Role of asparagine 1134 in glucosidic bond and transglycosylation specificity of reuteransucrase from Lactobacillus reuteri 121

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Glucansucrases from lactic acid bacteria convert sucrose into various \(\alpha\)-glucans that differ greatly with respect to the glucosidic bonds present (e.g. dextran, mutan, alternan and reuteran). This study aimed to identify the structural features of the reuteransucrase from Lactobacillus reuteri 121 (GTFA) that determine its reaction specificity. We here report a detailed mutational analysis of a conserved region immediately next to the catalytic Asp1133 (putative transition-state stabilizing) residue in GTFA. The data show that Asn1134 is the main determinant of glucosidic bond product specificity in this reuteransucrase. Furthermore, mutations at this position greatly influenced the hydrolysis/transglycosylation ratio. Changes in this amino acid expand the range of glucan and gluco-oligosaccharide products synthesized from sucrose by mutant GTFA enzymes.

Keywords
glucansucrase; Lactobacillus reuteri; product specificity; reuteransucrase; site-directed mutagenesis

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residues 1134–1136, 1138 and 1142 (GTFA numbering), located C-terminal to the putative transition state stabilizer D1133 (region IV, Fig. 1), determining the solubility of the glucan products and their glucosidic linkage ratios [9–12].

To investigate which combination of amino acids in this region, particularly residues 1134–1136, had the greatest effect on glucosidic bond product specificity in GTFA, we constructed various single and double mutants and characterized their respective oligosaccharide and glucan products. The results show that the identity of the amino acid at position 1134 in GTFA is a major determinant of glucosidic bond product specificity and the hydrolysis/transglycosylation activity ratio. Changes in this amino acid in GTFA expands the range of glucan and gluco-oligosaccharide products that can conveniently be synthesized from sucrose.

Results

Effects of single and double mutations in conserved region IV of GTFA on glucan structure

To investigate which of the three residue(s), N1134, N1135 or S1136, had most influence on the type of glucosidic linkages synthesized by GTFA, we constructed and examined six single and double mutants in this region. We expressed the six different variants in *Escherichia coli* BL21 DE3 star and purified them to homogeneity by Ni/nitrilotriacetate affinity chromatography followed by anion-exchange chromatography (data not shown). Wild-type and mutant enzymes were produced at comparable levels. Glucan polymers produced by these mutants were subjected to methylation and $^1$H-NMR analysis. The single mutant, N1134S, and the double mutants, N1134S/S1136V and N1134S/S1135E, displayed the largest shifts in glucosidic linkages present in their polymers (Table 1). All three variants containing the N1134S mutation synthesized more $\alpha$-(1 → 6) glucosidic linkages in their polymers, as previously observed for the GTFA triple mutant N1134S/S1135E/S1136V [12]. The other single and double mutants not containing the mutation N1134S showed a glucosidic linkage distribution in their glucan polymers similar to that of the wild-type. This indicates that, within this amino-acid triplet in conserved region IV, N1134 is the main determinant of glucosidic bond product specificity of GTFA (Table 1).

Effect of single amino-acid mutations in N1134 on GTFA activity

To better understand the involvement of N1134 in modulating glucan and oligosaccharide synthesis of GTFA, seven additional single amino-acid mutations, covering each of the different amino-acid classes, were introduced at this position in GTFA. The different variants were produced and purified to homogeneity (data not shown). Wild-type and mutant enzymes were produced at comparable levels.

Activity assays conducted with purified GTFA (mutant) enzymes showed that single mutations affected the specific total activity ($V_T$) to different extents (Table 2). In the case of Asn1134Gln, Asn1134Gly and Asn1134His, there was 45–75% loss of specific total activity. Changing Asn1134 to Tyr and Glu resulted in a somewhat increased specific total activity compared with the wild-type. Surprisingly, mutagenesis of Asn to Ala, Asp or Ser resulted in a twofold increase in specific activity.
Effects of single N1134 mutations on GTFA glucan synthesis

To investigate the effects of the single GTFA N1134 mutations on glucosidic bond product specificity, we analyzed their reaction products after prolonged incubation with sucrose (end point determination) (Table 2). The single mutants N1134Q, N1134E and N1134Y showed a greatly changed hydrolysis/transglycosylase product ratio. Compared with the wild-type, a twofold increase in their hydrolytic activity (increased release of glucose) and up to a 20% decrease in their glucan-synthesizing activity was observed. N1134H showed the largest change, its hydrolytic activity increasing threefold and only 50% of its glucan-synthesizing activity remaining. The other single mutants showed a product spectrum similar to the wild-type. As observed previously for the triple mutant N1134S/N1135E/S1136V, the seven single N1134 mutants also did not consume all the sucrose during incubation with 100 mM sucrose for 80 h [12].

