

Loop protein engineering for improved transglycosylation activity of a β -*N*-Acetylhexosaminidase

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ABSTRACT

Certain enzymes of the glycoside hydrolase family 20 (GH20) exert transglycosylation activity and catalyze the transfer of β -*N*-acetylglucosamine (GlcNAc) from a chitobiose donor to lactose to produce lacto-*N*-triose II (LNT2), a key human milk oligosaccharide backbone moiety. The present work is aimed at increasing the transglycosylation activity of two selected hexosaminidases, HEX1 and HEX2, to synthesize LNT2 from lactose and chitobiose. Peptide pattern recognition analysis was used to categorize all GH20 proteins in subgroups. On this basis, we identified a series of proteins related to HEX1 and HEX2. By sequence alignment, four additional loop sequences were identified that were not present in HEX1 and HEX2. Insertion of these loop sequences into the wild-type sequences induced increased transglycosylation activity for three out of eight mutants. The best mutant, HEX1_{GTEPG}, had a transglycosylation yield of LNT2 on the donor that was nine times higher than that of the wild-type enzyme. Homology modeling of the enzymes revealed that the loop insertion produced a more shielded substrate-binding pocket. This shielding is suggested to explain the reduced hydrolytic activity, which in turn resulted in the increased transglycosylation activity of HEX1_{GTEPG}.

KEYWORDS:

Loop Protein; Transglycosylation; β -*N*-Acetylhexosaminidase