Structure of the α -1,6/ α -1,4-specific glucansucrase GTFA from *Lactobacillus reuteri* 121

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The reuteransucrase GTFA from *Lactobacillus reuteri* 121, which belongs to glycosyl hydrolase family GH70, synthesizes branched α -glucans with both α -1,6- and α -1,4-glycosidic linkages (reuteran) from sucrose. The crystal structure of GTFA- Δ N, a 118 kDa fragment of GTFA comprising residues 745–1763 and including the catalytic domain, was determined at 3.6 Å resolution by molecular replacement. The crystals have large solvent channels and an unusually high solvent content of 85%. GTFA- Δ N has the same domain arrangement and domain topologies as observed in previously determined GH70 glucansucrase structures. The architecture of the GTFA- Δ N active site and binding pocket confirms that glucansucrases have a conserved substrate specificity for sucrose. However, this first crystal structure of an α -1,6/ α -1,4-specific glucansucrase shows that residues from conserved sequence motif IV (1128–1136 in GTFA- Δ N) contribute to the acceptor-binding subsites and that they display differences compared with other structurally characterized glucansucrases. In particular, the structure clarifies the importance of residues following the transition-state stabilizer for product specificity, and especially residue Asn1134, which is in a position to interact with sugar units in acceptor subsite +2.

1. Introduction

Glucansucrases (EC 2.1.4.5; glucosyltransferases; GTFs) catalyze the synthesis of high-molecular-mass (up to 50 MDa) α -glucan-type extracellular polysaccharides from sucrose (Kralj *et al.*, 2004; Monchois *et al.*, 1999; van Hijum *et al.*, 2006; Leemhuis *et al.*, 2012). Cleavage of sucrose results in the formation of a covalent glucosyl-enzyme intermediate and the release of fructose, after which the glucosyl moiety is transferred to a (growing) α -glucan chain. In addition to the synthesis of a wide variety of linear and branched α -glucan polymers, such as dextran, mutan, alternan and reuteran, they can also catalyze the transfer of glucosyl units to various other acceptors (Leemhuis *et al.*, 2012). Glucansucrases have been identified in Gram-positive bacteria such as *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Weissella* species (van Hijum *et al.*, 2006; Malik *et al.*, 2009; Bounaix *et al.*, 2010). GTFs are large extracellular enzymes of typically 120–200 kDa which have been classified into glycoside hydrolase family GH70 (Cantarel *et al.*, 2009) and share signature sequence motifs (I–IV) and reaction mechanisms with enzymes from the α -amylase superfamily (van Hijum *et al.*, 2006; Leemhuis *et al.*, 2012). In addition, the amylosucrase from the Gram-negative bacterium *Neisseria polysaccharea*, which synthesizes amylose-like polymers from sucrose (Skov *et al.*, 2000), can be considered as a glucansucrase belonging to glycoside hydrolase family GH13.

The extracellular polysaccharides produced by GTFs form a biofilm-like layer surrounding the bacteria, which is proposed to protect the bacteria against environmental stress such as desiccation, cold or attack by phagocytes and predatory microorganisms (Flemming & Wingender, 2010). Owing to the unique properties of the extracellular α -glucan polysaccharides and oligosaccharides, the products of glucansucrases have gained substantial interest in recent years. For example, they are used as viscosifying and water-binding

agents in food and nonfood applications (Badel *et al.*, 2011; Buchholz & Seibel, 2008; Naessens *et al.*, 2005). In addition, it is believed that α -glucans have health-promoting properties by stimulating the growth of beneficial endogenous microbiota in the gastrointestinal tract (German *et al.*, 1999; Welman & Maddox, 2003). On the other hand, the extracellular polysaccharide synthesized by *Streptococcus* strains in the oral cavity may promote dental plaque formation and cariogenesis (Badel *et al.*, 2011; Colby *et al.*, 1999). The development of anticaries agents may therefore be aimed at the specific inhibition of glucansucrases without inhibiting other carbohydrate-degrading enzymes in the oral cavity (Zhang *et al.*, 2011).

Although many α -glucan-producing bacterial strains have been identified, the number of confirmed and characterized glucansucrases has lagged behind. In particular, the molecular determinants of their different product specificities remain largely unknown. The key to understanding the differences in linkage type, the size of the polymeric product and the amount of branching must lie in their three-dimensional structures. For instance, the orientation of the non-reducing-end residue of the accepting oligosaccharide or growing α -glucan chain determines the type of glycosidic linkage that is made.

