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Site-specific mutagenesis at positions 272 and 273 of the *Bacillus* sp. SAM1606 α-glucosidase to screen mutants with altered specificity for oligosaccharide production by transglucosylation

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Abstract

The *Bacillus* sp. SAM1606 α-glucosidase catalyzes the transglucosylation of sucrose to produce theanderose (6-O^G-glucosylsucrose) as the major transfer product along with the other di-, tri-, and tetrasaccharides. To obtain an α-glucosidase variant(s) producing theanderose more abundantly, we carried out site-specific mutagenesis studies, in which an amino acid residue (Gly273 or Thr272) near the putative catalytic site (Glu271) of this α-glucosidase was replaced by all other naturally-occurring amino acids. Each mutant, whose concentration was set at 2.6 U/ml (sucrose-hydrolyzing units), was reacted at 60 °C and pH 6.0 with 1.75 M sucrose, and the course of the oligosaccharide production was monitored by HPLC to systematically analyze the effects of amino acid substitutions on the specificity of transglucosylation. The analysis clearly showed site- and residue-dependent differential effects of substitution near the catalytic site on the specificity of oligosaccharide production. For example, mutants with substitution at position 273 by aromatic amino acids or His virtually lost the ability to produce oligosaccharides by transglucosylation. Mutants with substitution at position 272 by amino acids that were bulkier than the wild-type Thr showed enhanced production of tetrasaccharides; whereas, mutants with substitution at position 273 by Lys and Arg exclusively produced disaccharidal transfer products. The highest specificity for theanderose formation (i.e. the highest content of theanderose in the reaction product) was obtained with the T272I mutant, which showed 1.74 times higher productivity (per sucrose-hydrolyzing unit) of theanderose than that of the wild-type enzyme.

Keywords: Transglucosylation; Mutagenesis; Oligosaccharide; α-glucosidase; Theanderose

1. Introduction

α-Glucosidase (EC 3.2.1.20) catalyzes the hydrolysis and transglucosylation of 1-*O*-α-D-glucopyranoside with a net retention of anomeric configuration [1] and is one of the enzymes which have frequently been used for the enzymatic syntheses of glycosides and

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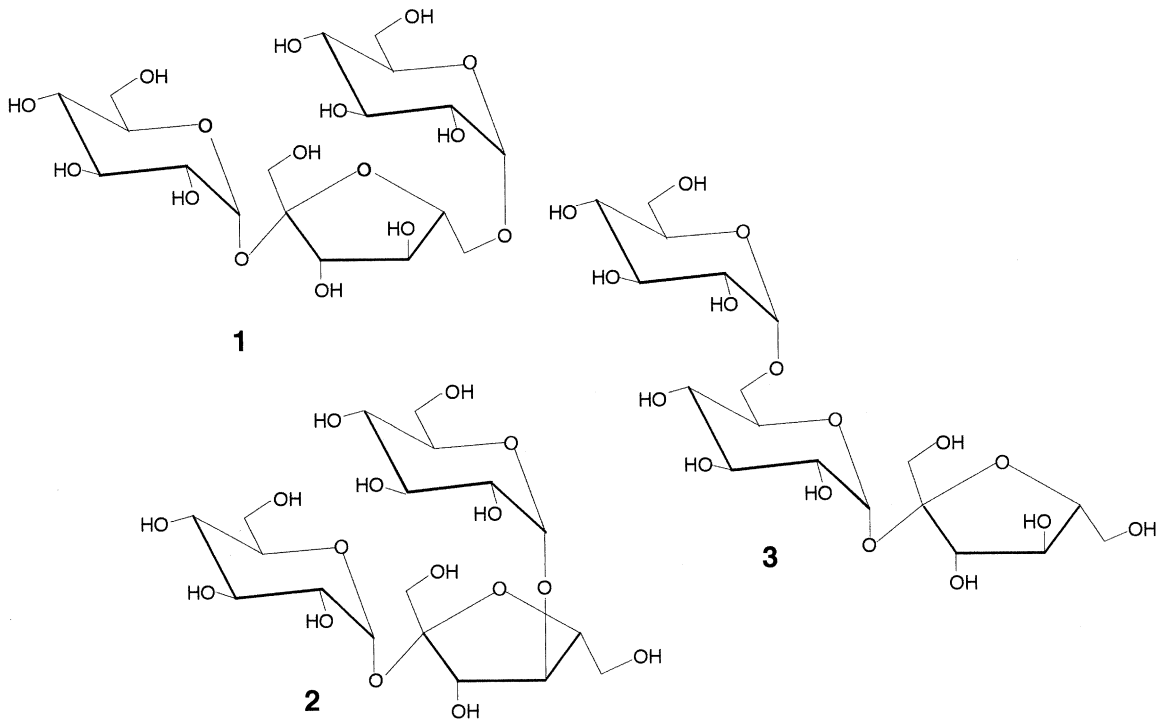

