Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 26 (2003) 157–165
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Catalytic function and affinity purification of site-directed mutant β-cyclodextrin glucanotransferase from alkalophilic *Bacillus firmus* var. *alkalophilus*

Kwang-Woo Lee, Hyun-Dong Shin, Yong-Hyun Lee*

Department of Genetic Engineering, College of Natural Sciences, Kyungpook National University, Daegu 702-701, South Korea

Received 18 March 2003; received in revised form 26 May 2003; accepted 26 May 2003

Abstract

Subsites −3 and −7 in the active site of β-cyclodextrin glucanotransferase (β-CGTase) from alkalophilic *Bacillus firmus* var. *alkalophilus* were modified through site-directed mutagenesis to obtain novel mutant CGTases. Four mutant CGTases, H59Q, Y96M, 90-PPI-92, and Δ(154–160) were constructed and produced using a recombinant *E. coli* with a secretive expression system extracellularly. The secreted mutant β-CGTases were purified by one-step affinity adsorption chromatography using a β-cyclodextrin (CD) polymer as an adsorbent to nearly homogeneous purity. The catalytic activities were modified significantly compared to the wild-type. In particular, the Y96M and Δ(154–160) mutants increased cyclization activity around 1.5 times without any significant reduction of coupling and hydrolyzing activities. Meanwhile, the Y96M and Δ(154–160) mutants exhibited a much higher conversion yield into CDs from 28.6 to 39% without any recognizable change in the CD ratio. The conversion yield into linear maltooligosaccharides was also significantly reduced. The catalytic functions of subsites −3 and −7 in the active site of β-CGTase would appear to be related to the overall productivity of CDs rather than the product specificity.

Keywords: β-Cyclodextrin glucanotransferase; *cgt* gene; Site-directed mutagenesis; Mutant β-CGTase; β-CD polymer affinity purification

1. Introduction

Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) is an enzyme that catalyzes the formation of α-, β-, and γ-cyclodextrins (CDs) from starch molecule through intra-molecular transglycosylation. It also catalyzes other enzyme reactions, such as coupling, disproportionation, and hydrolysis [1]. Since CD molecules can formulate inclusion complexes with a wide range of hydrophobic molecules, therefore, have many potential applications in the food, cosmetic, and pharmaceutical industries [2].

CGTase belongs to the glycosyl hydrolase family 13 that contains $(\beta/\alpha)_8$-barrel proteins, and is functionally closely related with α-amylase [3]. CGTase is composed of five domains (A–E), and the N-terminal domains (A–C) are structurally similar to the α-amylase domain. Domain E contains a raw-starch binding motif composed of two maltose-binding sites (MBS): one is a starch-binding site (MBS1) and the other guides the substrate to the active site (MBS2) [4,5]. The catalytic functions of CGTase, including its product specificity, cyclization activity, and pH characteristics, can be modified through the

* Corresponding author. Tel.: +82-53-950-5384; fax: +82-53-959-8314.
E-mail address: leeyh@knu.ac.kr (Y.-H. Lee).

1381-1177/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2003.05.005

site-directed mutagenesis of domain E potentially [6–8].

The structure of the active site in CGTase has already been intensively studied to understand the catalytic function and reaction mechanism of CGTase. The active site of CGTase from *Bacillus circulans* strain 251 is known to be composed of nine sugar-binding subsites (labeled −7 to +2), and the catalytic site between subsites −1 and +1 cleaves the glycosidic bond [9]. Crystallographic studies of CGTase have been also carried out after complexation with various substrates, such as maltohexaose (G6), maltoheptaose (G7), maltononaose (G9), and various CDs [10–12].

Since the binding conformations of substrates at subsites −3, −6, and −7 from the active site show distinctly different patterns, this has raised the suggestion that the size specificity of CDs can be controlled through modification of the above subsites. The ability to predict the size specificity of CDs and specific formation of certain CDs rather than a mixture of CDs have already been observed based on mutagenesis at the above subsites, however, the modification of CGTase has significantly reduced such specific activities [13–16].

In previous studies by the current authors, an alkalophilic *B. firmus* var. *alkalophilus* producing mainly β-CD without accumulation of α-CD was isolated and its enzymatic characteristics investigated after purification [17,18]. The β-CGTase gene (*cgt*) encoding 674 amino acids was cloned from the genomic DNA and its structural features analyzed after comparison with other CGTases from different sources [19]. The β-CGTase from *B. firmus* var. *alkalophilus* was modified by site-directed mutagenesis to clarify the conflict function of the E-domain, it was found that the E-domain was closely connected with the cyclization reaction of β-CGTase rather than the coupling or hydrolysis reactions [20]. Extracellular overproduction of β-CGTase was achieved when using a recombinant *E. coli* containing a secretive expression system [21].

