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Biochemical characterisation of a glycogen branching enzyme from *Streptococcus mutans*: Enzymatic modification of starch

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ABSTRACT

A gene encoding a putative glycogen branching enzyme (SmGBE) in *Streptococcus mutans* was expressed in *Escherichia coli* and purified. The biochemical properties of the purified enzyme were examined relative to its branching specificity for amylose and starch. The activity of the approximately 75 kDa enzyme was optimal at pH 5.0, and stable up to 40 °C. The enzyme predominantly transferred short maltooligosyl chains with a degree of polymerization (dp) of 6 and 7 throughout the branching process for amylose. When incubated with rice starch, the enzyme modified its optimal branch chain-length from dp 12 to 6 with large reductions in the longer chains, and simultaneously increased its branching points. The results indicate that SmGBE can make a modified starch with much shorter branches and a more branched structure than to native starch. In addition, starch retrogradation due to low temperature storage was significantly retarded along with the enzyme reaction.

1. Introduction

Glycogen, a major storage carbohydrate in bacteria, is a polysaccharide composed of α-1,4-linked glucans and highly branched by α-1,6-glycosidic linkages. A glycogen branching enzyme (GBE; α-1,4-glucan: α-1,4-glucan 6-glycosyltransferase; EC 2.4.1.18) is responsible for the formation of the α-1,6-linkages in the glycogen molecule (Preiss, 1984). Its branching catalysis is achieved by cleavage of the α-1,4-linkage, yielding a non-reducing end oligosaccharide chain, and subsequent attachment of the oligosaccharide to the α-1,6-position. Branching enzymes (BEs) are widely distributed in plant and animal tissues as well as microorganisms. Starch branching enzyme (SBE) is an analogue of GBE in plants that introduces α-1,6-branches into amylose and amylopectin (Borovsky, Smith, & Whelan, 1975). However, there are major differences in the BE actions of SBE and GBE; the degree of branching is 8–9% in glycogen and 3.5% in amylopectin, and the average chain-length of the branches is usually 10–12 glucose residues for glycogen and 20–23 glucose residues for amylopectin (Marshall, 1974; Myers, Morell, James, & Ball, 2000). The more branched structure of glycogen with shorter chains is thought to be primarily due to different specificities between GBE and SBE in terms of the size of transferred chains.

Prokaryotic GBEs are classified into two major groups on the basis of the amino acid alignment; the first group possesses an additional amino(N)-terminal stretch of more than 100 amino acids and the second group lacks it (Guan, Li, Imparl-Radesevich, Preiss, & Keeling, 1997). Truncation of the extra N-terminal region alters the enzyme branching specificity, causing it to transfer fewer short chains and a greater proportion of chains longer than degree of polymerization (dp) 12 of glucose units (Hilden, Leggio, Larsen, & Poulsen, 2000). GBE is known structurally to consist of three domains: an N-terminal domain, a carboxyl(C)-terminal domain, and a central $(\alpha/\beta)_8$ barrel catalytic domain (Jespersen, MacGregor, Henrissat, Sierks, & Svensson, 1993). While the C-terminal is assumed to be involved in substrate preference and catalytic capacity, the N-terminal appears to determine the size of the chain transferred (Kuriki, Stewart, & Preiss, 1997).

Reportedly, GBEs from *Bacillus stearothermophilus* and *Aquifex aeolicus* would attack amylopectin in waxy rice and waxy corn, resulting in increased branching and lower average molecular weight, but with no significant change in the chain-length distribution (Takata et al., 1994; Van der Maarel, Vos, Sanders, & Dkjkhuizen, 2003). The main product of the GBE reaction is thought to be a highly branched cyclic dextrin. In addition, microbial amylomaltases (4-α-glucanotransferase; EC 2.4.1.25) involved in the synthesis and degradation of glycogen have been shown to transform amylose to large cyclic glucan, or modify potato starch to have a broader distribution of shorter and longer side chains in amylopectin with the disappearance of amylose (Van der Maarel et al., 2005).

In this study, we cloned and expressed a putative GBE gene from *Streptococcus mutans*. The encoded enzyme contains four highly conserved regions of the α-1,4-GBE family and does not have the extra N-terminal stretch of the first bacterial GBE group. Here, we report the biochemical properties of the recombinant

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enzyme (referred to as SmGBE hereafter), with emphasis on its branching specificity for the modification of amylose and starch.

