Poster Presentation

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Structural studies on M. tuberculosis O6-methylguanine methyltransferase

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Mycobacterium tuberculosis (MTB) is an extremely well adapted human pathogen capable to survive for decades inside the hostile environment represented by the host's infected macrophages despite exposure to multiple potential DNA-damaging stresses. In order to maintain a remarkable low level of genetic diversity, MTB deploys different strategies of DNA repair, including multi-enzymatic systems, such as Nucleotide Excision Repair, and single-step repair. In particular, to counteract the mutagenic effects of DNA alkylation, MTB performs the direct alkylated-base reversal by sacrificing one molecule of a DNA-protein alkyltransferase, such as O6-methylguanine methyltransferase (OGT; orf: Rv1316c). We present here the biochemical and structural characterization of recombinant mycobacterial OGT (MtOGT) in its wild-type form along with its mutated variants mimicking the ones occurring in relevant clinical strains (i.e. MtOGT-T15S and MtOGT-R37L). Our studies reveal that MtOGT-R37L is severely impaired in its activity as consequence of its ten-fold lower affinity for modified double-stranded DNA (dsDNA) (1). Further investigations on a new structure-based panel of OGT versions, designed to explore different molecular environment at position 37, allowed us a better understanding of the functional role of the MtOGT Arg37-bearing loop during catalysis. Moreover, we solved the crystal structure of MtOGT in covalent complex with modified dsDNA that reveals an unprecedented MtOGT::DNA architecture, suggesting that the MtOGT monomer performing the catalysis needs assisting unreacted subunits during cooperative DNA binding. This work is supported by European Community FP7 program SYSTEMTB (Health-F4-2010-241587)

[1] *Miggiano R., et al. (2013) J Bacteriol. 195, 2728-2736*

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