Fig. 1. Structures of trisaccharides produced during transglucosylation of sucrose catalyzed by *Bacillus* sp. SAM1606 α-glucosidase. Sugars **1**, **2**, and **3** are isomelezitose, 4-O^F-glucosylsucrose, and theanderose, respectively.

oligosaccharides containing α-glucosidic linkage(s). The α-glucosidase of *Bacillus* sp. strain SAM1606 [2] is a member of family 13 of the glycosyl hydrolases [3,4] (also called "the α-amylase family" [5,6]) and shows a very broad substrate specificity of the enzymatic glucoside hydrolysis [2,7]. Also, the enzyme is one of the most thermostable α-glucosidases whose transglucosylation activities have been reported to date, and thus it has been used for the enzymatic synthesis of oligosaccharides of nutritional interest [2]. For example, reaction of the enzyme with sucrose produces 6-O^G-glucosylsucrose (theanderose; Fig. 1, compound **3**) as the major transfer product along with the other di- and trisaccharidal transfer products such as isomaltose, 6-O^F-glucosylsucrose (isomelezitose, compound **1**), and 4-O^F-glucosylsucrose (compound **2**). Theanderose is a selective growth-promoting factor for the genus *Bifidobacterium*, a benign bacterium that improves human fecal conditions [2,8] and also shows anti-dental caries activities by inhibiting the glucan synthesis by *Streptococcus mutans*, a primary causative agent of dental caries [2,9,10]. Therefore, the transfer products containing theanderose can be used as food material with health-giving functions. Importantly, the broad substrate specificity and high transglucosylation activity of this enzyme uniquely allow a simple and convenient preparation of this trisaccharide from one disaccharide, sucrose, used as a sole glucosyl donor and acceptor [2]. For comparison, theanderose had previously been produced by the action of fungal α-glucosidases using starch as a glucosyl donor and sucrose as a glucosyl acceptor [8].

We previously showed, by comparative site-directed mutagenesis studies, that replacement of Gly273 with Pro caused significant changes in the specificity of transglucosylation catalyzed by the enzyme [11,12]; the mutant having such substitution exhibits a significantly enhanced initial velocity of transglucosylation, yielding isomelezitose, instead of theanderose, as the major transfer product. Molecular modeling studies of the α-glucosidase on the basis of the published crystal structure of *B. cereus* oligo-1,6-glucosidase