Subsites −3 and −7 in the active site of CGTase were modified through site-directed mutagenesis to obtain four novel CGTase mutant, H59Q, Y96M, 90-PPI-92, and Δ(154–160), and the mutant β-CGTases were produced extracellularly in recombinant *E. coli* containing a secretive expression system. The secreted mutant β-CGTases were purified by one-step affinity adsorption chromatography using a β-cyclodextrin (CD) polymer as an adsorbent. The catalytic functions of subsites −3 and −7 in the active site of the CGTase from *B. firmus* var. *alkalophilus*, including cyclization, coupling, and starch-hydrolysis, were then analyzed, along with the conversion into CDs and maltooligosaccharides. The pH-dependent activity and thermostability of the mutant β-CGTases were also investigated.

2. Experimental

2.1. Bacterial strains and plasmids

The gene source of β-CGTase was *B. firmus* var. *alkalophilus* (KCTC 8528P) [19]. The high-stringency host *E. coli* BL21(DE3)pLysS [F-, *ompT*, *hsdSB*(*rB-mB*), *gal*, *dcm*, (DE3) pLysS] was used as the expression host of β-CGTase. The secretive expression vector pET-20b(+) (Novagen, Madison, USA) under the control of a T7 promoter was used for the extracellular over-expression of β-CGTase. The plasmid pECGT [21] harboring the *cgt* gene of *B. firmus* var. *alkalophilus* was used for the PCR template and site-directed mutagenesis.

2.2. Site-directed mutagenesis

The site-directed mutagenesis of the β-CGTase gene was carried out using the megaprimer PCR method [22]. The oligonucleotides used for the mutation of subsite −3 in the active site were H59Q, 5′-ATAGATCTC**CAG**AAGTATTGTTGTGGTGGG-3′; Y96M, 5′-GTCGAAAACGTT**ATG**GCCCTACA-CCCA-3′; and a double mutant replacing 90-QPV-92 with 90-PPI-92, 5′-TGGATTTCT**CCGCCAATC**GA-AAACGTT-3′. The mutated codons in the sequence are bolded, and the mismatched bases are underlined. The mutated PCR product of β-CGTase was cut and inserted into the *Hin*dIII site of the secretive expression vector pET-20b(+). The constructed plasmid harboring the mutant β-CGTase was then transformed into *E. coli* BL21(DE3)pLysS.

A deletion mutant Δ(154–160) removing seven amino acid residues from subsite −7 in the active site was constructed using two PCR primers: the

forward primer, 5′-GTTGAAAATGGGGCGATATA TGATAAT-3′ and reverse primer, 5′-ACGCCAAATC-ATTCATCACCGGCA-3′. A whole pECGT plasmid harboring the β-CGTase gene except for the seven amino acid residues between 154 and 160 was amplified, and transformed into *E. coli* BL21(DE3)pLysS after self-ligation.

The general DNA manipulations were performed according to the standard methods described in the *Molecular Cloning—A Laboratory Manual* [23]. The sequences mutated by the designed oligonucleotides were all confirmed using an ABI 377 automatic DNA sequencer (PE Applied Biosystem, CA, USA).

2.3. Cultivation of recombinants E. coli for production of mutant β-CGTases

Recombinants *E. coli* carrying the mutant β-CGTase genes were cultivated to over-express the mutant β-CGTases in a TB medium containing 1% (w/v) soluble starch supplemented with 50 μg/ml ampicillin and 34 μg/ml chloramphenicol as the selection marker at 30 °C up to an OD_{600} of 0.8. The culture broth was then supplemented with IPTG up to 0.4 mM to induce the T7 promoter and then further cultivated at 25 °C for 8 h.

2.4. Affinity chromatography of mutant β-CGTases and synthesis of β-CD polymer

The wild-type and mutant β-CGTases were purified by one-step affinity adsorption chromatography using a β-CD polymer as the adsorption matrix. To synthesize the β-CD polymer, the β-CD was dried at 70 °C for 4 h under a decompressed state to ensure complete water removal. The dried β-CD was the polymerized using hexamethylene diisocyanate (HDI) as the cross-linker and anhydrous *N*,*N*-dimethyl formamide (DMF) as the solvent at 80 °C for 24 h, as shown in Fig. 1. After precipitation with an excess amount of methanol, the precipitated β-CD polymer was collected by filtration and dried under a decompressed state at 50 °C [24].

