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Engineered *N*-acetylhexosamine-active enzymes in glycoscience

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ABSTRACT

Background: In recent years, enzymes modifying *N*-acetylhexosamine substrates have emerged in numerous theoretical studies as well as practical applications from biology, biomedicine, and biotechnology. Advanced enzyme engineering techniques converted them into potent synthetic instruments affording a variety of valuable glycosides.

Scope of review: This review presents the diversity of engineered enzymes active with *N*-acetylhexosamine carbohydrates: from popular glycoside hydrolases and glycosyltransferases to less known oxidases, epimerases, kinases, sulfotransferases, and acetylases. Though hydrolases *in natura*, engineered chitinases, β-*N*-acetylhexosaminidases, and *endo*-β-*N*-acetylglucosaminidases were successfully employed in the synthesis of defined natural and derivatized chitooligomers and in the remodeling of *N*-glycosylation patterns of therapeutic antibodies. The genes of various *N*-acetylhexosaminyltransferases were cloned into metabolically engineered microorganisms for producing human milk oligosaccharides, Lewis X structures, and human-like glycoproteins. Moreover, mutant *N*-acetylhexosamine-active glycosyltransferases were applied, e.g., in the construction of glycomimetics and complex glycostructures, industrial production of low-lactose milk, and metabolic labeling of glycans. In the synthesis of biotechnologically important compounds, several innovative glycoengineered systems are presented for an efficient bioproduction of GlcNAc, UDP-GlcNAc, *N*-acetylneuraminic acid, and of defined glycosaminoglycans.

Major conclusions: The above examples demonstrate that engineering of *N*-acetylhexosamine-active enzymes was able to solve complex issues such as synthesis of tailored human-like glycoproteins or industrial-scale production of desired oligosaccharides. Due to the specific catalytic mechanism, mutagenesis of these catalysts was often realized through rational solutions.

General significance: Specific *N*-acetylhexosamine glycosylation is crucial in biological, biomedical and biotechnological applications and a good understanding of its details opens new possibilities in this fast developing area of glycoscience.

Keywords: β-*N*-acetylhexosaminidase; chitinase; glycosynthase; site-directed mutagenesis;

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