Binding of MgtR, a Salmonella Transmembrane Regulatory Peptide, to MgtC, a Mycobacterium Tuberculosis Virulence Factor: A Structural Study

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Introduction

MgtC is a membrane protein and an important virulence factor for pathogens that replicate in human macrophages, such as *Mycobacterium tuberculosis* (*Mtb*), the causative agent for TB. MgtC has recently been shown to inhibit the all-important F_1F_0 ATPase that synthesizes ATP for the host. In *Salmonella* another small membrane protein, MgtR inhibits MgtC, but MgtR is not present in the genome of *Mtb*. Here, we describe the binding of *Salmonella* MgtR to a transmembrane helix of the *Mtb* MgtC using Oriented Sample Solid State NMR, Electron Paramagnetic Resonance and Restrained Molecular Dynamics, potentially opening a new approach for TB drug development [1].

Experiments

Peptides were chemically synthesized. Oriented Sample solid state NMR spectra were acquired on 400 MHz NMR spectrometer using an NHMFL Low-E triple-resonance biosolids probe. Native cysteines in MgtR and MgtC were spin labeled with MtSSL. All samples were prepared in DMPC lipid bilayers. Continuous wave EPR measurements were performed on a Bruker ELEXSYS E680 spectrometer at 150K and at the NHMFL.

Results and Discussion

Fig. 1 shows the ssNMR spectra characterizing the MgtR structure, additional ssNMR data was obtained for MgtC and for the complex. Fig. 2 shows EPR spectra of the components and complex providing a distance restraint between the two peptides, while Fig. 3 shows the restrained molecular dynamics refined structure.

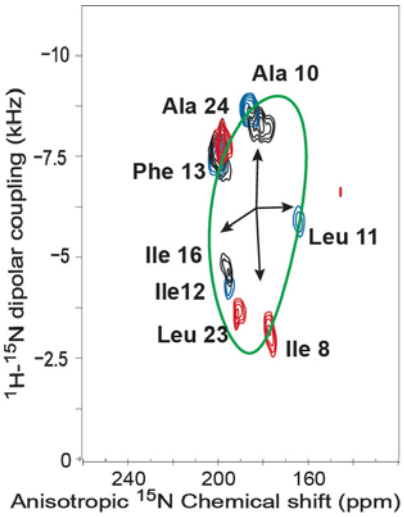

Fig. 1: Superimposed OS ssNMR spectra of three specific site isotopically labeled MgtR samples in liquid crystalline lipid bilayers.

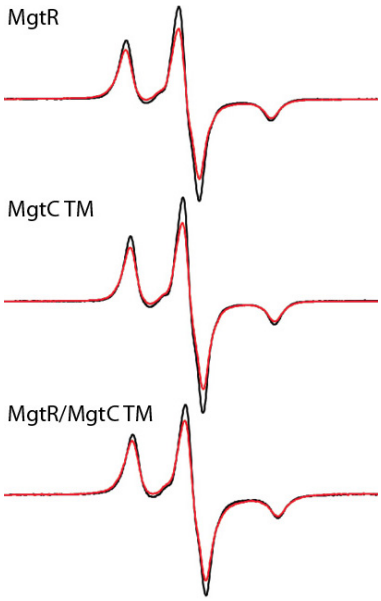

Fig. 2: EPR spectra of MgtR, MgtC TM helix #4 and the combination of the two. Red curves – spin labeled; Black curves – 30% labeled peptides in (a) and (b) and the sum single labeled peptides.

Conclusions

Salmonella MgtR binds to *Mtb* MgtC. The active site of MgtC is just a few residues from the N-terminus of the TM4 and therefore MgtR is in an ideal position to influence the function of MgtC raising the possibility that a peptide analog of MgtR could be developed as a new class of anti-TB drugs.

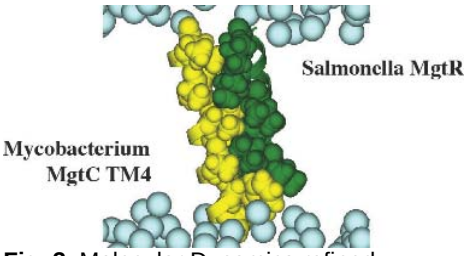

Fig. 3: Molecular Dynamics refined complex in DMPC bilayers showing the inter-digitation of the sidechains

Acknowledgements

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References

[1] Jean-Francois, F., *et al.,* J. Mol. Biol, **426**, 436-446 (2014).