The mutant β-CGTases secreted in the culture broth were purified by one-step β-CD polymer affinity adsorption/desorption chromatography as follows. One hundred milliliter of culture broth containing the extracellular wild-type or mutant β-CGTases was mixed with 1 g of the β-CD polymer. After adsorption at 4 °C for 18–21 h on the β-CD polymer, the β-CGTase adsorbed by the β-CD polymer was washed out with an excess amount of distilled water, 1 M NaCl, and distilled water, sequentially. The adsorbed β-CGTase was then eluted with a 5 mM β-CD solution dissolved in 0.5 M NaCl at room temperature for 3 h, dialyzed with distilled water for 12–14 h, then concentrated using an ultra-filtration MW of 50,000. The homogeneity of each purified β-CGTase was confirmed by SDS-PAGE.

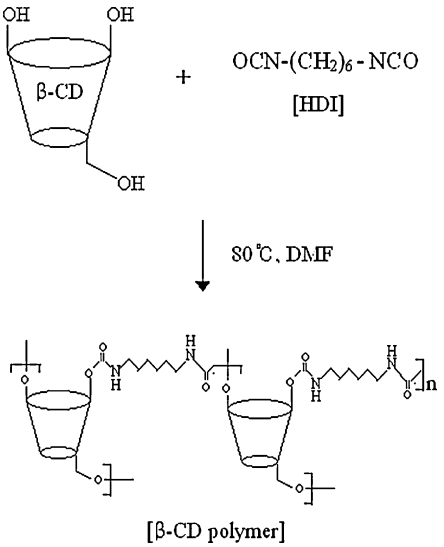

Fig. 1. Synthesis of β-CD polymer using hexamethylene diisocyanate as a cross-linker. The dried β-CD was polymerized using hexamethylene diisocyanate (HDI) as a cross-linker in anhydrous *N*,*N*-dimethyl formamide (DMF) as a solvent at 80 °C for 24 h.

2.5. Measurement of catalytic activities of CGTases

The cyclization activity was measured using the phenolphthalein method [25], and 1 U of CGTase was defined as the amount of enzyme that produced 1 μmol β-CD/min. The coupling activity was determined using the method of Shin et al. [20], and 1 U of activity was defined as the amount of enzyme coupling 1 μmol maltose with soluble starch per min. The hydrolyzing activity was determined by measuring the

reducing sugar liberated from soluble starch using the DNS method [7], and 1 U of activity was defined as the amount of enzyme that produced 1 μmol maltose/min.

2.6. Catalytic reaction of mutant β-CGTases as regards conversion into cyclodextrins

For the conversion reaction of starch, 5.0% (w/v) of soluble starch dissolved in a 50 mM Tris/maleic acid/NaOH buffer (pH 6.0) was reacted with each β-CGTase (0.1 U/ml cyclization activity) at 50 °C for 12 h, then the CDs and maltooligosaccharides were analyzed by HPLC (Gilson Medical Electronics Inc., Villers-le-Bel, Frances); a Cosmosil 5-NH_2 column (Nacalai Tesque Co., Kyoto, Japan), acetonitrile/water (65:35, v/v), 1.0 ml/min, and RI detector.

3. Results and discussion

3.1. Structural characteristics of sugar-binding subsites −3 and −7 in active site of β-CGTases from B. firmus var. alkalophilus

The amino acid sequences of sugar-binding subsites −3 and −7 in the active site of CGTases from various sources were compared according to the type of CGTase as shown in Fig. 2. The CGTase from alkalophilic *B. firmus* var. *alkalophilus* has been classified as β-type II along with the CGTase from *Bacillus* sp. KC 201, alkalophilus *Bacillus* sp. 1.1, and *B. ohbensis* C-1400. Most of the amino acid residues in subsites −3 and −7 in the active site of various CGTases exhibited an almost invariant pattern, however, certain amino acid residues, such as residues 59, 96, 90-QPV-92 and the residues between 154 and 160,