The first three-dimensional structure of a glucansucrase, GTF180- Δ N from *Lactobacillus reuteri* 180 (PDB entry 3klk; Vujčić-Žagar *et al.*, 2010), which synthesizes an α -glucan predominantly containing α -1,6- and α -1,3-linkages, revealed the presence of three domains (A, B and C) resembling those of GH13 α -amylases and two additional domains (IV and V). Four of the five domains appeared to be built up from discontinuous N- and C-terminal segments of the peptide chain; only domain C is formed from a single stretch of polypeptide. In the catalytic domain (A) of GTF180- Δ N, three acidic amino-acid residues (the nucleophile Asp1025, the general acid/base Glu1063 and the transition-state stabilizer Asp1136) constitute the catalytic machinery; they are present in the GH70 conserved sequence motifs II, III and IV, respectively (Fig. 1; Svensson, 1994; MacGregor *et al.*, 1996). Complexes of GTF180- Δ N with the substrate sucrose (bound in subsites -1 and +1) and the acceptor maltose (bound in subsites +1 and +2) confirmed the proposed double-displacement mechanism and revealed the acceptor-binding subsites. Two further glucansucrase structures have since been reported, showing a common domain organization and catalytic machinery: GTF-SI from *Streptococcus mutans* (Ito *et al.*, 2011), synthesizing a product with mainly α -1,3-linkages, and DSR-E Δ N123-GBD-CD2 from *Leuconostoc mesenteroides* NRRL B-1299 (Brison *et al.*, 2012), harbouring a catalytic site that only synthesizes the formation of α -1,2-branches on dextran acceptor molecules.

These structural investigations have given clues to some of the determinants that are responsible for the formation of α -1,2-, α -1,3- and α -1,6-glycosidic bonds, but not yet of α -1,4-glycosidic bonds. To further investigate the structure–function relationship of glucansucrases, we have determined the crystal structure of GTF- Δ N from *L. reuteri* 121. GTF- Δ N synthesizes a 50 MDa reuteran-type branched polymer with both α -1,4-linkages (46%) and α -1,6-linkages (34%); branching (α -1,4/6) and terminal sugar units constitute 12% and 9% of the polymer, respectively (Kralj *et al.*, 2004). Mutation studies with GTF- Δ N (Kralj *et al.*, 2005, 2006, 2008) revealed that residues from motif IV near the acceptor subsite +2 (GTF- Δ N residues 1128–1136; Fig. 1) are crucial for linkage-type specificity and the transglycosylation/hydrolysis ratio. The crystal structure of GTF- Δ N shows that these residues, and in particular residue Asn1134, are located near acceptor-binding subsite +2 and allows a rationalization of the effects of the mutations on product specificity.

2. Materials and methods

2.1. Expression, purification and characterization

Recombinant *L. reuteri* 121 GTF- Δ N (Met-Gly-741–1781-His₆) lacking the signal peptide and the N-terminal domain was over-expressed in *Escherichia coli* BL21 Star (DE3) and purified as described by Kralj *et al.* (2004). Protein concentrations were determined spectrophotometrically using a calculated specific absorbance of 1.186 ml mg⁻¹ cm⁻¹ at 279 nm. Dynamic light-scattering (DLS) experiments were performed at 277 K using 6 mg ml⁻¹ GTF- Δ N in 20 mM Tris-HCl pH 8.0, 0.2 M NaCl and a DynaPro MSTC-800 instrument; the results were analyzed with the DYNAMICS software package (Wyatt Instruments, Santa Barbara, USA). Analytical gel filtration was carried out at 283 K on a Superdex 200 PC3.2/30 column connected to a SmartSystem (GE Healthcare), with 25 mM acetic acid/sodium acetate pH 5.0, 0.1 M NaCl, 1 mM CaCl₂ as the running buffer at a flow rate of 50 μ l min⁻¹; gel-filtration markers (Bio-Rad) were used to determine the apparent molecular mass of the enzyme.