2. Materials and methods

2.1. PCR cloning and expression of SmGBE

S. mutans UA 159 ATCC 25175 was obtained from the American type culture collection. The genomic DNA of *S. mutans* was isolated by the spool method (Sambrook, Fritsch, & Maniatis, 1989). The nucleotide sequence of the gene, encoding a putative α-1,4-glucan branching enzyme (Q8DT52) in the bacteria, was retrieved from GenBank. The gene was amplified with PCR using *Pwo* DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany) and the genomic DNA as a template. The oligonucleotide primers for the 5′ and 3′-flanking ends of the gene were designed as 5′-GAGGTGGTACCATGAATGAGAGAGAAG-3′ (forward) and 5′-CAAATCTCGAGTTACTTTCTCAAACGA-3′ (reverse), and contained KpnI and XhoI restriction sites, respectively (underlined). PCR amplification was performed as described previously (Ryu, Park, Cha, Woo, & Lee, 2005). The 1.89 kb amplified DNA fragment was subsequently digested with KpnI and XhoI and ligated into the expression vector pET-30a(+) to finally construct pET-30a(+)-SmGBE. The *E. coli* BL21(DE3) transformant harbouring pET-30a(+)-SmGBE was grown in LB broth supplemented with 100 μg/ml of kanamycin at 37 °C until the attenuance at 600 nm reached 0.6 and then induced with isopropyl thiogalactoside (IPTG) for 6h, resulting in overproduction of the recombinant SmGBE. The nucleotide sequence of the PCR-generated gene was determined with the BigDye terminator cycle sequencing kit for the ABI 377 Prism (Perkin–Elmer, Norwalk, USA). The homology analysis of DNA and amino acid sequences was performed using CLUSTAL (Thompson, Higgins, & Gibson, 1994).

2.2. Enzyme purification

After induction with IPTG, the transformant cells were harvested by centrifugation (7000*g* for 30 min at 4 °C) and resuspended in 50 mM sodium phosphate lysis buffer (pH 8.0) containing 300 mM NaCl and 10 mM imidazole. A cell extract was obtained by sonication, followed by centrifugation (10,000*g*) for 30 min at 4 °C. The supernatant was pooled and His-tagged recombinant enzyme was purified by nickel–nitrilotriacetic acid (Ni–NTA) affinity column chromatography (Qiagen, Hilden, Germany). The purified protein was concentrated by ultrafiltration (Millipore Co., Bedford, MA, USA) after dialysis against 50 mM sodium acetate buffer (pH 6.0) and used for further investigation. The purity and molecular mass of the recombinant protein were estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) with a 10% (w/v) acrylamide gel. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI–TOF MS; Voyager–DE STR Biospectrometry Workstation, Applied Biosystems, Inc., Foster City, CA, USA) was also used to determine the molecular mass with 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) in 30% acetonitrile and 0.3% trifluoroacetic acid as a matrix.

2.3. Enzyme assay

The iodine staining assay for SmGBE was performed as described in the literature with small modifications (Takata et al., 1994). The assay was carried out in 50 mM sodium acetate buffer (pH 5.0) with amylose (1 mg/ml) at 37 °C as a standard condition. Fifty microlitres of the reaction mixture were quenched by 1 ml of iodine reagent containing 0.01% I_2, 0.1% KI, and 0.38% 1N HCl in water. After 15 min at room temperature for color stabilization, the absorbance at 620 nm was measured. Wavescans of the glucan–iodine complexes were also performed, from 400 to 900 nm, to determine changes in the shape and maximal wavelength. The protein concentration was determined according to Bradford's (1976) method with bovine serum albumin as a standard.

2.4. Effects of pH and temperature on SmGBE activity

To determine the optimal pH, enzyme activity was compared in 50 mM buffers of sodium citrate (pH 3.0 to 3.5), sodium acetate (pH 4.0 to 6.0), and Tris–HCl (pH 7.0 to 9.0). To examine the pH stability, the enzyme (0.5 mg/ml) was first incubated in the above pH buffers for 1 h at 37 °C, after which the remaining activity was measured under the standard conditions described above. To determine thermal stability, the enzyme (0.5 mg/ml) was pre-incubated for 1 h in the standard buffer at temperatures ranging from 25 to 60 °C. After each prescribed aliquot was taken and placed immediately in an ice-water bath, the residual hydrolyzing activity was determined under the standard conditions.