Effects of single N1134 mutations on GTFA oligosaccharide synthesis from 100 mM sucrose and 100 mM maltose

When sucrose (donor substrate) and maltose (acceptor substrate) were both present in the assay mixtures,
GTFA synthesized mainly panose and some maltotriose [12,13]. All GTFA-derived mutant enzymes tested, except N1134Q and N1134Y, were able to completely consume sucrose in the presence of maltose within 80 h. Wild-type GTFA and mutants converted 40–70% of the maltose initially present into gluco-oligosaccharides (Table 3). Interestingly, mutant enzymes N1134Q and N1134Y synthesized larger amounts of maltodextrins than wild-type GTFA. As previously observed, the mutant enzyme N1134S/N1135E/S1136V synthesized the tetrasaccharide α-d-glucopyranosyl-(1→6)-α-d-glucopyranosyl-(1→6)-α-d-glucopyranosyl-(1→4)-d-glucose [α-(1→6)-panose] from sucrose and maltose [12]. Wild-type GTFA and the mutant enzymes N1134Q, N1134E, N1134Y and N1134H did not produce α-(1→6)-panose at all. The N1134S and N1134A enzymes synthesized similar amounts of α-(1→6)-panose to the N1134S/N1135E/S1136V mutant. Interestingly, the two mutant enzymes N1134D and N1134G synthesized intermediate concentrations of α-(1→6)-panose (Table 3).

### Effects of single N1134 mutations on GTFA oligosaccharide synthesis from 100 mM sucrose and 100 mM isomaltose

When sucrose (donor substrate) and isomaltose (acceptor substrate) were both present in the assay mixtures, GTFA synthesized predominantly isopanose {α-D-glucopyranosyl-(1→4)-[α-D-glucopyranosyl-(1→6)]-d-glucose} and α-(1→6)-isopanose {α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→4)-[α-D-glucopyranosyl-(1→6)]-d-glucose} (Table 4) [5,13].

### Table 3. Product spectra of GTFA and derived site-directed mutants, after 80 h of incubation with 100 mM sucrose and 100 mM maltose.
The total and individual oligosaccharide yields indicate the amount of maltose consumed as a percentage of the total amount of maltose initially present in the incubation. The calibration curve of panose was used to calculate α-(1→6)-panose concentrations. ND, Not detectable.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Oligosaccharide yield (%)</th>
<th>Panose (%)</th>
<th>Maltotriose (%)</th>
<th>α-(1 → 6)-Panose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>54.9 ± 1.6</td>
<td>49.6 ± 1.7</td>
<td>5.3 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>N1134S</td>
<td>58.8 ± 2.1</td>
<td>35.2 ± 0.9</td>
<td>2.1 ± 0.1</td>
<td>21.4 ± 1.2</td>
</tr>
<tr>
<td>N1134D</td>
<td>67.6 ± 5.8</td>
<td>54.7 ± 4.0</td>
<td>2.1 ± 0.2</td>
<td>10.7 ± 1.6</td>
</tr>
<tr>
<td>N1134Q</td>
<td>35.8 ± 1.2</td>
<td>26.5 ± 0.9</td>
<td>9.3 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>N1134E</td>
<td>50.8 ± 9.3</td>
<td>46.7 ± 8.5</td>
<td>4.1 ± 0.8</td>
<td>ND</td>
</tr>
<tr>
<td>N1134G</td>
<td>62.1 ± 0.5</td>
<td>48.5 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>10.6 ± 0.6</td>
</tr>
<tr>
<td>N1134A</td>
<td>57.8 ± 1.6</td>
<td>37.4 ± 1.2</td>
<td>2.0 ± 0.1</td>
<td>18.4 ± 0.3</td>
</tr>
<tr>
<td>N1134Ya</td>
<td>13.3 ± 0.4</td>
<td>30.0 ± 0.4</td>
<td>11.3 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>N1134H</td>
<td>49.3 ± 0.1</td>
<td>40.8 ± 0.4</td>
<td>8.5 ± 0.5</td>
<td>ND</td>
</tr>
<tr>
<td>N1134S/N1135E/S1136V (SEV)</td>
<td>63.8 ± 3.8</td>
<td>40.7 ± 1.0</td>
<td>2.0 ± 0.2</td>
<td>21.0 ± 3.0</td>
</tr>
</tbody>
</table>

