Poster Presentation

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Crystal structure of the Mycobacterium tuberculosis transcriptional regulator FasR

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Mycobacteria have two fatty acid synthases (FAS I and FAS II) which work in concert to synthesize fatty acids and mycolic acids [1-2]. We identified a transcriptional regulator essential for mycobacterial viability: FasR, which specifically binds to fas promoter region and controls the de novo fatty acid biosynthesis [3]. The main purpose of our studies is to understand at the molecular level how mycobacteria exert a fine control over the biosynthesis of their membrane. The characterization of long chain acyl-CoAs that modulates the affinity of FasR for its target DNA was studied using electrophoretic mobility shift assay, SPR and in vitro transcription. In order to deeply understand the molecular bases of FasR activity; we performed experiments, based on sitting drop vapor diffusion, to obtain the crystal structures of FasR, FasR-DNA and FasR-effector. To identify crystallization conditions, full length protein was initially screened using commercial crystallization kits and FasR resulted in small crystals, although not suitable for structural determination. We then decided to choose alternative constructs in order to obtain truncated versions of the original ones, minimizing unstructured regions that would be causing solubility problems and/or hindering crystallization. By using sequence alignment and secondary structure prediction tools, we thus planned to remove an N-terminal disordered fragment of FasR from M. tuberculosis (FasRD-). Crystallization screenings using FasRD- identified two different sets of conditions producing crystals of FasRD- alone and in complex with acyl C20-CoA, both of which diffracted X rays at better than 1.8 Å resolution. We have recently been able to solve both crystal structures and the final refinement is underway. Electrophoretic mobility shift experiments using FasR mutants generated to prevent the binding of the ligand into the effector domain, designed from the structural analysis of FasRD- : C20-CoA complex, confirm the functionality of said domain, and the key role of the ligand in FasR-DNA interaction. In this work, we show that long-chain acyl-CoAs are key effector molecules that coordinate the expression of FAS system, by directly binding to FasR. Future efforts will be concentrated in obtaining crystals of FasR in complex with its cognate DNA oligonucleotide. The structural characterization of this novel transcriptional regulator will allow us to gain new insights into the transcriptional regulation to the fatty biosynthesis pathways in M. tuberculosis. Furthermore, this protein could represent an attractive target for the development of new antituberculosis drugs.

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