Poster Presentation

MS001.P10

From Structure to function: 'Smlt1473', a pH dependent Polysaccharide lyase

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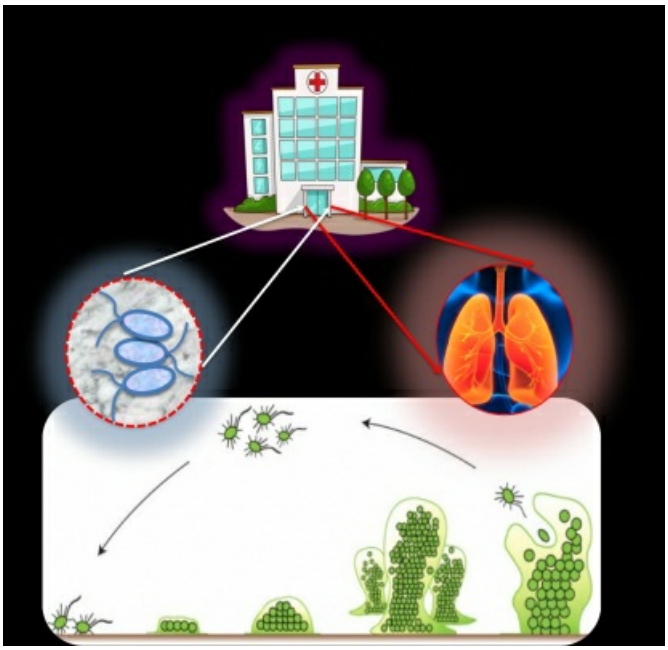
Polysaccharide lyases (PLs) are group of enzyme that cleaves diverse array of anionic polysaccharides via lytic β-elimination reaction mechanism. The end product is generated as an unsaturated hexenuronic acid at newly formed non-reducing end, with a concomitant cleavage of glycosidic bond. Depending upon the fold adopted by the secondary structure elements, which range from α/α barrels to β-helices, PLs are classified into 24 different classes (PL-1 to PL-24). PLs have been reported to catalyze the degradation of anionic polysaccharide of different size but within the same class. The study enzyme, Smlt1473 is a polysaccharide lyase from Stenotrophomonas maltophilia, which can cleave structurally and chemically different classes of anionic polysaccharides as a 'function of pH'. It can specifically cleave hyaluronic acid, poly-β-D-glucuronic acid and alginate at pH-5, 7, and 9 respectively. Hyaluronic acid being the major structural component of host extracellular matrix serves as potential target of 'smlt1473' for maintaining pathological and nutritional niche. Moreover, previous reports have already indicated a low airway surface pH (~5.5) in Cystic Fibrosis Patients (CFPs), which not only bottlenecks conventional antimicrobial treatment but also enhances the role of 'smlt1473' as a virulence factor in such infections. Here we have determined the crystal structure of 'substrate' and 'apo' bound form of smlt1473 at acidic and basic pH to understand the role of residues flanking around the catalytic center and the role of pH in imparting substrate specificity. Hanging drop vapor diffusion experiment was used to grow the protein crystals for X-ray diffraction work and was solved by Molecular Replacement method. Structural analysis has provided the possibility of Induce fit N-terminal lid loop motion in gating the entry of catalytic center. Furthermore, the specific arrangement of ionic, aromatic and hydrophobic residue flanking the catalytic center hints for the processive nature of enzymatic activity. Our results provide the mechanistic view of Smlt1473 pH dependent substrate specificity and will generate structural basis for designing specific and suitable inhibitors to combat pulmonary infections in CFPs caused by such bacterial pathogens.

[1] C. MacDonald, L. et al. (2014). The Journal of Biological Chemistry, 289:1, 312-325.
[2] C. MacDonald, L. et al. (2014). The Journal of Biological Chemistry, 289:26, 18022-18032.
[3] Pezzulo, Alejandro A. et al. (2012). Nature 487, 109-113.



Keywords: Polysaccharide lyase, Smlt1473, pH dependent substrate specificity.