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Rational design of a glycosynthase by the crystal structure of β-galactosidase from *Bacillus circulans* (BgaC) and its use for the synthesis of *N*-acetyllactosamine type 1 glycan structures

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ABSTRACT

The crystal structure of β-galactosidase from *Bacillus circulans* (BgaC) was determined at 1.8 Å resolution. The overall structure of BgaC consists of three distinct domains, which are the catalytic domain with a TIM-barrel structure and two all-β domains (ABDs). The main-chain fold and steric configurations of the acidic and aromatic residues at the active site were very similar to those of *Streptococcus pneumoniae* β(1,3)-galactosidase BgaC in complex with galactose. The structure of BgaC was used for the rational design of a glycosynthase. BgaC belongs to the glycoside hydrolase family 35. The essential nucleophilic amino acid residue has been identified as glutamic acid at position 233 by site-directed mutagenesis. Construction of the active site mutant BgaC-Glu233Gly gave rise to a galactosynthase transferring the sugar moiety from α-D-galactopyranosyl fluoride (αGalF) to different β-linked *N*-acetylglucosamine acceptor substrates in good yield (40–90%) with a remarkably stable product formation. Enzymatic syntheses with BgaC-Glu233Gly afforded the stereo- and regioselective synthesis of β1-3-linked key galactosides like galacto-*N*-biose or lacto-*N*-biose.

1. Introduction

The relevance of carbohydrates in numerous biological processes (Varki, 1993) motivated the development of synthetic strategies to advance the knowledge on protein–carbohydrate interactions (Schmaltz et al., 2011). Biocatalytic cascade reactions are a viable alternative to organic chemistry in the synthesis of complex carbohydrate structures due to their selectivity and product yields (Sauerzapfe and Elling, 2008). The advantage of enzyme-based processes lies in the one-step synthesis of glycan structures without stepwise protection and deprotection cycles of the hydroxyl groups (Flitsch, 2000; Palcic, 1999). Contrary to multiple chemical steps using distinct catalysts biocatalysis facilitates optimization of reaction conditions by biocatalysts engineering. Two disaccharides with particular importance as part of *N*- and *O*-glycans of glycoproteins as well as milk oligosaccharides are Gal(β1-3)GlcNAc (lacto-*N*-biose, *N*-acetyllactosamine 1 or LacNAc type 1) and Gal(β1-3)GalNAc (galacto-*N*-biose, TF-antigen or core 1) (Bode, 2012; Bojarova et al., 2013; Kobata, 2010). They have been synthesized by glycosyltransferases (Gao et al., 2013; Yi et al., 2008; Yu et al., 2010) and glycosidases (Fujimoto, 1997; Fujimoto et al., 1998; Hedbys et al., 1989; Vetere et al., 2000). The capability of glycoside hydrolases to achieve high stereoselectivity in transglycosylation reactions with cost-effective, purchasable substrates has led to several strategies of reaction engineering to improve their product yields and synthetic spectra (Bojarova and Kren, 2011). A promising prokaryotic β-galactosidase from *Bacillus*

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circulans (BgaC, GenBank accession no. BAA21669) which is clustered in glycoside hydrolase family 35 in the Carbohydrate Active enZYme (CAZy) database is referred to hydrolyse β1-3 linkages. In GH-35 around 800 database entries from different sources are described among them seven β-galactosidases with solved crystal structure (Henrissat and Davies, 1997). In terms of reaction mechanism BgaC is one of the retaining β-galactosidases. In a typical distance of 5 Å two carboxylic acid residues of glutamic acid within the $(\alpha/\beta)_8$ TIM barrel fold interact with an acceptor substrate in a double displacement reaction (Rye and Withers, 2000; White and Rose, 1997). A structure model relates Glu157 and Glu233 as acid/base and nucleophile catalyst, respectively. Using 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-D-galactopyranoside as reaction-based inhibitor the attacking carboxylate was identified as Glu233 for β-galactosidase from *B. circulans* (Blanchard et al., 2001). The potential of glycoside hydrolases for transglycosylation reactions is attenuated by the fact that hydrolysis is favored which results in moderate yields from 20 to 40% (Schmaltz et al., 2011). Employing organic solvents or ionic liquids increased product yields in transglycosylation reactions as demonstrated for β-galactosidases from *B. circulans* (Bayon et al., 2013; Kaftzik et al., 2002). In our previous work we could demonstrate that transglycosylation reactions with glycosidases gave access to nucleotide-activated oligosaccharides as a novel class of glycoconjugates (Kamerke et al., 2012; Nieder et al., 2003, 2004; Zervosen et al., 2001). Among them BgaC was used for the synthesis of UDP-activated core1 and LacNAc type 1 (Kamerke et al., 2013).