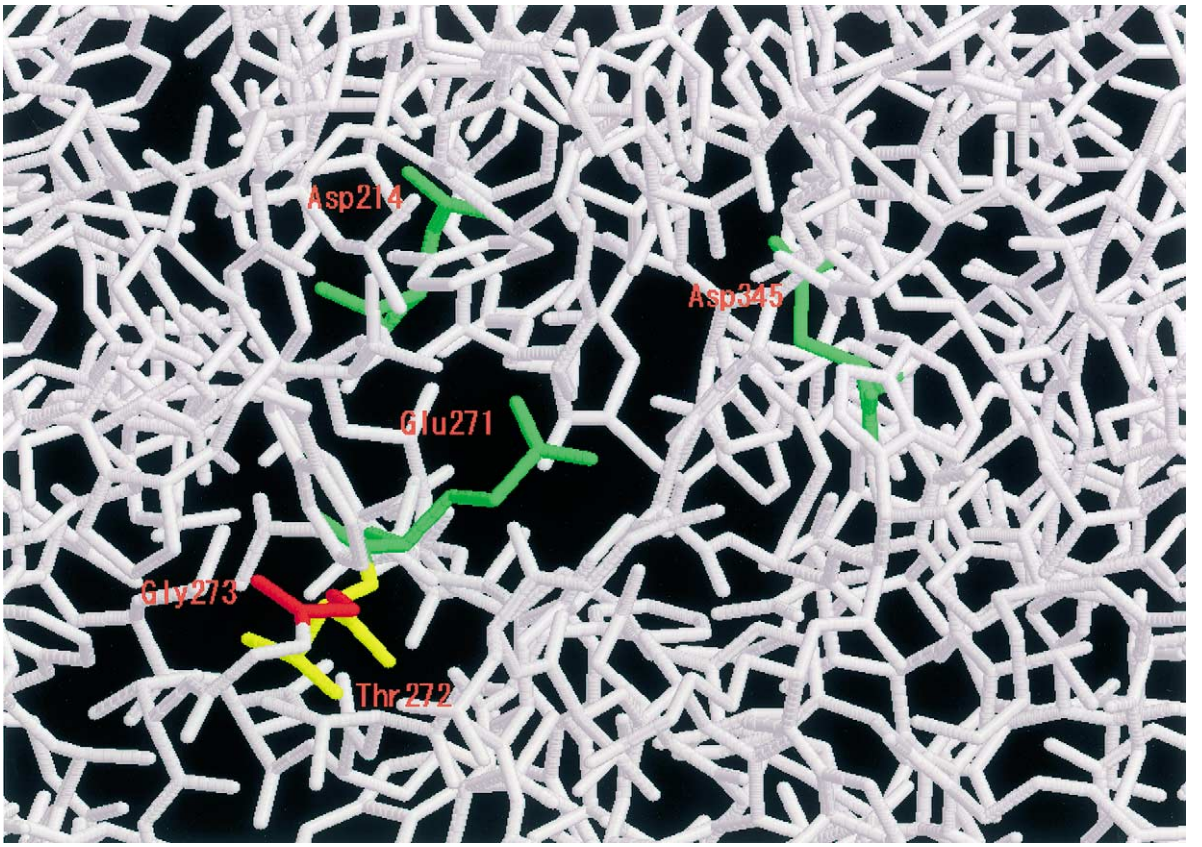

Fig. 2. A close-up view of the active site of SAM1606 α-glucosidase. Three-dimensional structures of the wild-type α-glucosidase were built based on the structure of oligo-1,6-glucosidase of *B. cereus* [13] which shows 67% sequence identity to the SAM1606 enzyme, using INSIGHT II HOMOLOGY software (Biosym Technologies, San Diego, CA). The model was subjected to limited energy refinement with the INSIGHT II DISCOVER software (Biosym Technologies, San Diego, CA). Putative catalytic residues (Asp214, Glu271, Asp345) are shown in green and Thr272 and Gly273 are shown in yellow and red, respectively.

(sequence identity, 67%) [13] showed that Gly273 is located at the active site, in close proximity to Asp214, Glu271, and Asp345, which are putative catalytic residues (Fig. 2).

The primary objective of this study is to obtain a variant(s) of the SAM1606 enzyme with an enhanced productivity of theanderose by engineering the active-site environment. Because structural requirements of the active site of glycosidases for enhancing the specificity of transglucosylation are not yet known, controlling these factors through rational protein engineering approaches is still difficult. An alternative strategy is a site-specific "randomization" approach, in which an amino acid residue near the catalytic site of this α-glucosidase is replaced by each of all other amino acids to screen a mutant enzyme of interest. Considering the suggested importance of an amino acid residue at position 273 of this α-glucosidase for the specificity of the transglucosylation as already described [11], the position 273 would be a good target for site-specific randomization. Also, position 272 may be an alternative target for randomization, because this site lies between one of the putative catalytic residues (Glu271) and Gly273 (see Fig. 2) so that amino acid substitution at this site potentially affects the specificity of transglucosylation.

In this study, we have created 19 mutants at each of positions 273 and 272 of this α-glucosidase, and individual mutants were analyzed for the specificity of the oligosaccharide production from sucrose. The

systematic analyses provide unequivocal examples of the site- and residue-dependent differential effects of substitution near the catalytic site on the specificity of transglucosylation and allowed us to obtain a mutant with enhanced production of theanderose.

2. Materials and methods

2.1. Materials

Sucrose was purchased from Nacalai Tesque, Kyoto, Japan. All other chemicals were of analytical grade. Daisopak SP-120-5-ODS-B was obtained from Daiso Co., Osaka, Japan.

2.2. Bacterial strain, plasmids, mutagenesis, and gene expression

In vitro mutagenesis [14] was carried out for creating each mutant with an amino acid substitution at position 273 or 272 using the Mutan K kit (Takara Shuzo, Kyoto, Japan) and the *Hin*cII/*Eco*RI fragment of the α-glucosidase gene (i.e. the fragment III of the plasmid pGBSU5 [12]) as a template according to manufacturer's guidance. Nineteen mutants at each of the positions 273 and 272 were individually prepared using specific mutagenesis oligonucleotides (not shown). Individual mutations were verified by DNA sequencing of the fragment III of the α-glucosidase gene. Replacement of the fragment III in the pGBSU5 with the mutated fragment III allowed us to obtain α-glucosidase genes having mutation at each of the positions 273 and 272. If Gly273 was replaced by alanine, for example, the resultant mutant was designated as the G273A mutant using the one-letter notation of amino acids. *Escherichia coli* strain W3110 was used as a host for expression of the wild-type and all of the mutant α-glucosidases under the control of the *icp* promoter of the insecticidal protein gene from *B. thuringiensis* subsp. *sotto* [7,11,12]. *E. coli* W3110 transformant cells were grown to the stationary phase at 37 °C in 3 l of L-broth containing 50 μg/ml ampicillin and the cells were collected by centrifugation. The wild-type and mutant enzymes were partially purified from crude extracts of the transformant cells by polyethyleneimine treatment, ammonium sulfate fractionation, and heat treatment as described previously [2] and were used for transglucosylation. The T272I mutant was purified according to the method described previously [12] to apparent homogeneity judging from sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [15].