2.5. Incubation of SmGBE with amylose and starch

A substrate solution (0.5%, w/v) of amylose or rice starch was prepared by dissolving it in 1 N NaOH (6 ml), followed by the addition of demineralized water (15 ml) and 200 mM sodium acetate buffer (3 ml, pH 5.0). The pH was adjusted to 5.0 with 1 N HCl (6 ml). Each substrate solution (0.5%, w/v) was gelatinized by incubation at 100 °C for 30 min and then incubated with 600 μl of SmGBE for 1–24 h at 37 °C. The reaction was stopped by boiling for 5 min. Three volumes of ethanol were added to the quenched reaction, and the mixture was then stored for 1 h at 4 °C. The resulting precipitate was collected by centrifugation (6000*g*, 10 min) and washed three times with 70% ethanol, followed by vacuum–drying. The dried glucan product was used in further experiments.

The glucan product of amylose by SmGBE incubation was investigated by both thin-layer chromatography (TLC) and mass analyses after α-amylolysis of the product. The 1% (w/v) solution of glucan product in 250 mM Tris–HCl buffer (pH 7.0, 900 μl) was incubated with 10 U of α-amylase (100 μl) for 24 h at 37 °C. TLC analysis of the hydrolyzed products by α-amylolysis was performed on Whatman K5F silica gel plates (Whatman, Kent, UK) with isopropyl alcohol–ethyl acetate–water (3:1:1, v/v/v) (Park et al., 1998). The α-limit glucan products were isolated by preparative paper chromatography onto Whatman 3 MM paper (23 × 55 cm) with a descending technique (Robyt & White, 1987). The spots on the paper were located using an $AgNO_3$ reagent to verify the separation of purified carbohydrates. The paper was sectioned and eluted with deionized water, and then lyophilized for the analysis. The molecular weights of the purified products were determined by MALDI–TOF MS with α-cyano-4-hydroxycinnamic acid as a matrix.

2.6. Measurement of reducing value with isoamylase treatment

Aliquots were taken, at various time points, from the reaction mixture of SmGBE with amylose or rice starch, and the glucan products were prepared as mentioned above. Each 10 mg of dried products was dissolved in 1 ml of 250 mM sodium acetate buffer (pH 3.5) and then incubated with 20 μl of isoamylase (1 U/μl, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) for 24 h at 37 °C. The reaction was stopped by boiling for 5 min. After centrifugation of the reaction mixtures, the supernatants were filtered with a 0.22 μm membrane filter (Millipore Co.). The amount of reducing sugars in the resulting filtrates was measured by the

copper-bicinchoninate method (Fox & Robyt, 1991) and compared to that of the glucan products before isoamylolysis.

2.7. Determination of branch chain-length distribution by HPAEC analysis

The distribution of branch chain-length in the glucan products periodically taken from the SmGBE reaction with amylose or rice starch was analyzed by high performance anionic-exchange chromatography (HPAEC) after isoamylase debranching as described above. The debranched glucan solution was filtered through a 0.22 μm membrane and analyzed by HPAEC, using a Dionex CarboPac PA100 column (0.4 × 25 cm, Dionex Co., Sunnyvale, CA) and an electrochemical detector ED40 (Dionex Co.) with a linear gradient of sodium acetate from 0 to 850 mM for 60 min and 150 mM sodium hydroxide at a flow rate of 1.0 ml/min (Park, Park, & Jane, 2007).

2.8. Molecular weight determination of glucans by HPSEC–MALLS–RI analysis

The average molecular weight of the glucan products from the SmGBE reaction with amylose or rice starch was determined by using high performance size exclusion chromatography (HPSEC) equipped with a multi-angle laser-light-scattering photometer (MALLS with He–Ne laser-light at 632 nm, Wyatt Technology, Santa Barbara, CA) and a differential refractive index detector (RI, Hewlett Packard, Valley Forge, PA, USA) (Yoo & Jane, 2002). The prepared dried glucan products were dispersed in 1 mM sodium nitrate solution and mechanically stirred while heating in a boiling water bath for 1 h. The hot sample solution was filtered through a nylon membrane filter (0.22 μm) and 100 μl of filtrate were immediately injected into the HPSEC system with a Shodex OH pak analytical column (KB-806, Showa Denko K.K., Tokyo, Japan). The weight-average molecular weight (M_w) of the glucan corresponding to each peak eluted was evaluated from the laser-light-scattering data using ASTRA 4.7.07 software (Wyatt Technology).