αSucrose consumed was 75–85% after 80 h of incubation.

### Table 4. Product spectra of GTFA and derived site-directed mutants, after 80 h of incubation with 100 mM sucrose and 100 mM isomaltose.
The total and individual oligosaccharide yields indicate the amount of isomaltose consumed as a percentage of the total amount of isomaltose initially present in the incubation. The calibration curve of panose was used to calculate isopanose and α-(1→6)-isopanose concentrations. ND, Not detectable.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Oligosaccharide yield (%)</th>
<th>Isopanose (%)</th>
<th>α-(1 → 6)-Isopanose (%)</th>
<th>Maltotetraose (%)</th>
<th>Isomalopentaose (%)</th>
<th>Isomaltohexaose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>35.5 ± 7.2</td>
<td>13.9 ± 1.5</td>
<td>14.3 ± 3.7</td>
<td>4.1 ± 1.2</td>
<td>1.2 ± 0.2</td>
<td>ND</td>
</tr>
<tr>
<td>N1134S</td>
<td>54.5 ± 4.2</td>
<td>5.3 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>28.3 ± 1.7</td>
<td>11.9 ± 0.8</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>N1134D</td>
<td>44.6 ± 0.6</td>
<td>14.8 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>20.8 ± 0.5</td>
<td>4.1 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>N1134Q</td>
<td>17.3 ± 3.7</td>
<td>10.8 ± 2.2</td>
<td>1.8 ± 0.4</td>
<td>3.1 ± 0.7</td>
<td>1.7 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>N1134E</td>
<td>23.1 ± 2.6</td>
<td>14.1 ± 1.5</td>
<td>1.5 ± 0.3</td>
<td>5.0 ± 0.5</td>
<td>2.4 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>N1134G</td>
<td>51.0 ± 0.5</td>
<td>22.0 ± 0.2</td>
<td>3.1 ± 0.1</td>
<td>20.8 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>ND</td>
</tr>
<tr>
<td>N1134A</td>
<td>51.6 ± 0.8</td>
<td>8.2 ± 0.5</td>
<td>4.1 ± 0.1</td>
<td>27.6 ± 0.5</td>
<td>9.1 ± 0.2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>N1134Ya</td>
<td>13.3 ± 5.3</td>
<td>6.8 ± 2.8</td>
<td>1.8 ± 0.5</td>
<td>3.1 ± 1.3</td>
<td>1.5 ± 0.8</td>
<td>ND</td>
</tr>
<tr>
<td>N1134H</td>
<td>20.3 ± 0.1</td>
<td>13.8 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>N1134S/N1135E/S1136V (SEV)</td>
<td>57.3 ± 2.8</td>
<td>7.7 ± 0.3</td>
<td>ND</td>
<td>26.5 ± 0.5</td>
<td>19.1 ± 1.4</td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>

αSucrose consumed was 50–90% after 80 h of incubation. bData from [12].
N1134Y, and N1134H were not able to completely consume sucrose in the presence of isomaltose within 80 h. Wild-type GTFA and mutants converted 15–60% of the isomaltose initially present into glucose-oligosaccharides (Table 4). The N1134S and N1134A mutant GTFA enzymes showed up to sevenfold increased synthesis of isomaltotriose. These two mutants also showed increased isomaltotetraose synthesis and decreased synthesis of \( \alpha-(1 \rightarrow 6) \)-isopanose. Both mutants also synthesized isomaltopentaose and isomaltohexaose. The N1134S, N1134D, N1134G, and N1134A mutants displayed clearly increased glucose-oligosaccharide yields (two-fold) from sucrose and isomaltose. A similar change was observed previously for N1134S/N1135E/S1136V [12]. Compared with the wild-type, mutants N1134Q, N1134E, N1134Y and N1134H showed 1.5–2-fold decreased glucose-oligosaccharide yields (Table 4).

