Poster Presentation

MS009.P56

Structural and functional studies on Mycobacterium tuberculosis ribonucleotide reductase

Lumbini Ramraj Yadav[1], Shekhar Mande[1]
[1]*Lab 2NB, National Centre For Cell Science, Pune, India*
E-mail: lumbini.y@gmail.com

Tuberculosis a disease of dearth and negligence is caused by Mycobacterium tuberculosis. According to WHO reports, about 10.4 million people developed TB and 1.4 million died from it in the year 2015. India is amongst those 15 countries in which highest estimated TB incidence rates are reported. TB is a curable disease, but its management is complicated by the ubiquitous appearance of drug-resistant strains. So, new approaches and new targets are necessary for the development of anti-tuberculosis drug therapy. Ribonucleotide reductases (RNR) enzyme is a potential drug target for new antituberculous agents, as formation of deoxynucleotides cannot be bypassed through complementary enzyme activity

RNR is the enzyme that catalyzes the reduction of ribonucleotides to deoxyribonucleotides, required for DNA replication and repair. Based on the cofactors required and radical initiation pathway RNR are classified into 3 classes Class1 (1a and 1b), Class II and Class III. Our focus is on class 1b RNR that is functional under oxidative stress and iron-limited condition. Class 1b is the functional and only indispensable RNR in Mycobacterium tuberculosis (Mowa et al., 2009). Class 1b RNR is composed of two homodimeric subunits; 1. α subunit encoded by nrdE that has the catalytic site for substrate reduction, an allosteric site for regulation and redox active thiols/disulfide required for catalysis. 2. β subunit encoded by nrdF2 which has oxygen linked di-ferric iron centre and tyrosyl radical. NrdI a flavodoxin-like protein is reported to form oxidants by oxygen reduction for the radical generation. NrdI is therefore considered essential for the enzyme activity as it is involved in cofactor biosynthesis and maintenance in NrdF2 (Boal et al., 2010). The cluster of genes that codes for RNR have an oxidoreductase Rv3049c that exist between them, the role of which is yet to be explored. Most of the literature till date, about RNR, is from the study of RNR operon in E. coli (Nordlund & Reichard, 2006).

We aim at understanding the protein-protein interaction among different subunits and gain structural insights into the reaction mechanism in Mycobacterial species. To understand this reduction process, genes associated in mycobacterial species (NrdE, NrdF, NrdI, and Rv3049c) were cloned and purified. For coexpression and pull down study, constructs with different resistance marker and affinity tags were coexpressed. Complex purification of M. thermoresistible (M.th) NrdF-NrdI was successfully accomplished. Till date, it is known that NrdE-NrdF and NrdF-NrdI form complex to assist NrdE in the reduction process. Interestingly, in our pull-down experiment, we for the first time could see the pull down of M.th NrdI and NrdFI with NrdE and M.tb NrdF2 with Rv3049c. This has led us to rethink of whether it is a cascade of reaction or it's a multiprotein complex that associates with each other to perform the function. Preliminary crystallisation screening yielded microcrystals for M.th NrdI and complex crystals of M.th NrdF-NrdI and M.tb NrdF2- Rv3049c

1. Boal, A. K., Cotruvo, J. A., Jr., Stubbe, J. & Rosenzweig, A. C. (2010). Science 329, 1526-1530.
2. Mowa, M. B., Warner, D. F., Kaplan, G., Kana, B. D. & Mizrahi, V. (2009). Journal of bacteriology 191, 985-995.
3. Nordlund, P. & Reichard, P. (2006). Annual review of biochemistry 75, 681-706.

Keywords: Ribonucleotide reductase , Protein-protein interaction , Mycobacteria