2.9. Measurement of retrogradation by differential scanning calorimetry (DSC)

Thermal analysis was performed using a differential scanning calorimeter (DSC120, Seiko Instrument Inc., Tokyo, Japan) (Kim & Jung, 2006; Kohyama, Matsuki, Yasui, & Sasaki, 2004). Each 1 mg of sample and 9 mg of distilled water were directly weighed into a silver pan, dispersed by a needle, sealed hermetically, and left for 3 h to equilibrate. The pan containing native or SmGBE-treated rice starch sample was initially heated from 20 to 110 °C (5 °C/min) to complete gelatinization. Immediately after the first run, the pan was quenched to room temperature and then stored at 4 °C for 10 days. A pan containing 9 mg of distilled water was used as a reference. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy change (ΔH_r) of retrogradation were measured by scanning the samples from 20 to 110 °C at 1.0 °C/min. Each sample was measured twice.

3. Results and discussion

3.1. Cloning and sequence alignment of GBE from S. mutans

The homology analysis showed that SmGBE with 628 amino acids had a similarity to reported prokaryotic GBEs in the range of 39–77% sequence identity, including *S. pneumoniae* (77%, Q8DPS6), *Bacillus caldolyticus* (44%, P30537), *Bacillus stearothermophilus* (44%, P30538), *Bacillus subtilis* (43%, P39118), *E. coli* (39%, P07762), *Aquifex aeolicus* (44%, O66936), and *Haemophilus influenzae* (40%, P45177). Multiple alignments of amino acid sequences of GBEs from *S. mutans* and other bacterial species demonstrated that SmGBE shares four highly conserved sequences with the branching enzymes (data not shown). In addition, the enzyme does not have the extra N-terminus extended by 100–150 amino acid residues that exists only in the first group of the prokaryotic GBEs, including *E. coli* GBE (Hilden et al., 2000). Thus, SmGBE belongs to the second group in the phylogenetic classification of prokaryotic GBEs, which includes GBEs from *Bacillus stearothermophilus* and *B. caldolyticus*.

3.2. Expression and characterization of SmGBE

The enzyme was efficiently expressed and purified by Ni–NTA affinity chromatography, and was seen as a dense protein band on SDS–PAGE gels (Fig. 1A). The molecular mass was confirmed to be approximately 74 kDa by MALDI–TOF mass spectrometry (data not shown), which was close to the expected size deduced from the primary amino acid sequence of the protein. The pH and temperature ranges at which SmGBE was active and stable were determined using the iodine staining assay with amylose. As shown in Fig. 1B, the optimal activity of SmGBE was observed at pH 5.0, and most of the activity was retained in a narrow pH range (pH 4.5–6.0). The enzyme was relatively stable during 1 h of incubation in the pH range between 4.0 and 8.0. As expected, the enzyme showed no thermal stability and began to be inactivated at temperatures above 40 °C for 1 h of incubation at pH 5.0.

The branching action of SmGBE on amylose was investigated by analyzing the iodine–glucan complex and reducing value of the glucan product after isoamylolysis. With the progression of the enzymatic reaction, the ability of amylose to form the iodine complex decreased rapidly, which was observed as a decrease of absorbance at the optimal wavelength (λ_{max}), 660 nm, of the iodine–amylose complex (Fig. 2A). At the same time, the reducing value of the corresponding glucan product, after ethanol precipitation and isoamylase treatment, was 10- and 15-fold higher for 6 and 12 h reactions, respectively (data not shown). Because the amylose used was virtually free of α-1,6-branching points, the increase of reducing ends of the glucan product after isoamylase debranching indicated the formation and expansion of newly introduced branching points on the amylose as a result of the SmGBE reaction. Meanwhile, α-limit dextrins of the glucan product after extensive α-amylase hydrolysis were detected by TLC analysis (Fig. 2B). The resulting α-limit maltooligosaccharides (indicated by arrows in lane 2, Fig. 2B), which were expected to be larger than at least