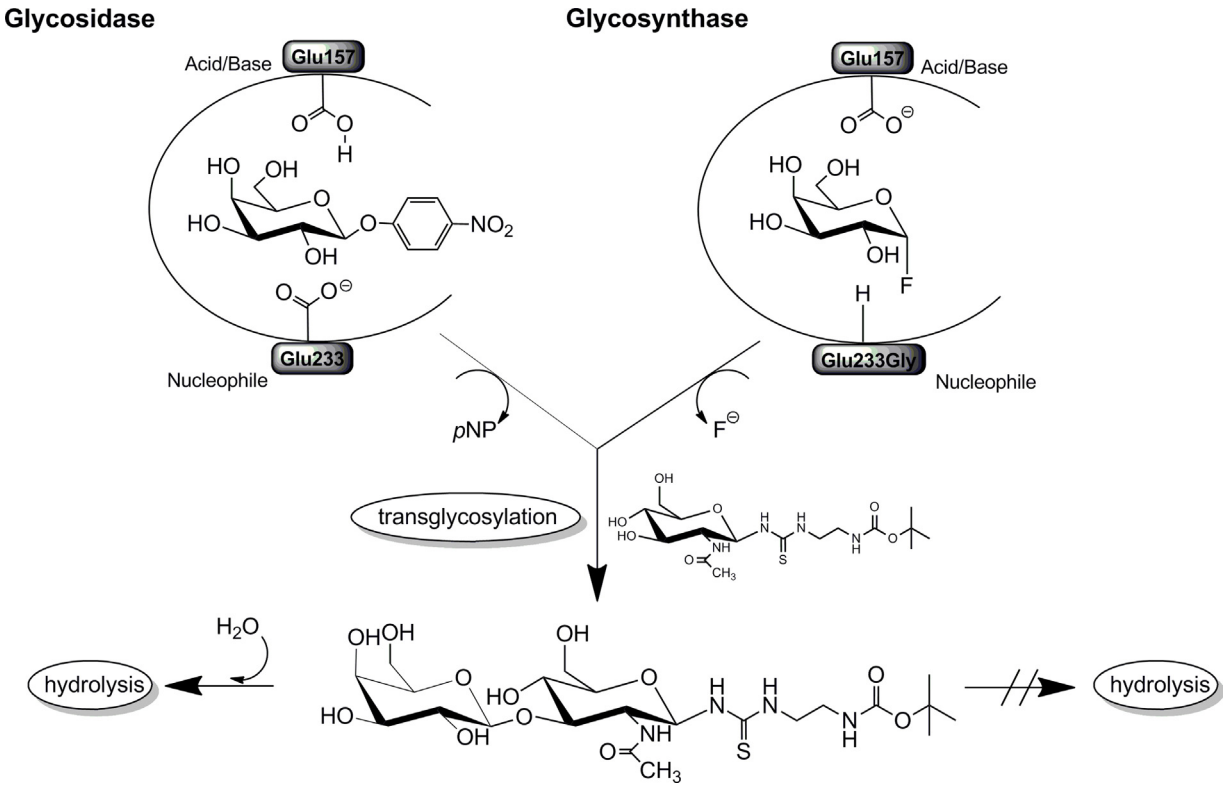
The concept of glycosynthases requires the knowledge of protein structure and was first published by Withers (Mackenzie et al., 1998) and Planas (Malet and Planas, 1998). Endo- and exoglycosidases with mutations in their proton donor site were generated and referred to as glycosynthases as a new class of biocatalysts. Their lacking hydrolytic activity renders them as novel biocatalyst tools for the synthesis of oligosaccharides in nearly quantitative yields. The application of glycosynthases requires activated sugars (glycosyl fluoride, glycosyl azide) which mimic the glycosyl–enzyme intermediate (Mackenzie et al., 1998). Glycosynthases of several GH families have been generated to produce a range of building blocks (Cobucci-Ponzano and Moracci, 2012; Cobucci-Ponzano et al., 2011a; Hancock et al., 2006; Perugino et al., 2004). In the GH family 35 mutation of the acid/base in the active site of the β-galactosidase from *Xanthomonas manihotis* gave rise to a thioglycoligase (Kim et al., 2006, 2007). In the course of our studies presented here, the generation of a glycosynthase from BgaC for enzymatic synthesis of aryl-substituted lacto- and galacto-*N*-biose was reported (Li and Kim, 2014).

In our previous studies we focused on the combinatorial synthesis of poly-LacNAc type 2 with two recombinant glycosyltransferases, human β1,4-galactosyltransferase and β1,3-*N*-acetylglucosaminyltransferase from *Heliobacter pylori* (Adamiak et al., 2012; Kupper et al., 2012; Rech et al., 2011; Sauerzapfe et al., 2009). The amino-*t*Boc and azide-linked GlcNAc acceptor substrates provide versatile functional groups for coupling poly-LacNAc onto biomaterial surfaces and functional testing of galectins for the setup of an artificial ECM (Beer et al., 2012, 2013; Kupper et al., 2013). In this respect, we aim for the synthesis of linker-functionalized lacto-*N*-biose (LacNAc type 1) as potential ligand of galectins. We report here the crystal structure of *B. circulans* β3-galactosidase (BgaC) from GH35 which lays the basis for the generation of a galactosynthase to synthesize LacNAc type 1 starting with linker-conjugated GlcNAc acceptor substrates (Scheme 1).

2. Materials and methods

2.1. Strains and substrates

Escherichia coli BL21(DE3) and *E. coli* Nova Blue were obtained from MerkMillipore (USA). The GlcNAc-linker-*t*Boc was kindly provided by Prof. Dr. Křen (Academy of Science of the Czech Republic). The synthesis of *O*-linked amino-, propargyl- or azide-conjugated



Scheme 1. Synthesis of lacto-*N*-biose (LacNAc type 1) by recombinant BgaC from *Bacillus circulans* and the associated glycosynthase.