2.2. Crystallization

GTF- Δ N was crystallized using a manual hanging-drop vapour-diffusion setup at 293 K. A successful condition from the Wizard II screen (Emerald BioSystems, USA) was optimized to yield crystals of typically 0.15–0.25 mm in size. Drops were prepared by mixing 1–2 μ l protein solution (5–12 mg ml⁻¹ in 20 mM Tris-HCl pH 8.0, 0.2 M NaCl) with an equal volume of reservoir solution (1.0 M ammonium

Figure 1 Sequence alignment of conserved sequence motifs I–IV of GH70 glucansucrases, adapted from Leemhuis *et al.* (2012) and prepared with *ESPrpt* (Gouet *et al.*, 1999). NU, nucleophile; A/B, general acid/base; TS, transition-state stabilizer. Residues interacting with subsites -1, +1 and +2 are indicated with black boxes. The three residues from motif IV following the transition-state stabilizer are shown in yellow; the residue immediately following the transition-state stabilizer (Asn1134 in GTF- Δ N) is indicated by a red triangle. LrGTF, *Lactobacillus reuteri* 121 GTF; LrGTF180, *L. reuteri* 180 GTF180; LrGTFO, *L. reuteri* ATC 55730 GTF; LmDSRS, *Leuconostoc mesenteroides* NRRL B-512F dextranucrase; LmDSRBCB4, *L. mesenteroides* B-1299 DSRB CB4 dextranucrase; LmASR, *L. mesenteroides* B-1355 alternansucrase; SmGTF-SI, *Streptococcus mutans* glucosyltransferase GTF-SI; SoGTFR, *S. oralis* ATC10557 glucosyltransferase R; LmDSREG2, *L. mesenteroides* NRRL B-1299 DSR-E (glucan-binding domain and catalytic domain CD2).

sulfate, 0.1 M citric acid/Na₂HPO₄ buffer pH 4.2). The crystals were cryoprotected using 1.2 M ammonium sulfate, 30% (v/v) glycerol, 50 mM NaCl, 50 mM citric acid/Na₂HPO₄ buffer pH 4.2.

2.3. Data collection and processing

Despite extensive efforts to optimize the crystallization conditions, all tested GTFA-ΔN crystals diffracted poorly and anisotropically, even at room temperature and in the absence of cryoprotectant; only one useful data set was collected at 100 K on beamline ID14-2 of the ESRF (Grenoble, France). Diffraction data were indexed, integrated and scaled with *XDS* (Kabsch, 2010). Data were analyzed with *SCALA* using anisotropy correction (Winn *et al.*, 2011) and with *phenix.xtriage* (Adams *et al.*, 2010).

2.4. Structure determination and refinement

The crystal structure of GTFA-ΔN was solved by molecular replacement with *Phaser* (McCoy *et al.*, 2007). Search models were generated with the *FFAS03* (Jaroszewski *et al.*, 2005) and *SCWRL* (Canutescu *et al.*, 2003) servers based on the structure of the N-terminally truncated glucanase GTF180-ΔN from *L. reuteri*

Figure 2
(a) Dynamic light-scattering analysis of GTFA-ΔN; a regularization analysis histogram of the hydrodynamic radius (R_h) is shown. (b) Analytical gel-filtration profile of GTFA-ΔN on Superdex 200 PC3.2/30. The elution positions of the molecular-mass markers are indicated with their molecular masses (in kDa).

Table 1

Data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data statistics	ID14-2, ESRF
Beamline	0.933
Wavelength (Å)	ADSC Q4
Detector	340
Crystal-to-detector distance (mm)	70
No. of images	1.0
Oscillation range (°)	10
Exposure time (s)	<i>P</i> 6 ₂ 22
Space group	$a = b = 183.9$, $c = 374.0$, $\alpha = \beta = 90$, $\gamma = 120$
Unit-cell parameters (Å, °)	0.18
Mosaicity (°)	49.07–3.60 (3.79–3.60)
Resolution range (Å)	44105
Total reflections	6287
Unique reflections	8.5 (8.6)
Multiplicity	22.0 (95.3)
$R_{\text{merge}}^{\dagger}$ (%)	8.0 (34.3)
$R_{\text{p.i.m.}}^{\ddagger}$ (%)	6.7 (2.1)
$\langle I/\sigma(I) \rangle$	100.0 (100.0)
Completeness (%)	73.3
Wilson <i>B</i> (Å ²)	
Final model	
$R_{\text{cryst}}/R_{\text{free}}^{\S}$ (%)	24.0/26.0
R.m.s. deviation from ideality	
Bond lengths (Å)	0.005
Bond angles (°)	0.76
Contents of the asymmetric unit	
Protein atoms	8087
Ca ²⁺ ion	1
Average <i>B</i> factor, protein atoms (Å ²)	123
<i>B</i> factor, Ca ²⁺ ion (Å ²)	105
Ramachandran plot	
Favoured regions (%)	93.2
Additionally allowed regions (%)	6.4
Outliers [¶] (%)	0.4
MolProbity	
Clashscore/percentile	8.6/97th
MolProbity score/percentile	1.91/100th

