Poster Presentation

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Characterization of proline utilization pathway in Mycobacterium tuberculosis

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The proline utilization pathway in Mycobacterium tuberculosis (Mtb) has been recently identified as an important factor in Mtb persistence in vivo, suggesting that this pathway could be a valuable therapeutic target against tuberculosis (TB). In Mtb, two distinct enzymes perform the conversion of proline into glutamate; the first step is the oxidation of proline into Δ1-pyrroline-5-carboxylic acid (P5C) by the flavoenzyme proline dehydrogenase (PruB) and the second reaction involves converting the tautomeric form of P5C (glutamate-γ-semialdehyde) into glutamate using the NAD+-dependent Δ1-pyrroline-5-carboxylic dehydrogenase (PruA). Here we describe three-dimensional structures of Mtb-PruA, determined by X-ray crystallography both in its apo state and in complex with NAD+ at 2.5 and 2.1 Å resolution, respectively. The structure reveals a conserved NAD+ binding mode, common to other related enzymes. Conformational differences in the active site, however, linked to changes in the dimer interface, suggest possibilities for selective inhibition of Mtb-PruA despite reasonably high sequence identity with other PruA enzymes. Using recombinant PruA and PruB, the proline utilization pathway in Mtb has also been reconstituted in vitro. Functional validation using a novel NMR approach has demonstrated that the PruA and PruB enzymes are together sufficient to convert proline to glutamate, the first such demonstration for monofunctional proline utilization enzymes.

[1] *Berney, M., Weimar, M. R., Heikal, A. & Cook, G. M. (2012). Mol. Microbiol. 84, 664-681,* ***[2]*** *Zhang, Y. J., Ioerger, T. R., Huttenhower, C., et al. (2012). PLoS Pathog. 8, e1002946.,* ***[3]*** *Griffin, J. E., Gawronski, J. D., Dejesus, M. A., et al. (2011). PLoS Pathog. 7, e1002251.*

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