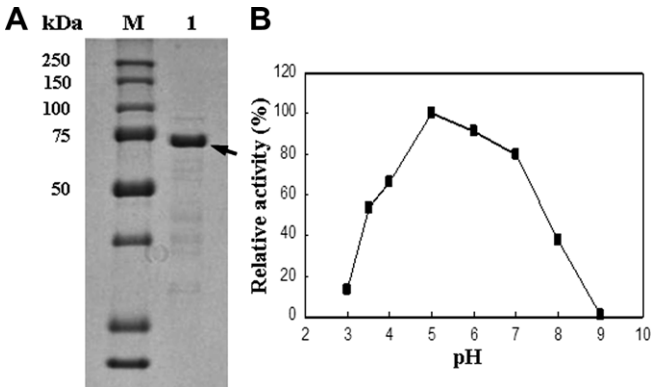

Fig. 1. SDS–PAGE (A) and optimal pH (B) of recombinant SmGBE. (A) Lane 1, cell extract after sonication; lane 2, supernatant after centrifugation; lane 3, purified SmGBE (arrow) after Ni–NTA affinity chromatography. (B) The relative activity was measured and is shown as a percentage of the activity (100%) at pH 5.0.

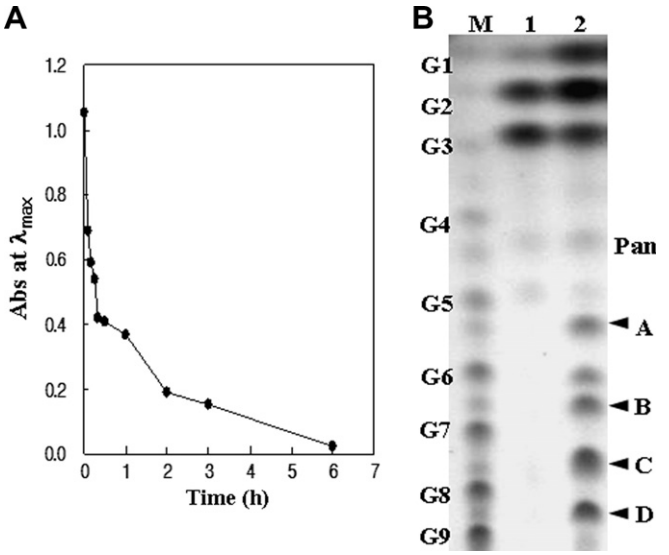

Fig. 2. Time-dependent changes in maximal iodine absorption (A) and TLC analysis after α-amylolysis (B) of glucan product from SmGBE reaction with amylose. (A) Absorbance at 660 nm (λ_{max} of intact amylose–iodine complex) was measured. (B) Lane M, maltodextrins of G1 to G9; lane 1, α-amylase hydrolysis of amylose ; lane 2, α-amylase hydrolysis of glucan product derived from 12 h incubation of SmGBE with amylose.

maltopentaose, judging from their positions on the TLC plate, were isolated by paper chromatography. From the mass spectra of four purified products, four peaks clearly appeared at *m*/*z* 689.3, 851.3, 1013.3, and 1175.4 ($[M + Na]^+$), which, respectively, corresponded to the calculated molecular masses of sodium ion adducts of maltooligosaccharides ranging from maltotetraose to maltoheptaose. However, each purified product exhibited a mass smaller by about one glucose unit than expected, just as in the case of a panose. Generally, a panose exhibits a slower mobility due to its α-1,6-linkage than the same molecular weight of linear α-1,4-linked maltotriose on an upward TLC (lane 2 in Fig. 2B) (Cho et al., 2000). Consequently, this result also confirmed that SmGBE catalyzed the formation of an α-1,6-linked branched structure in the glucan product.