[†] $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$. [‡] $R_{\text{p.i.m.}} = \frac{\sum_{hkl} [1/\{N(hkl) - 1\}]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$. [§] $R_{\text{cryst}} = \frac{\sum_{hkl} |F_{\text{obs}} - |F_{\text{calc}}||}{\sum_{hkl} |F_{\text{obs}}|}$, where F_{obs} is the observed structure factor and F_{calc} is the calculated structure factor. R_{free} is the same as R_{cryst} except calculated using 5% of the data that were not included in any refinement calculations. [¶] Of the four Ramachandran plot outliers, residue Glu1646 is in a loop with less well defined electron density between domains B and IV. The other three, Thr1154, Ile1251 and Ser1582, are in loops near or at the enzyme surface.

180 (PDB entry 3klk; Pijning *et al.*, 2008; Vujičić-Žagar *et al.*, 2010). Refinement with *REFMAC* (Murshudov *et al.*, 2011) included TLS refinement, for which the parameters were generated with the *TLSDM* server (Painter & Merritt, 2006), and isotropic *B*-factor refinement. 5% of the reflections were set aside for calculation of the free *R* factor. Cycles of refinement (including *B*-factor map sharpening) were alternated with map inspection and manual rebuilding in *Coot* (Emsley & Cowtan, 2004). Secondary structure was assigned using *DSSP* (Kabsch & Sander, 1983); the quality of the refined model was analyzed with *MolProbity* (Chen *et al.*, 2010). Figures of the atomic structures were prepared with *PyMOL* (Schrödinger LLC). Atomic coordinates and structure factors have been deposited in the Protein Data Bank with accession code 4amc.

3. Results and discussion

Analysis of GTFA-ΔN (theoretical molecular mass 117.9 kDa) by dynamic light scattering (DLS) revealed particles with a hydrodynamic radius (R_h) of 4.6 nm and an estimated molecular mass of 121 kDa in solution, a low polydispersity (12.5%) and less than 0.1% (w/w) detected aggregates (Fig. 2a). In gel-filtration experiments, GTFA-ΔN eluted at an apparent molecular mass of 94 kDa

(Fig. 2*b*); the delayed elution giving rise to a lower apparent molecular mass may arise from binding of the enzyme to the dextran-based column matrix. Together, these results indicate that GTFA- Δ N is monomeric in solution.

The crystal structure of N-terminally truncated and C-terminally His-tagged reuteransucrase GTFA from *L. reuteri* 121 (residues 741–1781), hereafter referred to as GTFA- Δ N, was solved by molecular replacement (for details, see Table 1). Diffraction data to 3.60 Å resolution were used, guided by an $\langle I/\sigma(I) \rangle$ of 2.1; although the R_{merge} in the highest resolution shell is rather high (95.3%), the $R_{\text{p.i.m.}}$ value (34.3%) indicated that it was valid to use the data to 3.60 Å resolution (Weiss, 2001). *Phaser* found only one GTFA- Δ N molecule in the asymmetric unit of the $P6_222$ unit cell; although there is space to fit several more molecules in the asymmetric unit, the values of the R_{cryst} and R_{free} factors after refinement (see Table 1) indicated that only one molecule was present. In the resulting crystal lattice (Fig. 3*b*), two main types of intermolecular contacts are observed: ‘head-to-tail’ interactions between domains C and V, and ‘side-by-side’ interactions between A domains. The presence of large solvent channels, resulting in a remarkably high solvent content of 85% and a Matthews coefficient (V_M) of 8.4 Å³ Da^{−1} (Matthews, 1968), may explain the poor diffraction properties of the GTFA- Δ N crystals.

The final GTFA- Δ N model (Fig. 4*a*) comprises residues 745–1763 (Table 1); the four N-terminal and 25 C-terminal residues (including the His tag) were not visible in the electron density and were omitted from the model. GTFA- Δ N is structurally most similar to GTF180- Δ N, with which it also shares the highest sequence identity (70%) among available three-dimensional structures. The three α -amylase-like domains (A, B and C) and the two additional domains (IV and V) (Fig. 4*b*) all have a very similar topology to the corresponding GTF180- Δ N domains. As can be expected at 3.60 Å resolution, not all amino-acid side chains were well defined in the electron-density map; Fig. 5 shows a representative part of the structure with electron density. Based on biochemical data (Kralj *et al.*, 2004) and sequence and structural homology with GTF180- Δ N (Vujičić-Žagar *et al.*, 2010), a calcium (Ca²⁺) binding site was identified at the same position as in GTF180- Δ N.

