Poster Presentation

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Structural characterization of heparinase III from Bacteroides thetaiotaomicron

T. Ulaganathan[1], M. Cherney[1], D. Yao[1], G. Li[2], L. Li[2], R. Linhardt[2], M. Cygler[1]

[1]University of Saskatchewan, Department of Biochemistry, Saskatoon, Canada, [2]Rensselaer Polytechnic Institute, Biocatalysis and Metabolic Engineering, Troy, USA

Glycosaminoglycans (GAGs) are linear polysaccharides comprised of disaccharide repeat units, a hexuronic acid, glucuronic acid (GlcA) or iduronic acid (IdoA), linked to a hexosamine, N-acetylglucosamine (GlcNAc) or N-acetylgalactosamine (GalNAc). GAGs undergo further modification such as epimerization and sulfation. These polysaccharides are abundant in the extracellular matrix and connective tissues. GAGs function in stabilization of the fibrillar extracellular matrix (ECM), control of hydration, regulation of tissue, organism development by controlling cell cycle, cell behavior, and differentiation. Niche adapted bacteria expresses enzymes called polysaccharide lyases (PL), which degrade GAGs for their nutrient content. Polysaccharide lyases have been classified into 23 sequence-related families. Comparison of three-dimensional structures of the prototypic members of these families allowed identification of distant evolutionary relationships between lyases that were unrecognized at the sequence level and identified occurrences of convergent evolution. We have characterized structurally and enzymatically Heparinase III (HepIII) from Bacteroides thetaiotaomicron, which is classified within the PL12 family. HepIII is a 72.5KDa protein. We will present the X-Ray structures of two crystal forms of HepIII of resolution 1.8 Å and 2.6 Å. HepIII contains two domains, the N-terminal α-helical domain forming a toroid and the C-terminal β-sheet domain. Comparison with recently determined structures of two other heparinases from the same PL12 family allowed us to identify structural flexibility in the arrangement of the domains indicating open-close movement. Based on comparison with other GAG lyases we identified Tyr301 as the main catalytic residue and confirmed this by site-directed mutagenesis. We have characterized substrate preference of HepIII toward sulfate poor heparan sulcate substrate.

Keywords: Polysaccharide lyase, Heparan sulfate