3.3. Changes in branch chain-length and molecular weight distributions of amylose and starch by SmGBE

The side-chain-length of the branched glucans produced over time from the SmGBE reaction with amylose was determined by HPAEC analysis after isoamylase debranching (Fig. 3). Branch chains of dp 6 and 7 were prominently accumulated from the early incubation. As the reaction proceeded, a much smaller population of chains, ranging from dp 8 to over 20, slowly developed, with an optimum peak at dp 12. The short chains of dp 6 and 7 increased more rapidly and were the most abundant of the branch chains of the glucan products. The relative amounts of both dp 6 and 7 were maintained at 76 and 52–54% at 1 and 3–9 h of incubation, respectively, in comparison with that of the other remaining chains. Reportedly, the extra amino terminus present in group I GBEs acts to limit the short size of chains transferred (Binderup, Mikkelsen, & Preiss, 2002). However, SmGBE was able to preferentially transfer the short chains of dp 6 and 7, even though it lacked the extra N-terminal stretch. Therefore, the extra N-terminal sequence might not be a general prerequisite for the transfer of short chains in bacterial GBE catalysis. On the other hand, between the two isoforms of SBE, SBE II preferentially transfers glucose chains of dp 6–7, which is similar to the action of SmGBE (Andersson et al., 2002). In contrast, the production ratio of both dp 6 and 7

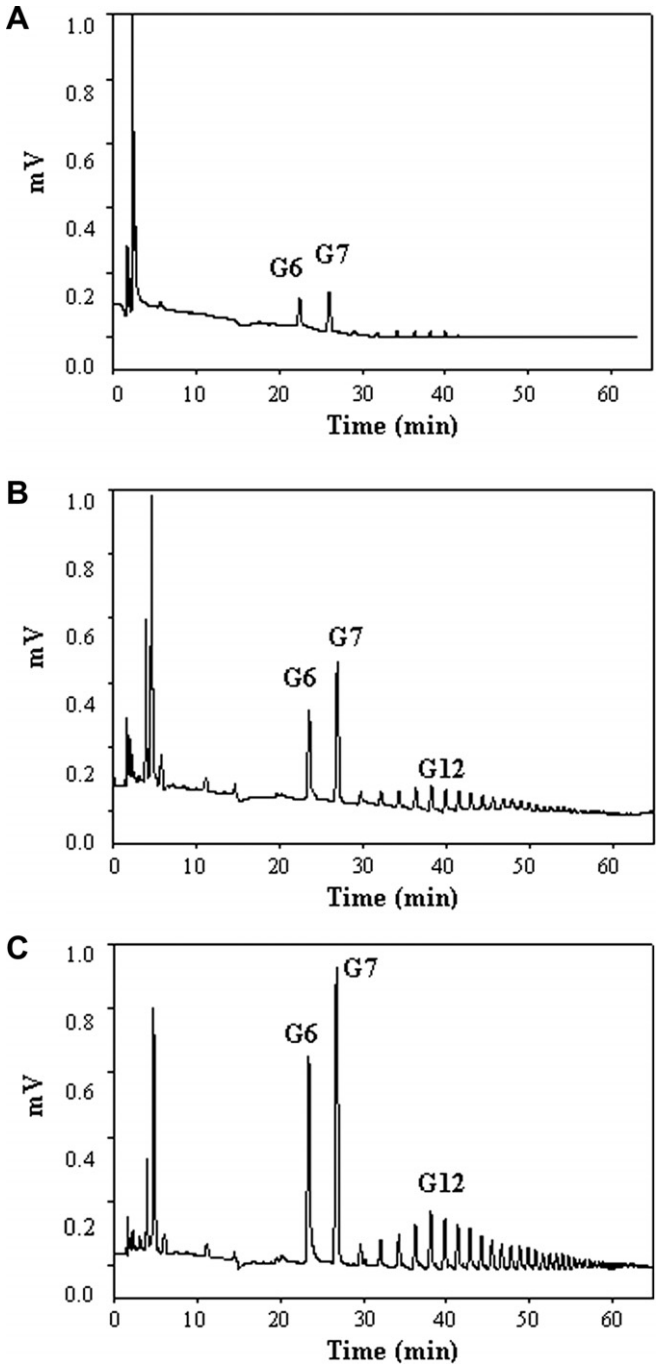

Fig. 3. HPAEC analysis of the side chain distribution of branched glucan products from SmGBE reaction with amylose for 1 h (A), 3 h (B), and 6 h (C).

in the branches of glucan product is only about 20–22%, and longer chains are highly composed. Accordingly, SmGBE is better at generating branched glucans that are very rich in maltohexaose and maltoheptaose in the branches. In fact, SmGBE has a lower similarity to the whole amino acid sequence of *E. coli* GBE (39%) and maize or potato SBE II (25%), but shows higher homology in the four conserved regions of the branching enzymes. Thus, the differences in side-chain transfer by BEs are very likely due to subtle differences in the active site architectures of the enzymes (Abad et al., 2002). Further studies are needed to provide detailed structural insights that may explain the differences in the chain transfer patterns of the BEs.