2.3. Enzyme assay

The enzymatic hydrolysis of sucrose was assayed at 55 °C as described previously [12]. Briefly, the reaction mixture contained 5.0 μmol of sucrose, 1.0 μmol of sodium phosphate buffer (pH 6.0), and the enzyme in a final volume of 100 μl. The mixture without the enzyme was brought to 55 °C. The reaction was started by the addition of enzyme. After incubation at 55 °C for 10 min, the reaction was stopped by heating at 100 °C for 5 min. Glucose that was produced in the reaction mixture was determined by the method of Pütter and Becker [16]. The blank did not contain the enzyme. One unit of the enzyme is defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmol of sucrose per minute at 55 °C.

2.4. Transglucosylation

The reaction of 1.75 M sucrose (Fig. 3) with the SAM1606 α-glucosidase was monitored by high performance liquid chromatography (HPLC) [11]. The standard assay mixture contained 1.75 M (60% (w/v)) sucrose, 200 μmol of sodium phosphate buffer (pH 6.0), and the enzyme in a final volume of 3 ml. The mixture without the enzyme was brought to 60 °C. The reaction was started by the addition of the enzyme (7.8 U). At appropriate time intervals, an aliquot of the reaction mixture was withdrawn and heated at 100 °C for 5 min to inactivate the enzyme followed by freezing for storage at −40 °C until analysis. After appropriate dilution of the mixture with H_2O, the reaction mixture was analyzed using a Hitachi 655A-11 HPLC system equipped with a Daisopak SP-120-5-ODS-B column (10 mm × 250 mm; column temperature, 37 °C; mobile phase, H_2O, isocratic; flow rate, 1.8 ml/min). The sugars were monitored by analyzing the changes in the refractive index of the eluates using a model JASCO RI-930 refractometer. Retention times of sugars under these HPLC conditions were as follows: glucose, 8.66 min; fructose, 9.00 min; disaccharides, 9.73, 10.17, and 10.47 min; isomelezitose,

11.11 min; sucrose, 12.03 min; 4-O^F-glucosylsucrose, 13.69 min; theanderose, 16.75 min; and tetrasaccharides, 13.19, 15.73, and 26.42 min.

3. Results and discussion

3.1. Preparation of site-directed mutants at position 273 or 272 and analysis of oligosaccharide production by mutants

One of the key objectives of this study is to analyze the effects of amino acid replacement near the catalytic site on the specificity for oligosaccharide production from sucrose and to obtain a variant(s) of the SAM1606 enzyme with enhanced production of theanderose whose physiological activities are well established. To achieve this, single amino acid substitution was introduced at position 273 or 272 of SAM1606 α-glucosidase by the method of Kunkel [14] and individual mutant enzymes were expressed under the control of the *icp* promoter [7,12] in the *E. coli* transformant cells. Among 38 site-directed mutants created, all except for the T272R mutant showed enzyme activities, being consistent with the notion that amino acid residues at these positions should not be directly involved in the primary catalysis [7,12]. These mutants were stable at 60 °C and pH 7.0 for prolonged periods of time (more than 1 h). Enzyme assays based on sucrose hydrolysis showed that crude extracts of the mutants had distinct specific activities from each other, probably due to alterations of molecular activity of the enzyme upon mutation and/or due to different levels of gene expressions. To analyze the oligosaccharide formation catalyzed by these mutants, the enzyme amount used for transglucosylation was expressed on the basis of hydrolytic activity, as in the case of most enzymatic synthesis studies, which use a crude or immobilized form of the enzymes. Thus, in all cases, enzyme concentration in the reaction mixture was set at 2.6 U/ml based on sucrose-hydrolyzing activity. The enzymes were allowed to react with 1.75 M sucrose at pH 6.0 and 60 °C and the sugar composition of reaction mixtures was analyzed at time intervals by HPLC. The specificity of oligosaccharide production was analyzed by the amounts and structures of oligosaccharides formed and was expressed in terms of "oligomer specificity" (distribution of di-, tri-, and tetrasaccharides in the transfer product) and "trisaccharide specificity" (i.e. regiospecificity of trisaccharide formation) (see Tables 1 and 2). Also, to compare the ability of the enzymes to produce oligosaccharides from sucrose, we have introduced the R_{trans} (%) value:

$$R_{trans} = \left(\frac{A_{mutant}}{A_{wild}}\right) \times 100$$

where A_{mutant} and A_{wild} are the maximum amount (expressed as g/100 ml) of total transfer products obtained during reaction for 48 h using 2.6 U/ml of the mutant and wild-type enzymes, respectively. The R_{trans} value represents the "relative activity" of oligosaccharide production by mutant in comparison with the value of the wild-type enzyme which was taken to be 100%. This comparison is based on the enzyme amount normalized by sucrose-hydrolyzing activity (not by weight) because enzyme amount has conveniently been expressed on the basis of hydrolytic activity in many synthetic studies using enzymatic transfer activities and the relative magnitude of enzyme's transfer activity over hydrolytic activity must be as high as possible. It is important to note, however, that high R_{trans} value of a mutant does not necessarily mean that the mutant has a high specific activity (per milligram protein).