Domains A, B, C and IV of GTFA- Δ N (residues 794–1632) can be superimposed on the corresponding domains of GTF180- Δ N (Fig. 4*a*) with an r.m.s.d. of 1.04 Å (for 838 C α atoms). Domain V, composed of an N-terminal and a C-terminal segment, has a somewhat different position in GTFA- Δ N with respect to the other four domains, resulting in a shift of about 20 Å at the ‘top’ of domain V. This may be explained by an inter-domain hinge between domains IV and V located near residues 793 and 1646 (Fig. 4*a*). Domain V is structurally similar to the same domain in GTF180- Δ N and harbours the same type of sequence repeats, which have been proposed to be involved in glucan binding (Monchois *et al.*, 1999; van Hijum *et al.*, 2006; Vujičić-Žagar *et al.*, 2010).

The active site of GTFA- Δ N (subsite −1) is most similar to that of GTF180- Δ N, with the catalytic residues (Asp1024, Glu1061 and Asp1133) as well as most of the other residues surrounding subsite −1 being conserved (Fig. 6*a*). This conservation also extends to other glucansucrases (Fig. 1). Thus, one may expect the binding mode of the substrate sucrose at subsites −1 and +1 to be similar in these enzymes.

In contrast, differences are observed in subsites +1, +2 and above, which contain the amino-acid residues that are responsible for the binding mode of acceptor sugars. In the complexes of GTF180- Δ N and GTF-SI with maltose [PDB entries 3kl1 (Vujičić-Žagar *et al.*, 2010) and 3aib (Ito *et al.*, 2011)], the glucosyl unit in subsite +2 makes a hydrophobic stacking interaction with a tryptophan two residues downstream of the general acid/base (Fig. 1) which is conserved in

most glucansucrases (Monchois *et al.*, 1999; Leemhuis *et al.*, 2012), while the subsite +1 glucosyl unit is oriented with its ⁶OH oriented towards the −1 subsite for α -1,6-linkage formation. A superposition of GTFA- Δ N with GTF180- Δ N (Fig. 6*b*) shows that a similar binding mode is feasible in GTFA- Δ N, with Trp1063 being likely to provide the same hydrophobic stacking platform for acceptor sugars. However, while the conserved tryptophan residue provides rather nonspecific interactions, the precise orientation of acceptor sugars must be determined by more specific interactions with surrounding residues. For example, in the GTF180- Δ N–maltose complex, a direct hydrogen bond is observed between ⁴OH (subsite +1) and Asn1029. Since the equivalent residue in GTFA- Δ N is also an asparagine (Asn1028), a similar binding mode for acceptor sugars seems possible for GTFA- Δ N, thus explaining its α -1,6-linkage specificity. As for the α -1,4-linkage specificity of GTFA- Δ N, other interacting residues, which do not necessarily need to be conserved, may contribute. Interestingly, residues 1134–1136 of GTFA- Δ N, immediately following the transition-state-stabilizing residue Asp1133, have been found to be critical for linkage-type specificity. For example, a triple mutation (N1134S/N1135E/S1136V) in motif IV converted GTFA- Δ N from a mainly reuteran-producing enzyme with 45% α -1,4-glycosidic linkages to a mainly dextran-synthesizing enzyme with 80% α -1,6-glycosidic linkages in the product (Kralj *et al.*, 2005).

Figure 3
(*a*) Typical crystal of GTFA- Δ N. (*b*) Crystal packing of GTFA- Δ N crystals viewed along the *c* axis of the $P6_222$ cell. One of the molecules is shown in cartoon representation (for domain colouring, see the legend to Fig. 4).