When SmGBE reacted with rice starch, the chain distribution of the resulting products was analyzed after complete debranching by isoamylase. As a result, the branch chain-length distribution of rice starch was largely changed to smaller branches (Fig. 4). The optimum chain-length in the composition was decreased by dp 6 from dp 12 to 6. The long chains from dp 8 were less present and chains longer than about dp 25 were almost completely absent after 24 h of reaction with SmGBE. The short chains from dp 3 to 5 were new additions. In addition, the differential reducing value before and after debranching for SmGBE-treated rice starch was approximately 7- and 10-fold higher for 12 and 24 h reactions, respectively, as compared to that for the corresponding native starch in the same amount. These results imply that rice starch could be modified by SmGBE to be a glycogen-like glucan with much shorter and more numerous branches.

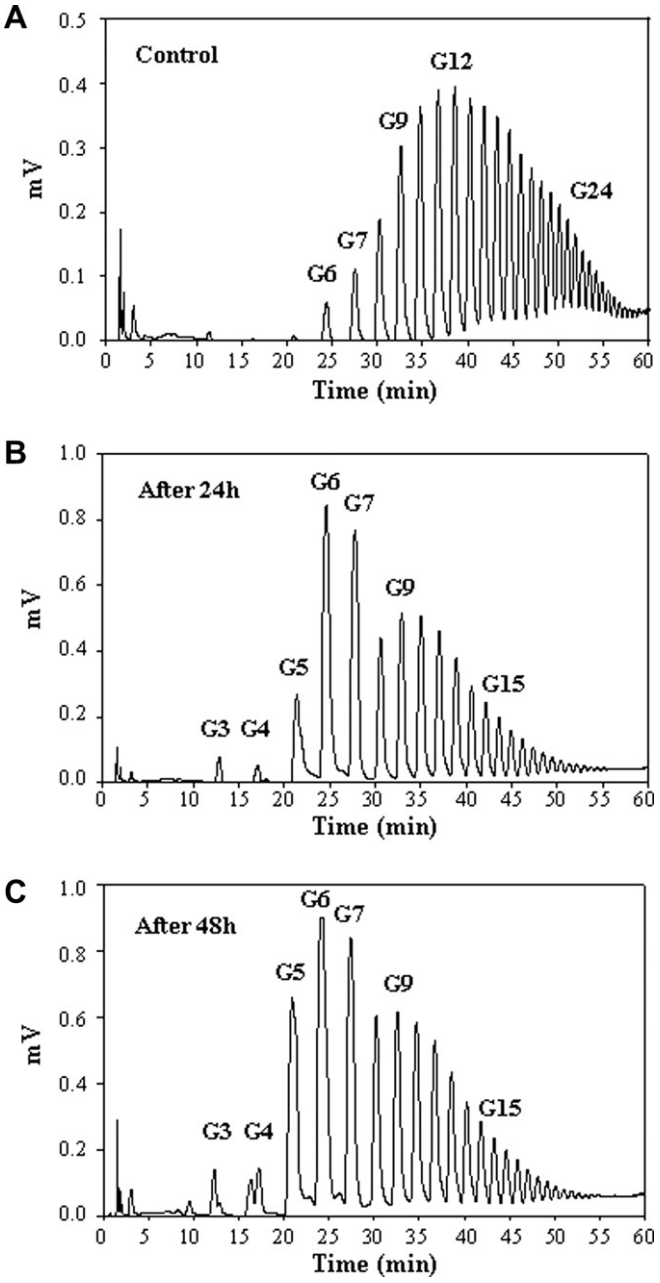

Fig. 4. HPAEC analysis of the side chain distribution of glucan products from SmGBE reaction with rice starch for 0 h (A), 24 h (B), and 48 h (C).

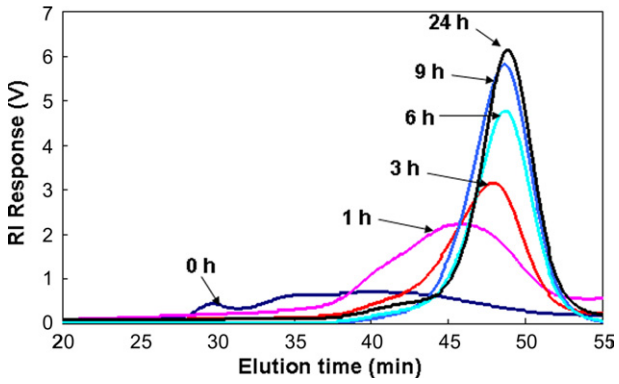

Fig. 5. HPSEC elution profile of glucan products from rice starch after various reaction times with SmGBE.