The analysis showed that amino acid substitution at position 273 or 272 caused perturbations in the specificity of oligosaccharide production, as summarized in Table 1 (for substitution at position 273) and Table 2 (for substitution at position 272).

3.2. Effects of amino acid substitution at position 273 on the specificity of oligosaccharide production

The wild-type enzyme catalyzes the intermolecular transglucosylation of sucrose to produce theanderose along with the other trisaccharidal (i.e. isomelezitose and 4-O^G-glucosylsucrose), disaccharidal, and tetrasaccharidal transfer products (see [2,11] and Table 1 and Fig. 4A). The amounts of these transfer products gradually increased with time during the reaction [11] (Fig. 3A). At 48 h, the amount of total oligosaccharides was 17.6 g/100 ml, and 35% (w/w) of this was occupied by theanderose.

Amino acid substitution at position 273, in many cases, caused a decrease in the R_{trans} values (Table 1). Particularly, replacement by an aromatic amino acid

Table 1
Effects of amino acid substitution at position 273 of SAM1606 α-glucosidase on the specificities of the enzymatic oligosaccharide production from sucrose[a]

Amino acid	R_{trans} (%)[b]	Product specificities						Amount of theanderose formed (g/100 ml)[c]
		Oligomer specificity (relative %)[d]			Trisaccharide specificity (relative %)[e]			
		Di-	Tri- (A)	Tetra-	**1**	**2**	**3** (B)	
Wild-type (Gly)	100	23	73	4	40	12	48	6.1 (100)
Aliphatic								
Ala	90	22	77	1	51	14	35	4.2 (69)
Ile	64	23	77	0	74	0	26	2.0 (33)
Leu	32	11	89	0	64	0	36	1.8 (30)
Val	78	18	80	2	47	15	38	3.9 (64)
Imino acid								
Pro	72	0	100	0	73	2	25	
Hydroxy								
Ser	80	24	75	1	50	10	40	4.3 (70)
Thr	70	28	70	2	57	7	36	2.9 (48)
Sulfur-containing								
Cys	64	47	52	1	50	5	45	2.6 (43)
Met	24	28	72	0	58	0	42	1.8 (21)
Acidic								
Asp	63	45	54	1	46	15	38	2.4 (39)
Glu	39	74	26	0	71	7	22	0.4 (7)
Amide								
Asn	69	58	40	2	36	6	58	2.6 (43)
Gln	35	68	32	0	74	7	18	0.4 (7)
Basic								
Arg	81	99	1	0		nd		
His	7		nd			nd		
Lys	110	99	1	0		nd		
Aromatic								
Phe	8		nd			nd		
Tyr	5		nd			nd		
Trp	11		nd			nd		

[a] The values are based on the analysis of the reaction mixture at 48 h of the transglucosylation reaction using 2.6 U/ml of enzyme, except for the values for the Pro mutant which is based on that at 10 min, nd: not determined.

[b] see Section 3.1, for definition.

[c] Values in parentheses indicate percentage amount of theanderose relative to that produced by the wild-type enzyme.

[d] Relative percentage of concentrations (g/100 ml) of disaccharides (other than sucrose, di-), trisaccharides (tri-), and tetrasaccharides (tetra-) determined by HPLC. Total concentration of these oligosaccharides was taken to be 100%.

[e] Relative percentage of concentrations (g/100 ml) of isomelezitose (**1**), 4-O^F-glucosylsucrose (**2**), and theanderose (**3**) determined by HPLC. Total concentration of these trisaccharides was taken to be 100%.

(i.e. Phe, Tyr, and Trp) or His caused a significant diminution of the R_{trans} value of the enzyme, indicating the selective loss of transglucosylation activity in these mutants: these mutants virtually did not produce transfer products but yielded only hydrolytic products, i.e. glucose and fructose, even at high concentration (1.75 M) of sucrose (see also Figs. 3B and 4B for the case of G273Y mutants). Thus, these single amino acid substitutions converted the α-glucosidase with high transglucosylation activity into a hydrolytic enzyme virtually lacking transfer activity.

