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STRUCTURAL BASIS FOR THE TRANSGLYCOSYLATION ACTIVITY OF ENDO-β-N-ACETYLGLUCOSAMINIDASE A

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Endo-β-N-acetylglucosaminidase A (Endo A) is a 72 kDa glycohydrolase that, unlike other endoglycosidases such as Endo H, is capable of transglycosylation: the transfer of released oligosaccharides to hydroxyl groups other than water. The enzyme has been used in the enzymatic synthesis of neoglycoconjugates. The crystal structure of Endo A, in complex with a core pentamannoside, has been determined to 2.2 Å resolution. The molecule consists of three domains: an endoglycosidase domain with typical (β/α) 8-fold; a plant lectin-like all-β-domain; and a fibronectin type III domain. The role of the two additional domains is unclear because they are not positioned correctly for interaction with the substrate. The active site of the enzyme differs from that of Endo H and related endoglycosidases in that the primary catalytic residue, Glu173, is in hydrogen bonding contact with Trp216, a residue that has been shown to be essential for transglycosylation. In addition, the aspartic acid that has been shown to be involved in stabilizing the intermediate in the substrate-assisted catalysis mechanism of family-18 glycohydrolases is replaced by an asparagine. The interaction of the pentamannoside with the enzyme reveals that the high-mannose substrate specificity of Endo A is defined by positive recognition of the α(1-3)-linked mannose on the α(1-6)-linked branch. However, the detailed molecular architecture in this area of the molecule differs greatly from that of other high-mannose specific endoglycosidases such as Endo H and Endo F1.
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Keywords: GLYCOHYDROLASE TRANSGLYCOSYLATION PROTEIN-CARBOHYDRATE INTERACTION

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INSIGHTS INTO THE FUNCTIONAL ARCHITECTURE OF THE CATALYTIC CENTER OF A MAIZE β -GLUCOSIDASE Zm-p60.1

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The maize β-glucosidase Zm-p60.1 has been implicated in regulation of plant development by the targeted release of free cytokinins from cytokinin-O-glucosides, their inactive storage forms. The crystal structure of the enzyme indicated that the enzyme specificity toward substrates with aryl aglycones is determined by aglycone aromatic system stacking with W373, and interactions with edges of F193, F200 and F461 located opposite W373 in a slot-like aglycone-binding site. Recently, these aglycone-active-site interactions were hypothesized to determine substrate specificity in inactive enzyme-substrate complexes of ZMGlu1, an allozyme of Zm-p60.1. We tested this hypothesis by kinetic analysis of F193I/Y/W mutants. The decreased Km of all mutants confirmed the involvement of F193 in determining enzyme affinity towards substrates with an aromatic aglycone. Unexpectedly, a 30-fold decrease in kcat was found in F193I mutant compared to the wild type. Kinetic analysis and computer modeling demonstrated that the F193-aglycone-W373 interaction not only contributes to aglycone recognition as hypothesized previously but also co-determines catalytic rate by fixing the glucosidic bond in an orientation favorable for attack by the catalytic pair, E186 and E401. The catalytic pair was confirmed by kinetic analysis of E186D/Q and E401D/Q mutants. Unexpectedly, the E401D as well as C205S and C211S mutations dramatically impaired the assembly of a catalysis-competent homodimer suggesting novel links between the active site structure and dimer formation.

Keywords: **GLYCOSIDASE CYTOKININ GLYCOSYL HYDROLASES**

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BIOCHEMICAL AND STRUCTURAL ASSESSMENT OF THE 1-N-AZASUGAR GALNAC-ISOFAGOMINE AS A POTENT FAMILY 20 β-HEXOSAMINIDASE INHIBITOR

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Azasugar inhibitors of the isofagomine class are potent competitive inhibitors of configuration-retaining β-glycosidases. This potency results from the formation of a strong electrostatic interaction between a protonated endocyclic nitrogen at the 'anomeric' center of the inhibitor and the catalytic nucleophile of the enzyme. Whereas the majority of retaining beta-glycosidases use a mechanism involving a carboxylate residue as a nucleophile, Streptomyces plicatus β-N-acetylhexosaminidase (SpHEX) and related family 20 glycosidases lack such a catalytic residue and use instead the carbonyl oxygen of the 2-acetamido group of the substrate as a nucleophile to attack the anomeric center. Thus, a strong electrostatic interaction between the inhibitor and enzyme is not expected to occur; nonetheless, the 1-N-azasugar (2R,3R,4S,5R)-2-acetamido-3,4-dihydroxy-5-hydroxymethyl-piperidinium chloride (GalNAc-isofagomine HCl), which was synthesized and assayed for its ability to inhibit SpHEX, was found to be a potent competitive inhibitor of the enzyme (Ki = 2.7 uM). A crystallographic complex of GalNAc isofagomine bound to SpHEX (1.75 Å resolution) revealed a novel hydrogen-bonding interaction between the equatorial proton of the azasugar ring nitrogen of GalNAc-isofagomine and the carboxylate of the general acid-base residue Glu314 of SpHEX. This hydrogen bond appears to compensate for the lack of a strong electrostatic interaction that would occur between the endocyclic ring nitrogen atom of an isofagomine inhibitor and a glycosidase containing an enzymic nucleophile. Hence, this novel hydrogen-bonding interaction appears to contribute to the unexpected potency of GalNAc-isofagomine toward the family 20 glycosidase.

Keywords: **GLYCOSIDASE FAMILY 20 INHIBITOR**

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STRUCTURAL INSIGHTS INTO THE MECHANISM OF THYMIDYLATE SYNTHASE COMPLEMENTING PROTEINS

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Thymidylate synthase (TS) catalyzes the synthesis of deoxythymidine monophosphate. TS inhibition stops DNA production, arresting the cell cycle and eventually leading to 'thymineless' cell death. Thymidylate synthase complementing proteins (TSCP) are encoded by the thy1 gene and are not homologous to TS enzymes encoded by thyA and thyB genes. The mechanism by which TCSP dependent organisms make survival without exogenous thymidylate is completely unknown.
Here we present the first structure of a TSCP from *Thermotoga maritima*. This structure exhibits a novel fold, and structural studies with various compounds reveal an interesting mechanism for this family of enzymes. In contrast to the classical dimeric nature of TS enzymes, the TSCP structure revealed a tetramer with four interconnected active sites. Four flavin adenine dinucleotide (FAD) molecules are present in the active site.
Due to the widespread occurrence of thy1 gene among many pathogenic bacteria TSCP inhibitors are potential antibacterial drugs. The unexpected binding of FAD is mechanistically interesting. From our current results, we propose a mechanism similar to the folate-dependent ribothymidyl synthase. Details of the structures and an overview of the mechanism of TSCP catalysis will be presented.
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