Residue Asn1134 was found to be the major determinant for these drastic changes in linkage type (Kralj *et al.*, 2006). The GTFA- Δ N structure shows that residues 1134–1136 and especially residue Asn1134 are located near subsite +2 and may interact with a bound acceptor sugar, in particular with the O1 atom of the sugar (Fig. 6*b*). In other glucansucrases, the same region was also found to be important for product specificity. For instance, mutation of the equivalent residues (S642N/E643N/V644S) in DSRBCB4 from *L. mesenteroides* B1299CB4 reduced α -1,6-linkage formation by 30%

(Kang *et al.*, 2011) and introduced a significant amount of α -1,4-linkages into the product. In DSRS from *L. mesenteroides* NRRL B-512F, the mutations S663Y, S663N/E664N/V665S and S663Y/E664D/V665A resulted in a drastically reduced activity and changed the acceptor product profiles (Moulis *et al.*, 2006). Mutations of the residue following the transition-state stabilizer in *S. oralis* GTFR (S628D and S628R), corresponding to residue 1134 of GTFA- Δ N, abolished polymer synthesis (Hellmuth *et al.*, 2008). For the S628D mutation in GTFR, the kinetic parameters remained similar to the

Figure 4
(a) Overall structure of GTFA- Δ N (coloured domains) superposed on the crystal structure of GTF180- Δ N (grey; PDB entry 3klk; Vujičić-Žagar *et al.*, 2010). Colour scheme: GTFA- Δ N domain V (residues 745–791 and 1643–1763), red; domain IV (residues 792–925 and 1610–1642), yellow; domain B (residues 926–988, 1517–1554 and 1596–1609), green; domain A (residues 989–1234, 1382–1516 and 1555–1595), blue; domain C (residues 1235–1381), magenta. The three catalytic residues in domain A are shown in stick representation and the calcium ion (Ca²⁺) bound between domains A and B is shown as a green sphere. The largest differences between GTFA- Δ N and GTF180- Δ N are observed for domain V; the hinge between domains IV and V is indicated with asterisks (residues 793 and 1646). (b) Schematic domain arrangement of GTFA- Δ N. The first 740 residues (signal peptide and N-terminal domain) are not present in the crystallized construct. Each domain consists of two (N-terminal and C-terminal) stretches of polypeptide (the N-terminal stretches are more darkly coloured), with the exception of domain C.

Figure 5
Stereo figure of σ_A -weighted electron-density maps; shown is part of the active site of GTFA- Δ N. Grey, $2mF_o - DF_c$ map contoured at 1.0σ ; green/red, $mF_o - DF_c$ map contoured at $\pm 4.0\sigma$. Positive densities in the $mF_o - DF_c$ map may represent glycerol molecules from the cryoprotectant; however, these were not included in the model because of the limited resolution of the diffraction data.

Figure 6

Comparison of the active site of GTFA-ΔN (blue, catalytic domain A; green, domain B) and GTF180-ΔN. Relevant residues from GTFA-ΔN are shown with blue or green C atoms and black labels and residues from GTF180-ΔN are shown with white C atoms and grey labels. (a) Superposition of GTFA-ΔN with the GTF180-ΔN-sucrose complex (subsites -1 and +1; PDB entry 3hz3; Vujičić-Žagar *et al.*, 2010). The three catalytic residues and the tyrosine residue below subsite -1 are shown in stick representation. The sucrose bound to GTF180-ΔN is shown with yellow C atoms. (b) Superposition of GTFA-ΔN with the GTF180-ΔN-maltose complex (subsites +1 and +2; PDB entry 3klj; Vujičić-Žagar *et al.*, 2010). Maltose from the GTF180-ΔN complex, with its reducing-end glucosyl unit (subsite +2) stacking with a conserved tryptophan residue, is shown with yellow C atoms. The O1 atom of the acceptor sugar residue, which possibly interacts with Asn1134 in GTFA-ΔN, is indicated with an asterisk (*).

wild-type levels but the transglycosylation product spectrum changed drastically: with glucose as the acceptor substrate, the isomaltose yield (47%) was increased 25-fold compared with that of the wild-type enzyme. Addition of fructose as the acceptor substrate significantly enhanced the yield of leucrose (64%) and palatinose (21%). Thus, taken together, the residue that follows the transition-state stabilizer (Asn1134 in GTFA-ΔN) is a key residue for the different orientations of acceptor sugars, leading to different glycosidic bond specificities in various glucansucrases. Notwithstanding the accumulated evidence on the importance of the residue that follows the transition-state stabilizer, understanding the molecular details of the interactions of the enzyme with substrate and product, and explaining the effect of the various mutations on the product specificity of the enzymes, requires the elucidation of crystal structures of glucansucrases with bound substrates and products. Such investigations are under way.

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