His-tagged FtsX has been over expressed using *E. coli* and purified using detergent and nickel affinity chromatography. The protein can be reconstituted into a membrane-like environment using POPC and POPG (4:1 ratio) in a protein to lipid ratio of 1:150. For preliminary NMR experiments, the protein has been ^{15}N uniformly labeled and specifically labeled using ^{15}N isoleucine. This protein was used to prepare mechanically aligned samples. ^{15}N-^{13}C uniformly labeled FtsX loop from *E. coli* has been over expressed and purified as stated before. Samples of the loop have been used for initial characterization using 2D HSQC at different pHs.

Results and Discussion

Optimization of conditions for obtaining high quality mechanically aligned samples of FtsX is still in progress. Emphasis has been put on detergent removal during reconstitution, and optimizing the amount of protein and lipid. Despite that, a PISEMA spectra suggests a tilt angle for the transmembrane helices close to 15° (Figure 1A). Initial screening of *E. coli* FtsX loop shows no significant difference of the HSQC spectra at pH between pH 6.0 and 7.5. Figure 1B shows a HSQC spectra obtained at pH 7.5. It is possible to observe a small dispersion of the signals, which is consistent with a CD spectra that indicates this loop contains disordered regions. Additional spectra are been collected for sequential assignment.

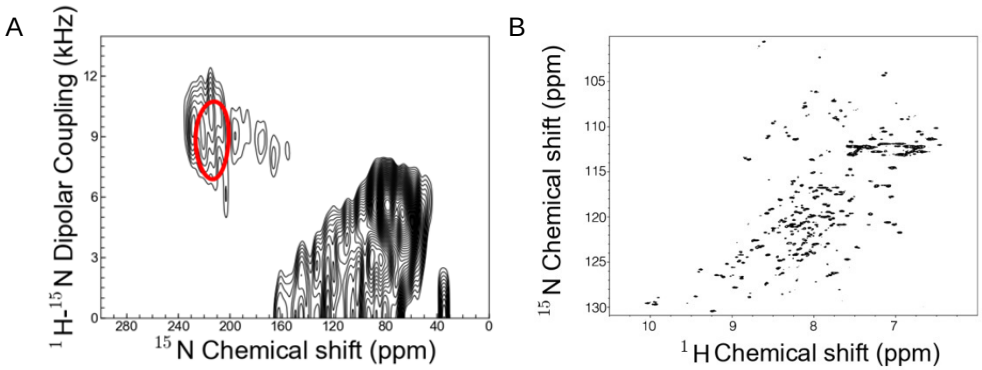

Figure 1**: Solution and solid state NMR spectra of FtsX. (A)** 2D PISEMA spectra of a ^{15}N uniformly labeled FtsX aligned sample. A calculated pisa wheel for a tilt angle of 15° is represented in red. The spectra was collected using 4096 scans and 25 increments at 15°C (600 MHz magnet). **(B)** 2D HSQC spectra of ^{15}N uniformly labeled FtsX from *E. coli*. This spectra was collected using 256 scan and 256 increments at 25° C (800 MHz magnet).

Conclusions

From these results it is possible to conclude that improvements on aligned samples have been done and more work is required to prepare specifically aligned samples for further characterization of the transmembrane domain of FtsX.

Acknowledgements

This study is supported by NIH P01AI-074805 and the NHMFL (State of Florida and NSF DMR 1157490), as well as, by the Fulbright Scholarship awarded to C. Escobar.

References

[1] Yang, D. C., *et al*., PNAS, **108(45)**, E1052-60 (2011).
[2] Ahmad Mir, M., *et al*., Arch Microbiol, **185**, 147–158 (2006).