Other remarkable effects of substitution at position 273 were those of replacement by a basic amino acid (i.e. Lys and Arg) on the specificity of oligosaccharide

Table 2
Effects of amino acid substitution at position 272 of SAM1606 α-glucosidase on the specificities of the enzymatic oligosaccharide production from sucrose[a]

Amino acid	R_{trans} (%)[b]	Product specificities (%)						Amount of theanderose formed (g/100 ml)[c]
		Oligomer specificity (relative %)[d]			Trisaccharide specificity (relative %)[e]			
		Di-	Tri- (A)	Tetra-	**1**	**2**	**3** (B)	
Wild-type (Thr)	100	23	73	4	40	12	48	6.1 (100)
Aliphatic								
Ala	93	20	77	3	39	11	50	6.2 (108)
Gly	125	15	73	12	40	13	47	7.6 (125)
Ile	139	8	65	27	26	7	67	10.6 (174)
Leu	134	8	69	23	40	9	51	8.1 (133)
Val	125	9	75	16	39	8	53	8.7 (143)
Imino acid								
Pro	132	16	76	8	41	18	41	7.2 (118)
Hydroxy								
Ser	121	22	73	5	40	13	47	7.3 (120)
Sulfur-containing								
Cys	106	19	76	5	38	12	50	7.2 (118)
Met	53	4	92	4	52	8	40	3.5 (57)
Acidic								
Asp	125	16	75	9	46	12	42	6.7 (113)
Glu	149	16	75	9	51	10	29	7.6 (125)
Amide								
Asn	173	24	67	9	40	14	46	9.4 (154)
Gln	181	21	60	9	49	13	38	7.2 (118)
Basic								
Lys	163	27	52	21	43	16	41	6.1 (100)
His	171	23	59	18	42	15	43	7.7 (126)
Aromatic								
Phe	145	15	63	22	35	16	49	7.9 (130)
Tyr	142	19	58	23	37	16	47	6.7 (110)
Trp	77	7	77	16	49	7	44	4.6 (75)

[a] The values are based on the analysis of the reaction mixture at 48 h of the transglucosylation reaction using 2.6 U/ml of enzyme except for the values for Lys mutant which is based on that at 10 h, nd: not determined.
[b] see Section 3.1 for definition.
[c] Values in parentheses indicate percentage amount of theanderose relative to that produced by the wild-type enzyme.
[d] Relative percentage of concentrations (g/100 ml) of disaccharides (other than sucrose, di-), trisaccharides (tri-), and tetrasaccharides (tetra-) determined by HPLC. Total concentration of these oligosaccharides was taken to be 100%.
[e] Relative percentage of concentrations (g/100 ml) of isomelezitose (**1**), 4-O^F-glucosylsucrose (**2**), and theanderose (**3**) determined by HPLC. Total concentration of these trisaccharides was taken to be 100%.

production. G273K and G273R mutants were found to lack the ability to produce the trisaccharides and exclusively produced disaccharides as transfer products under the same conditions (see Figs. 3C and 4C). Because the R_{trans} values of these mutants were comparable with that of oligosaccharide production by the wild-type enzyme (110 and 81% for G273K and G273R mutants, respectively), the inability of these mutants to produce tri- and tetrasaccharides should not arise from a decrease in transglucosylation activity but should be due to a shift in the product (oligomer) specificity of transglucosylation.

The unique initial "burst" kinetics of isomelezitose formation followed by the rapid degradation of

