Protein-Lipid Interactions of Mycobacterium Division Proteins FtsQ and ChiZ

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Introduction

Mycobacterium tuberculosis is reported to be the leading cause of death by infectious disease with an estimated 10 million new infections resulting in over 1.3 million deaths annually[1]. Cellular division is an essential step in the life cycle of *M. tuberculosis* which requires a high degree of structural rearrangement at the septum[2]. The membrane proteins FtsQ and ChiZ are involved in this process as the localize to the septum at division. It has been suggested that both FtsQ and ChiZ interact with each other as well as other proteins involved in the division process including FtsZ[*3*–5].

Experimental

Proteoliposome samples were prepared in the Chemical Sciences Laboratory of Florida State University. Solid-State samples include full-length ChiZ and the N-terminal 99 residues of FtsQ ($FtsQ_{N\text{-}Term}$) reconstituted in vesicles composed of either natively extracted *Mycobacterium smegmatis* lipids or synthetic POPE/POPG lipids. Solution NMR samples include full-length ChiZ embedded in DPC micelles, DMPC/DHPC bicelles or POPC/POPG nanodiscs.

Solid-state NMR experiments include ^{13}C detected CP-DARR and INEPT-TOBSY. Solution-state NMR experiments include ^{1}H detected HSQC, HNCA, HNCO, HNCACB and HN(CO)CB.

Results and Discussion

$FtsQ_{N\text{-}Term}$ remains soluble in aqueous buffers and can be measured by solution NMR. Chemical shift analysis of assigned residues indicates that $FtsQ_{N\text{-}Term}$ remains dynamic and primarily disordered with a short stretch displaying alpha-helical character. A titration of $FtsQ_{N\text{-}Term}$ with FtsZ led to signal intensity reduction amongst residues 59-77 and chemical shift perturbations localized amongst the first 40 residues. These perturbations are attributed to FtsZ-FtsQ interactions.

$FtsQ_{N\text{-}Term}$ shows no interaction with POPC vesicles but binds tightly to Vesicles containing 40-80% negatively charged POPG lipids as well as vesicles formed from native lipid extractions. These samples can be measured using Solid-State NMR. Comparison of INEPT and CP based ^{13}C-^{13}C correlation spectra indicate that much of the $FtsQ_{N\text{-}Term}$ remains highly mobile and dynamic despite being bound to vesicles. The only residues clearly visible by CP, and therefore rigid-like, appear to be arginines, alanines and glutamic acids.

Full-length ChiZ can also be measured by solid-state NMR when reconstituted in vesicles. Contrary to FtsQ, the majority of ChiZ signals are seen in the CP based experiment, indicating more widespread rigidity in the structure.

Conclusions

Despite binding tightly to negatively charged membrane surfaces, the N-terminal domain of $FtsQ_{N\text{-}Term}$ remains primarily unstructured. While this may not remain true in a crowded cell environment, it may maintain flexibility to facilitate interactions with partners at the inner leaflet surface. The interactions with the negatively charged lipids are driven by electrostatics and may be dependent on unique arginine motifs. The motivating forces of FtsZ interaction are less clear but appear to be limited to N-terminal 1-40 residues and while ChiZ exhibits a more rigid structure, work remains to study its interaction with FtsQ.

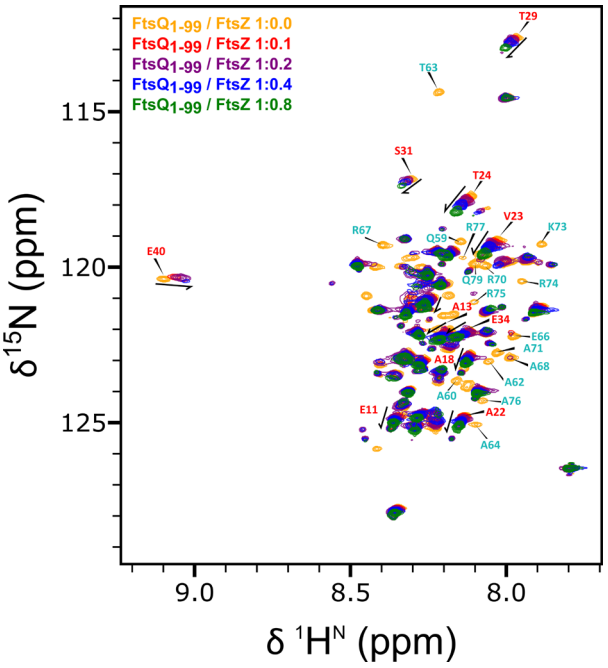

Fig.1 $FtsQ_{N\text{-}term}$ titrated with FtsZ by solution-state NMR. Residues of interest are labeled with those experiencing chemical shift perturbations (red) and those with attenuated signal (blue)

Acknowledgements

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