Elucidation of ENGase Glycan Recognition Motifs Using Non-natural Core Trisaccharides

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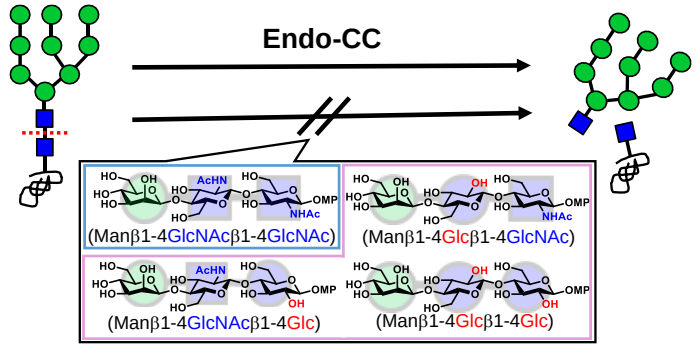
Endo-β-*N*-acetylgucosaminidase (ENGase) is a glycoside hydrolase that acts between the core GlcNAcβ1-4GlcNAc moiety of *N*-linked glycans. The corresponding enzymes have been found in various species and exhibit different substrate specificities. Their hydrolysis activity has been utilized for structural analysis of glycoprotein oligosaccharides. Furthermore, some ENGases are known to have not only glycoside hydrolase activity but also glycosyltransferase activity that transfers glycans to GlcNAc residues as a reverse reaction. Thus, ENGases are attracting attention as bioengineering tools towards glycoproteins and glycopeptides. Various studies on the substrate specificity of ENGases have revealed their branch specificity for *N*-linked glycan. On the other hand, substrate specificity focusing on the core structure of *N*-linked glycans is still unclear. Because the GlcNAcβ1-4GlcNAc core structure is cleaved after ENGase treatment, co-crystal structure analysis for core structure recognition by the enzyme is difficult. In this study, we synthesized non-natural core trisaccharide-type inhibitors designed based on the core structure of *N*-linked glycan, and examined the recognition specificity of ENgases focusing on the core glycan structure from their inhibitory activity. We focused on the acetamido group of GlcNAc residue and synthesized a series of non-natural core trisaccharides in which two acetamido groups in the core glycan structure were systematically replaced with hydroxy groups. The importance of the acetamide group in glycan recognition was then evaluated from the inhibitory activity of each core trisaccharide against ENGase glycoside hydrolysis. The inhibition assays were performed using four core trisaccharides inhibitors (see Figure) against Endo-CC, which belongs to the GH85 ENGase family. Moreover, docking simulations of the inhibitors to Endo-CC were also performed to predict the mode of inhibition. In this presentation, we would like to discuss the recognition properties of ENGase for the core glycan structure, especially the importance of the acetamide group.



Trisaccharide Type Inhibitor