**Effects of single N1134 mutations on size and structure of GTFA glucan products**

The sizes of the glucans produced by the single mutants N1134S, N1134E, N1134Q, and N1134Y were similar to that of the wild-type (Table 1). N1134G, N1134A, and N1134H synthesized relatively large polymers compared with the wild-type. Methylation and \( ^1 \)H-NMR analysis (Table 1) of the polymers produced by the N1134 mutant enzymes showed that N1134S produced a polymer with a greatly increased level of \( \alpha-(1 \rightarrow 6) \) linkages and a decreased amount of \( \alpha-(1 \rightarrow 4) \) linkages. N1134Q, N1134H, and N1134Y had a linkage distribution in their glucan polymers similar to that of wild-type GTFA. Clear shifts from \( \alpha-(1 \rightarrow 4) \) glucosidic linkage specificity towards \( \alpha-(1 \rightarrow 6) \) specificity were observed for N1134S, N1134A, N1134D, N1134G, and N1134E (magnitude of change in decreasing order).

**Discussion**

Mutagenesis of a tripeptide (N1134S/N1135E/S1136V), in conserved region IV, converted GTFA from a mainly reuteran \([ \approx 45\% \], \( \alpha-(1 \rightarrow 4) \)\) into a mainly dextran \([ \approx 80\% \], \( \alpha-(1 \rightarrow 6) \)\) synthesizing enzyme. A similar shift in glucosidic linkages was also observed for the oligosaccharides produced by GTFA from sucrose using maltose or isomaltose as acceptor substrate [12]. The aim of this study was to identify which of these GTFA amino-acid residue(s) was the strongest determinant of glucosidic bond product specificity.

For amylosucrase and cyclodextrin glucanotransferase (GH13 family), the different sugar-binding acceptor subites have been mapped out on the basis of 3D structural information, and labeled \(-2, -1, +1, +2, \) etc., according to the definition of Davies et al. [14]. Glucansucrases of the GH70 family and members of the GH13 family have similar structural features [7,15]. We postulate that members of both enzyme families have a similar acceptor subsite organization. In amylosucrase and acarviosyltransferase, both members of the GH13 family, the amino acid next to the transition-state stabilizer (D394 and M329, respectively; corresponding to GTFA residue N1134) [16] is part of acceptor-binding subsite +1, and involved in the correct positioning of the glucosyl residue at this site. Mutagenesis of this residue results in changes in the amylosucrase product spectrum [17] and, for acarviosyltransferase, in a changed reaction specificity [18].

Detailed investigations of the NNS triplet in region IV of GTFA showed that, in GTFA of the GH70 family, N1134, immediately next to the putative transition-state stabilizer D1133, was also the main determinant of GTFA glucosidic bond product specificity (this study; Table 1). The single mutant, N1134S, and the double mutants, N1134S/S1136V and N1134S/N1135E, displayed the largest shift in glucosidic linkages present in their polymers (Table 1). To better understand the involvement of N1134 in modulating glucan as well as oligosaccharide synthesis of GTFA, we introduced seven additional single amino-acid mutations, covering each of the different amino-acid classes, at this position in GTFA.