The molecular weight distribution of the glucan products from reaction with SmGBE was determined by HPSEC equipped with MALLS and RI detectors. The HPSEC profile of SmGBE-treated rice starch is shown in Fig. 5. The peak shape of the glucan products gradually became narrower and higher, and the maximum peak moved toward a longer elution time (around 50 min), along with the enzyme reaction. High and mid-size weight-average M_w of peaks with 0.4–3.4 $\times$ 10^8 and 6.8 $\times$ 10^5 g/mol, which were detected in the untreated starch, gradually disappeared and finally converged to a new peak with weight-average M_w of 1.3–1.8 $\times$ 10^5 g/mol. These results suggest that amylopectin and amylose in rice starch with high and mid-size M_w were largely modified into some cluster units of branched glucans by the degradation and branching actions of SmGBE. The degradation and large M_w reduction of starch by bacterial GBEs has been previously described and produces highly-branched cyclic glucans by intramolecular α-1,6-branching activity with no significant side-chain distribution (Takata et al., 1994; Van der Maarel et al., 2003). In contrast, the glucan products formed by SmGBE in this study were hydrolyzed by glucoamylase almost to glucose (data not shown), implying that there was no cyclic glucan product from the SmGBE reaction with amylose or rice starch. Consequently, it appeared that SmGBE only catalyzed intermolecular α-1,6-branching, partly with endo-acting α-1,4-hydrolysis.

3.4. Effects of SmGBE reaction on starch retrogradation

The turbidity of the rice starch solution (5%, w/v) was 20–40% lower after reaction with SmGBE for 6–12 h, based on spectrophotometric measurement at 600 nm. This finding indicated that the glucan products formed by SmGBE became more soluble than the rice starch substrate, possibly due to the change in the branching structure and size (Van der Maarel et al., 2005). DSC thermal analysis showed that the enzymatic treatment caused an increase in the T_o and T_p, while the T_c was lower (Table 1). In addition, the ΔH_r of rice starch gradually dropped, along with the enzyme reaction, ultimately to levels up to 6-fold lower. Accordingly, the retro-

Table 1
Thermal properties of native and SmGBE-modified rice starches after storage for 10 days at 4 °C

Enzyme reaction time (h)	Rice starch			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_r (J/g)
0	37.5 ± 1.5	49.8 ± 0.2	61.7 ± 1.2	3.5 ± 0.2
1	45.4 ± 0.9	53.1 ± 0.5	58.2 ± 0.1	1.9 ± 0.3
3	46.1 ± 0.7	52.8 ± 0.2	59.7 ± 0.4	1.6 ± 0.4
24	44.9 ± 0.5	53.2 ± 0.3	58.6 ± 1.0	0.55 ± 0.15

gradation of rice starch was considerably retarded by the SmGBE treatment. Generally, starch retrogradation would easily develop in the presence of amylose and longer chains of amylopectin (Kohyama et al., 2004; Varavinit, Shobsngob, Varanyanond, Chinachoti, & Naivikul, 2003). Partial amylolytic hydrolysis of starch is known to induce the retardation of its retrogradation (Morgan, Gerrard, Ross, & Gilpin, 1997). Therefore, the significant reduction of retrogradation in this study may have been mainly attributed to the conversion of amylose and amylopectin to shortly-branched glucans with reduced molecular weights by the branching action of SmGBE.

4. Conclusions

SmGBE efficiently transformed amylose to branched glucans with the main chains exhibiting dp of 6 and 7. The enzyme also acted on rice starch in a way that modified the chain-length distribution, yielding shorter branches with a more limited range in length. The M_w of branched glucans became significantly lower than that of intact rice starch through the enzymatic reaction. SmGBE could be used to produce more soluble glucan products from starch, with a glycogen-like structure containing shorter and more numerous branches. Gelling properties of the glucan products from starch are currently under investigation.

Acknowledgments

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