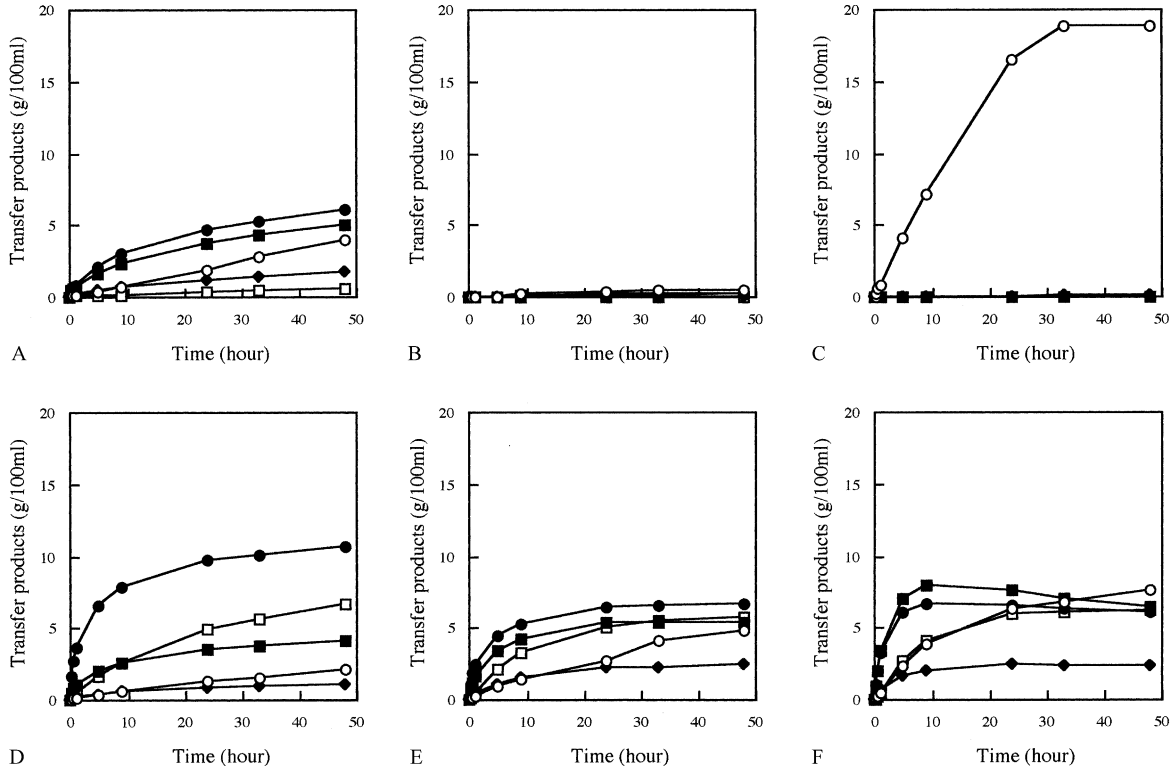

Fig. 3. Progress curves of oligosaccharide production during the reaction of 1.75 M sucrose at pH 6.0 and 60 °C with wild-type enzyme (panel A), G273Y (panel B), G273K (panel C), T272I (panel D), and T272Y (panel E), and T272K mutant (panel F). Sugar concentrations ((■) isomelezitose; (◆) 4- O^F-glucosylsucrose; (●) theanderose; (○) disaccharides; and (□) tetrasaccharides) are found by Daisopak SP-120-5-ODS-B HPLC.

this trisaccharide (not shown here) was observed only with the Pro mutant [11]. Other mutants produced trisaccharides as major transfer products, as does the wild-type enzyme. However, the yields of theanderose produced by these mutants were lower than that with the wild-type, because the R_{trans} values or the oligomer and regioisomer specificities for theanderose formation (i.e. A and B, respectively; see Table 1), or both, were lower than that of the wild-type enzyme.

3.3. Effects of amino acid substitution at position 272 on the specificity of oligosaccharide production

Effects of substitution at this position on the specificity of oligosaccharide production (Table 2) were in contrast to those observed with substitution at position 273. None, except for substitution by Met, caused significant diminution of the R_{trans} value. Substitution by amino acids (Leu, Ile, Lys, His, Phe, Tyr, and Trp) that are bulkier than the wild-type amino acid (Thr) caused an increase in the relative contents of tetrasaccharides in the transfer products, although trisaccharides were yet the most abundant transfer products. These observations may be, at least in part, related to the increased R_{trans} values of these mutants. Enzymes with substitution by amino acids (i.e. Ala, Cys, and Ser) which are similar, in size and nature, to the wild-type amino acid showed a specificity for oligosaccharide production which was similar to that of the wild-type enzyme. A remarkable shift of specificity toward theanderose formation was observed with the T272I mutant (Figs. 3D and 4D). This mutant also showed an increased R_{trans} value ($R_{trans} = 139$), resulting in the maximum yield of theanderose production (10.6 g/100 ml), that was 1.74 times higher than that obtained with the wild-type enzyme. Relative content of theanderose in the transfer