Where studied, the linkage specificity of glucansucrases is conserved in glucose-oligosaccharide synthesis, and oligosaccharides are elongated at their nonreducing end [13]. For the single mutants investigated, the change in glucosidic linkage ratio observed in glucan products was also reflected in their glucosyl-oligosaccharide products. Mutants N1134S and N1134A showed the largest increases in \( \alpha-(1 \rightarrow 6) \) glucosidic bond specificity in their glucan products compared with wild-type GTFA (Table 1). Both mutants also synthesized the largest amounts of \( \alpha-(1 \rightarrow 6) \)-panose from maltose, and isomaltohexaose from isomaltose (Tables 3 and 4). N1134D and N1134G synthesized intermediate levels of \( \alpha-(1 \rightarrow 6) \) glucosidic bonds in both their glucan and oligosaccharide products, intermediate amounts of \( \alpha-(1 \rightarrow 6) \)-panose from maltose, and isomaltohexaose from isomaltose (Tables 1, 3 and 4). However, the ratio of glucosidic linkages introduced by N1134Q, N1134Y and N1134H in their glucan and oligosaccharides did not coincide. Although the linkage introduced in the glucan was similar to the wild-type, these mutants synthesized a larger amount...
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of maltotriose from maltose and sucrose than the wild-type (introduction of more $\alpha$-(1 $\rightarrow$ 4) glucosidic linkages). Maltose and also isomaltose are relatively poor acceptor substrates for N1134Q and N1134Y compared with wild-type GTFA (Tables 3 and 4). This suggests that, in these mutant proteins, the affinity at acceptor subsites (+1, +2) for (oligo)saccharides had decreased. This may, at least partly, explain why for these two mutants the ratio of glucosidic linkages synthesized in their oligosaccharide products (especially with sucrose and maltose as acceptor substrate) does not agree with those in their glucan products (Tables 1, 3 and 4).

It remains difficult to explain how changes in the Asn (amide) residue at position 1134 into a Ser (hydroxyl) and Ala (aliphatic), Asp (acidic) and Gly (small aliphatic), Gln (amide), Tyr (aromatic) and His (basic) residues can have similar effects on glucosidic linkage distribution in GTFA products. Local structural disturbances may explain some of the variations, and also some general features can be observed. The Asn to Asp mutation clearly changed GTFA glucosidic bond specificity towards more $\alpha$-(1 $\rightarrow$ 6) linkages. As the two amino acids are of similar size, the different side chain of Asp must determine the change in glucosidic linkage product specificity. It remains unknown how Asp and Gly can have a similar effect on glucosidic bond specificity. The Asn to Gln, Glu and Tyr mutations resulted in double the hydrolytic activity, and the change into His in a three times increase in hydrolytic activity (end point conversion) (Table 2). The much higher hydrolytic than transglycosylation activity of these mutants indicates that the binding of sugar acceptor substrates is hindered in particular. Indeed these mutants also show a reduced oligosaccharide yield with isomaltose as acceptor substrate (Table 4). GTFO also has Asn at position 1134 and displays an even higher hydrolytic activity (>55%) than mutant N1134H (>50%) or wild-type GTFA (>15%) (end point conversions [5]). This indicates that other amino acids are also important in determining the hydrolysis/transglycosylation ratio. Elucidation of high-resolution 3D structures of wild-type and mutant GTFA proteins, ideally with bound substrates/products, may provide a more detailed explanation for the observed biochemical changes in the hydrolysis/transglycosylation ratio and in oligosaccharide and glucan product specificity.

The different amino-acid mutations at position 1134 strongly affected the rate of glucan synthesis and hydrolysis of sucrose. The oligosaccharide yields with maltose and isomaltose were also greatly affected. This indicates that position 1134 in region IV is directly involved in glucosyl transfer from sucrose to acceptor substrates, consistent with its location next to D1133, the residue suggested to act as transition-state stabilizer [7,13]. This study also shows that the amino acid immediately next to the transition-state stabilizer is at an important position for tailoring the structure of glucan and oligosaccharide products in glucansucrases of the GH70 family. Furthermore, modification of the corresponding residue in glucansucrases that synthesize glucan polymers with different types of glucosidic linkages may further expand the range of glucans and gluco-oligosaccharides. However, this is not the only region important for determining glucosidic linkage specificity. There are other examples of glucansucrases that possess the triplet N1134S/N1135E/S1136V (SEV) in region IV which synthesize either mutan or dextran polymers; the higher amount of $\alpha$-(1 $\rightarrow$ 4) glucosidic linkages synthesized by GTFO compared with GTFA also cannot be explained by differences in this region. Indeed other regions (close to the nucleophile and the N-terminus of the catalytic core) that influence the ratio of glucosidic linkage in oligosaccharide products [12,19] and the structure of the glucan product [20] have also been identified.

