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# Transglycosylation of Asparagine-linked Complex-type Oligosaccharides from Glycoproteins by Endo-β-*N*-acetylglucosaminidase HS

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Endo-β-*N*-acetylglucosaminidases hydrolyze *N*,*N*´-diacetylchitobiose linkages of asparagine-linked oligosaccharides. They can also cleave the linkage with suitable agents having hydroxyl groups and transfer the released oligosaccharides to the agents. Thus endo-β-*N*-acetylglucosaminidases are very useful for synthesis of neoglycoconjugates having asparagine-linked oligosaccharides. On the other hand, the structures of asparagine-linked oligosaccharides are divided into three groups, high-mannose type, hybrid type and complex type. We discovered a novel endo-β-*N*-acetylglucosaminidase (Endo), named Endo HS. Endo HS can specifically hydrolyze bi-, tri- and tetraantennary complex-type oligosaccharides from glycoproteins. We have investigated the transglycosylation reaction by Endo HS. Endo HS transferred the biantennary complex type oligosaccharide from human transferrin to *p*-nitrophenyl (PNP)-β-D-Glc and PNP-β-D-Gal. Endo HS was strictly distinct from other enzymes in transferring oligosaccharide to the Gal moiety. The amount of the transglycosylation product increased depending on the concentration of the acceptors. Endo HS also transferred the oligosaccharide to PNP-α-D-Glc, PNP-α-D-Gal, PNP-β-D-Man, PNP-β-D-Xyl, PNP-β-D-GlcNAc and PNP-glycerol. The amount of the transglycosylation product successively increased and became constant and then barely decreased. No apparent difference in the $K_m$ value for human transferrin as an oligosaccharide donor was observed using different acceptors such as PNP-β-D-Glc and PNP-glycerol. Endo HS also transferred the triantennary complex-type oligosaccharide from calf fetuin and the bi-, tri- and tetraantennary complex-type oligosaccharides from

human $\alpha_1$-acid glycoprotein to PNP-β-D-Glc. In addition to glycoproteins, Endo HS transferred biantennary complex-type oligosaccharide from glycopeptides. Furthermore, Endo HS transferred bi- and triantennary complex-type oligosaccharides from glycoasparagines to various monosaccharides, oligosaccharides, sugar alcohols and glycosides. The addition of polar organic solvents was also effective for the transglycosylation efficiency. The results demonstrate that Endo HS is a useful tool for synthesis of neoglycoconjugates having a wide variety of complex-type asparagine-linked oligosaccharides from glycoproteins.

**Key words:** endo-β-*N*-acetylglucosaminidase HS, endo HS, asparagine-linked oligosaccharide, glycoprotein, transglycosylation



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