Studies on the Enzymatic Characterization of Novel Endo-β-N-acetylglucosaminidase from Bacteroides nordii

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Title　：Studies on the Enzymatic Characterization of Novel Endo-β-*N*-acetylglucosaminidase from *Bacteroides nordii*
(*Bacteroides nordii* 由来の新規 Endo-β-N-アセチルグルコサミニダーゼの酵素的特性に関する研究)

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Thesis Summary

Endo-β-*N*-acetylglucosaminidases (ENGases) are enzymes that hydrolyze the *N*-linked oligosaccharides on glycoproteins which are found in various organisms from bacteria, up to humans. Many ENGases have already been identified and characterized. However, there are still a few enzymes that have hydrolytic activity towards multibranched complex-type *N*-glycans on glycoproteins. In this study, one novel ENGase from *Bacteroides nordii* (Endo-BN) species was identified and characterized. The recombinant protein was prepared and expressed in *Escherichia coli* cells. This Endo-BN exhibited optimum hydrolytic activity at pH 4.0. High performance liquid chromatography (HPLC) analysis showed that Endo-BN preferred core-fucosylated complex-type *N*-glycans, with galactose or α2,6-linked sialic acid residues at their non-reducing ends. This enzyme can also hydrolyze multi-branched (tri-antennary or tetra-antennary) complex-type-*N*-glycans. The hydrolytic activities of Endo-BN were also tested on different glycoproteins from high-mannose type to complex-type oligosaccharides. The reaction with human transferrin, fetuin, and α1-acid glycoprotein subsequently showed that Endo-BN is capable of releasing multi-branched complex-type *N*-glycans from these glycoproteins. The domain of Endo-BN was also analyzed and results showed that deletion of the T9SS C-terminal (a.a.948-1021) retained its enzymatic activity. However, deletion of an additional 200 amino acids (a.a. 748-947) resulted in 100% loss of activity. This means that the T9SS is not essential for the Endo-BN. Furthermore, the transglycosylation activity of Endo-BN was analyzed and it showed that the wild type Endo-BN could not transfer the sialobiantennary type oligosaccharide onto the deglycosylated RNase B. To obtain Endo-BN mutants with desired transglycosylation activity, mutation analysis was performed on the active site residues Asn-188 (N188), by individually replacing it with another amino acid residue. Transglycosylation analysis showed that N188H and N188Q mutants exhibited higher activity towards glycoprotein acceptors with sugar oxazolines for transferring the biantennary-complex-type oligosaccharide.

The substrate specificity of this ENGase will be useful in *N*-glycan analysis and/or *N*-glycan remodeling. Findings in this study will not only be useful in basic research for analyzing the oligosaccharide contents of antibodies but also in biomedical applications for developing pharmaceutical drugs.