**Experimental procedures**

**Bacterial strains, plasmids, media and growth conditions**

*E. coli* TOP 10 (Invitrogen, Carlsbad, CA, USA) was used as host for cloning purposes. Plasmid pBPE1500, containing the 3' part of the catalytic core of the *gtfA* gene of *L. reuteri* 121 was used as template for mutagenesis, and plasmid pBGTF2, with the full-length *gtfA* gene including a C-terminal His tag, was used for cloning purposes [13]. Plasmid pET15b (Novagen, Madison, WI, USA) was used for expression of the different (mutant) *gtf* genes in *E. coli* BL21 Star (DE3) (Invitrogen). *E. coli* strains were grown aerobically at 37 °C in Luria–Bertani medium [21]. *E. coli* strains containing recombinant plasmids were cultivated in Luria–Bertani medium with 100 $\mu$g/mL ampicillin. Agar plates were made by adding 1.5% agar to the Luria–Bertani medium.

**Molecular techniques**

General procedures for restriction, ligation, cloning, PCR, *E. coli* transformations, DNA isolation and manipulations, isolation of DNA fragments from gel, and agarose gel electrophoresis were as described [13]. Primers were obtained
from Eurogentec (Seraing, Belgium). Sequencing was performed by GATC (Konstanz, Germany).

Construction of plasmids for site-directed mutagenesis experiments

The QuickChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and appropriate primer pairs were used to introduce mutations in pBPE1500. After successful mutagenesis (confirmed by DNA nucleotide sequencing), the pBPE1500 derivative was digested with PstI and EcoRV and exchanged for the corresponding fragment of pBGTF2 [13]. The resulting 5.3-kb fragment was introduced, using Ncol/BamHI restriction sites, in the expression vector pET15b (Novagen) [13].

Enzyme activity assays and enzyme purification

GTFA (mutant) proteins were produced and purified as described previously [13]. All reactions were performed at 50 °C in 25 mM sodium acetate buffer, pH 4.7, containing 1 mM CaCl₂ and 30 mM purified (mutant) GTFA. The total glucansucrase activity (V₅) was determined as initial rate by measuring fructose release (enzymatically) from sucrose conversion (six data points over a period of 6 min, using 100 mM sucrose). One unit of enzyme activity is defined as the release of 1 μmol monosaccharide·min⁻¹ [13,22].

Characterization of the glucans produced

Polymers were produced by incubation of (mutant) enzyme preparations with 146 mM sucrose for 7 days, using the conditions described above in enzyme activity assays, and addition of 1% Tween 80 and 0.02% sodium azide. Glucans produced were isolated by precipitation with ethanol as described previously [22].

Methylation analysis was performed as described by permethylation of the polysaccharides using methyl iodide and dimethyl sulfoxide at room temperature [23].

One-dimensional ¹H-NMR spectra were recorded on a 500-MHz Varian Inova NMR spectrometer (Palo Alto, CA, USA) at a probe temperature of 303 K. Before NMR spectroscopy, samples were dissolved in 99.9 atom percent-D₂O (Aldrich, St Louis, MO, USA). Chemical shifts are expressed in p.p.m. by reference to external acetone (δ = 2.225). Proton spectra were recorded in 8000 datasets, with a spectral width of 8000 Hz. Before Fourier transformation, the time-domain data were apodized with an exponential function, corresponding to 0.8-Hz line broadening.

Molecular mass analysis was performed as described previously, using high-performance size-exclusion chromatography coupled on-line with multi angle laser light scattering and differential refractive index detection [4].

Analysis of products synthesized from sucrose

After complete depletion of sucrose (100 mM, 80 h at 50 °C) by 30 nM GTFA (mutant) enzymes, the concentrations of fructose, glucose, isomaltose and leucrose in the reaction medium were determined using anion-exchange chromatography (Dionex, Sunnyvale, CA, USA) [13].

Oligosaccharides synthesized from sucrose and (iso)maltose as acceptor substrates

After complete depletion of sucrose (100 mM, 80 h at 50 °C) by 30 nM GTFA (mutant) enzymes, incubated with the acceptor substrates maltose or isomaltose (100 mM each), the oligosaccharides synthesized were analyzed by anion-exchange chromatography (Dionex) as described previously [12].

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References


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