Poster Presentation

MS009.P30

Lipid metabolism enzymes ECH & R-domains maintain mycobacterial lipid diversity

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The biosynthesis machinery [polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS) along with fatty acid synthases (FAS)] and the degradation machinery [FadAB along with enoyl-CoA hydratases/isomerases (ECH)] help in maintaining the remarkably diverse cell wall lipids of Mycobacterium tuberculosis (Mtb). Here, we report how FadAB-ECH functional complex and R-domains of NRPS show strategic adaptations in common folds that have helped establish and maintain the lipid diversity in Mtb.

The studies on degradation machinery showed that Mtb FadB cannot assimilate cis-unsaturated fatty acids due to a constricted fatty acid binding pocket with a volume of 256 Å3 as compared to orthologs (>317 Å3) and hence is dependent on isomerase function of ECH. A 2.06 Å resolution crystal structure of ECH (RWork/RFree 0.178/0.215) helped in classifying the 21 paralogs of Mtb ECH, that share only 10-15% sequence identity with orthologs, into two sub-classes, namely mono-functional (isomerase-only) and bi-functional (isomerase and hydratase). Such a classification is based on the distribution of negatively charged amino acids in the active site identified by structure-based sequence analysis, where a single negative charge indicates mono-functional while two or more negative charges indicate bi-functional enzymes. It is also shown that each of the isomerase-only ECH paralogs makes a functional complex with FadAB to a varying efficiency in assimilating cis-octenoyl fatty acids. These studies seem to suggest that different FadAB-ECH functional complexes help recycle cis-unsaturated fatty acids of varying lengths depending on their bioavailability during different stages of pathogenesis [1].

We also present structural studies of the R-domain from Mtb that is involved in the termination step of biosynthesis by NRPS. A combination of X-ray crystallography, small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR) spectroscopy revealed a unique evolutionary variation that helps the R-domains to release products by a [2+2]e– reduction to alcohols. The first crystal structure of R-domain solved to a resolution of 2.3 Å (RWork/RFree 0.229/0.278) showed that these enzymes belonged to a common short-chain dehydrogenase (SDR) family of enzymes. The structure also revealed that the R-domains have a conserved SDR fold but have also evolved a C-terminal domain that shows conformational changes identified through SAXS upon NADPH binding with a change in radius of gyration from 22 Å to 24 Å and also confirmed by chemical shift perturbations in NMR spectroscopy [2]. In pursuit of an NADPH-bound crystal structure, a new crystal form of apo R-domain was solved to a resolution of 1.81 Å (RWork/RFree: 0.202/0.245), which helped in the identification of changes specifically in the loop regions near the NADPH-binding site of R-domains, viz., Gating and Catalytic loops. These loops form a malleable network of interactions that prevent NADPH binding until carrier protein domains are loaded with fully mature products [3]. Thus, the Mtb R-domains work in precise coordination with other domains during biosynthesis to avoid wasteful expenditure of energy.

[1] Srivastava, S. et al. (2015). Chem. Biol. 22:1577-1587.
[2] Chhabra, A. et al. (2012). Proc. Natl. Acad. Sci. USA. 109:5681-5686.
[3] Kinatukara, P. et al. (2016). J. Struct. Biol. 194:368-374.

Keywords: Enoyl CoA hydratase/isomerase (ECH), Reductase domains (R-domains), virulent lipids