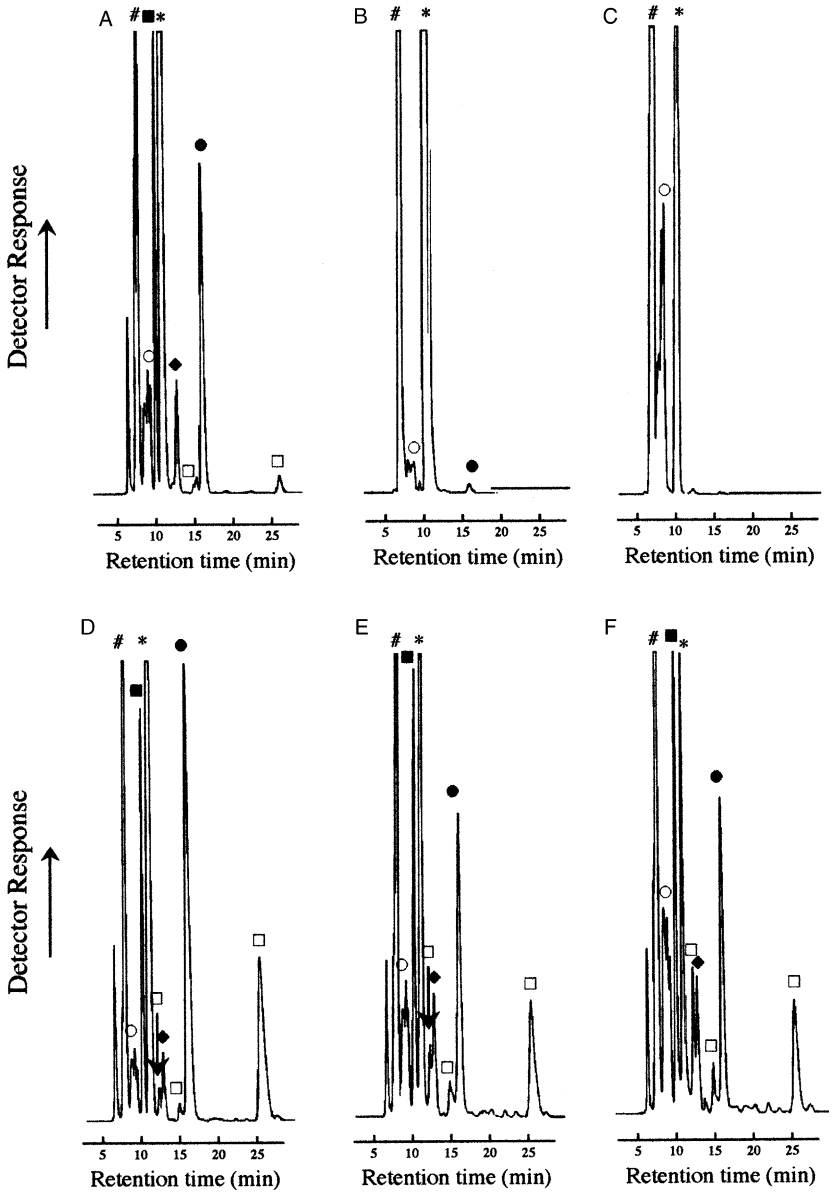

Fig. 4. The HPLC analysis of oligosaccharides produced from sucrose. (A) Wild-type enzyme; (B) G273Y; (C) G273K; (D) T272I; (E) T272Y, and (F) T273K. The HPLC profiles of reaction mixtures at 48 h (for A, B, C, D, and E; see Fig. 3) and at 10 h (for F) are shown. For detailed HPLC conditions, see Section 3.1. Sugar peaks are indicated as follows: (■) isomelezitose; (◆) 4-O^F-glucosylsucrose; (●) theanderose; (○) disaccharides; (□) tetrasaccharides; (#) fructose; and (∗) sucrose.

products was highest with T272I mutant (44% (w/w), see also Table 2 and Figs. 3D and 4D). The T272I mutant was purified to apparent homogeneity judging from SDS-PAGE by a combination of ammonium sulfate fractionation, heat treatment, and ion-exchange as well as gel filtration chromatographies as described previously [12]. It showed a specific activity for sucrose hydrolysis of 5.21 U/ml, which was 9.2% of that of the wild-type enzyme [12]. Because the site of amino acid substitution was in close proximity to one

of the putative catalytic residues (Glu271), it would be likely that this substitution may not only cause the alteration of specificity of oligosaccharide production from sucrose but also cause the alteration of enzyme's specific activity.

It should be emphasized that effects of amino acid residue at position 273 on the specificity of oligosaccharide production were in striking contrast with those observed at position 272. For example, an oligomer specificity with preference for tetrasaccharide formation was observed with replacement at position 272 by Lys (Figs. 3F and 4F) and this contrasts with the observation with replacement at position 273 by Lys which resulted in a shift of oligomer specificity toward disaccharide formation (Figs. 3C and 4C). Also, substitution by Tyr at position 272 caused a shift of oligomer specificity toward tetrasaccharide formation (Figs. 3E and 4E) and this contrasts with the observation with substitution by the same amino acid at position 273, which virtually abolished the transglucosylation activity (Figs. 3B and 4B). In this connection, an importance of an aromatic amino acid residue near the active site for lowering transglucosylation activity has been proposed in the *Saccharomycopsis fibuligera* α-amylase, a family-13 enzyme [17]. Molecular modeling studies suggested that, in this α-amylase, Tyr83 should play roles in the binding of oligosaccharide substrates through the stacking interaction and in the indirect fixation of catalytic water through hydrogen bonding with the hydroxyl of the bound substrates. Mutations of this aromatic residue to non-aromatic residues are proposed to cause changes in the binding pattern of substrates to favor transglucosylation over hydrolysis. However, molecular modeling studies of the SAM1606 α-glucosidase did not predict that the amino acid residue at position 273 of SAM1606 α-glucosidase plays roles equivalent to those of positions 83 of *S. fibuligera* α-amylase. Elucidation of three-dimensional structure of the glucosylenzyme intermediate as well as the glucosyl acceptor site of the SAM1606 α-glucosidase should help to understand the details of the mechanisms underneath the results obtained in this study.

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