MUTANT GLYCOSYLTRANSFERASES AND HEXOSAMINIDASES IN THE SYNTHESIS OF ADVANCED GLYCOMATERIALS

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The β-*N*-acetylhexosaminidase (EC 3.2.1.52, CAZy GH20) from the filamentous fungus *Talaromyces flavus* has been shown to possess unique substrate flexibility and remarkable synthetic ability. However, the yields of the transglycosylation reactions are significantly lowered by the undesired hydrolysis of the glycosidic bonds of the substrate and product. To overcome this problem, **mutant variants of *T. flavus* β-*N*-acetylhexosaminidase** were designed based on a computational model of the active site of this enzyme, aiming at diminishing its hydrolytic activity and retaining transglycosylation activity at the same time, *i. e.* at the preparation of a transglycosidase [1]. All of the **mutant variants featured strongly (ca 200-fold) reduced hydrolytic activity** while maintaining high transglycosylation activity reaching extraordinary conversion rates. This synthetic toolbox was used for the preparation of chitooligomeric spacers terminated with a β4-linked β-*N*-acetylgalactosaminyl-(1→4)-*N*-acetylglucosamine (LacdiNAc) epitope using human glycosyltransferases β4GalT, β4GalTY284L [2]. These ligands presented in the form of a multivalent neoglycoconjugate on BSA proved to be inhibitors of human galectin-3 (Gal-3). Chitooligomeric spacer revealed that this approach is very good nature-like solution for the presentation of prepared Gal-3 glycan epitopes, surpassing the performance of commonly used synthetic spacers. Tyr470Asn mutant of β-*N*-acetylhexosaminidase was employed for synthesis of azide-terminated chitooligomeric ligands that proved to have (sub-)nanomolar affinity to wheat germ agglutinin upon multivalent presentation at the polymeric matrix (*N*-(2-hydroxypropyl) methacrylamide - HPMA) [3]. These examples clearly demonstrate high utility of this mutant glycosidase for preparatory synthesis of functionalized chitooligomers acting as more efficient biomimetic spacers for multivalent carbohydrate presentation in biomaterials.

References

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