α-CGTase			
KLEPN	$_{72}$NLKKYT ··· $_{105}$PPIENVNNT	KLEPN	$_{172}$ANDEN——E
BMAC2	$_{72}$NLKLYF ··· $_{105}$QPVENITAV	BMAC2	$_{172}$ASSTDPSFAE
TBNOVO	$_{72}$SLKKYF ··· $_{105}$QPVENIYAV	TBNOVO	$_{172}$ASETDPTYGE
TABIUM	$_{72}$SLKKYF ··· $_{105}$QPVENIYAV	TABIUM	$_{172}$ASETDPTYAE
BLICH	$_{79}$NLKLYC ··· $_{112}$QPVENVFSV	BSTEA	$_{178}$AMETDTSFME
β-CGTase I			
B1018	$_{72}$NLRLYC ··· $_{105}$QPVENIYSI	B1018	$_{172}$ASSDDPSFAE
B382	$_{72}$NLRLYC ··· $_{105}$QPVENIYSV	B382	$_{172}$ASSDDPSFAE
BC251	$_{72}$NLRLYC ··· $_{105}$QPVENIYSI	BC251	$_{172}$ASSDQPSFAE
B1011	$_{72}$NLRLYC ··· $_{105}$QPVENIYSV	B1011	$_{172}$ASSDDPSFAE
β-CGTase II			
BCGFA	$_{57}$DLHKYC ··· $_{90}$QPVENVYAL	**BCGFA**	$_{153}$ALETNPNYDF
BKC201	$_{92}$DLHKYC ··· $_{125}$QPVENVYAL	BKC201	$_{188}$ALETNPNYVE
BSP11	$_{92}$DLHKYC ··· $_{125}$QPVENVYAL	BSP11	$_{188}$ALETNPNYVE
BOHB	$_{70}$DLHKYC ··· $_{103}$QPVENVYAL	BOHB	$_{166}$ALETDPSYAE
λ-CGTase			
BF290	$_{53}$DLTKYC ··· $_{86}$PPIENVNEL	BF290	$_{148}$VD————IE

Fig. 2. Sequence alignment of region around sugar-binding subsite −3 and −7 in CGTase from various sources. The mutated amino acid residues in this work are in bold. KLEPN, *K. pneumoniae* M5al [26]; BMAC2, *B. macerans* [27]; TBNOVO, *Thermoanaerobacter* sp. ATCC53.627 [13]; TABIUM, *T. thermosulfurigenes* EM1 [28]; BLICH, *B. licheniformis* [29]; B1018, *B.* sp. B1018 [30]; B382, Alkalophilus *B.* sp. 38.2 [31]; BC251, *B. circulans* 251 [4]; B1011, Alkalophilus *B.* sp. 1011 [32]; BCGFA, *B. firmus* var. *alkalophilus* [this study]; BKC201, *Bacillus* sp. KC201 [33]; BSP11, Alkalophilus *B.* sp. 1.1 [34]; BOHB, *B. ohbensis* C-1400 [35]; BF290, *B. firmus*/*lentus* 290-3 [36].

varied distinctly according to the type of CGTase. CD product specificity among α-, β-, and γ-CGTases seems to be closely connected with this amino acid sequence in subsites −3 and −7.

3.2. Construction of mutant β-CGTases and their production after transformation into recombinant E. coli

The amino acid residues 56, 96, and 90-QPV-92 at subsite −3, and seven amino acid residues 154-LET-NPNY-160 at subsite −7 in alkalophilic *B. firmus* var. *alkalophilus* were modified through site-directed mutagenesis to improve the catalytic functions of the CGTase, including the productivity and size specificity of CDs. Four mutant genes, H59Q replacing His59 with a short side chain yet strongly (+) charged Gln, Y96M replacing Tyr96 with a non-aromatic Met, 90-PPI-92 replacing 90-QPV-92 with a PPI at subsite −3, and Δ(154-160) deleting seven amino acid residues at subsite −7, were constructed by site-directed mutagenesis as illustrated in Fig. 3.

The constructed mutant β-CGTase genes were ligated with the secretive expression vector pET20b(+) and transformed into *E. coli* BL21(DE3)pLysS. The recombinants *E. coli* harboring the mutant β-CGTase genes were cultivated in a TB medium to overproduce the mutant β-CGTases extracellularly. As shown in Table 1, the volumetric and specific β-CGTase activities substantially increased in the recombinants *E. coli* harboring the Y96M and Δ(154–160) mutant genes compared to the wild-type. Meanwhile, the β-CGTase activities in the H59Q and 90-PPI-92

Table 1
Production of wild-type and mutant β-CGTases of *B. firmus* var. *alkalophilus* using recombinant *E. coli* having secretive expression system

β-CGTase	Cell growth (A_{600nm})	Volumetric cyclization activity (U/ml)	Specific cyclization activity (U/mg)
Wild-type	3.2	7.5	20.8
H59Q	3.0	5.2	13.9
Y96M	3.6	9.5	29.0
90-PPI-92	3.1	3.0	10.5
Δ(154–160)	3.5	9.1	29.2

Recombinants *E. coli* were cultivated in TB medium at 37 °C after inducing IPTG for 24 h.

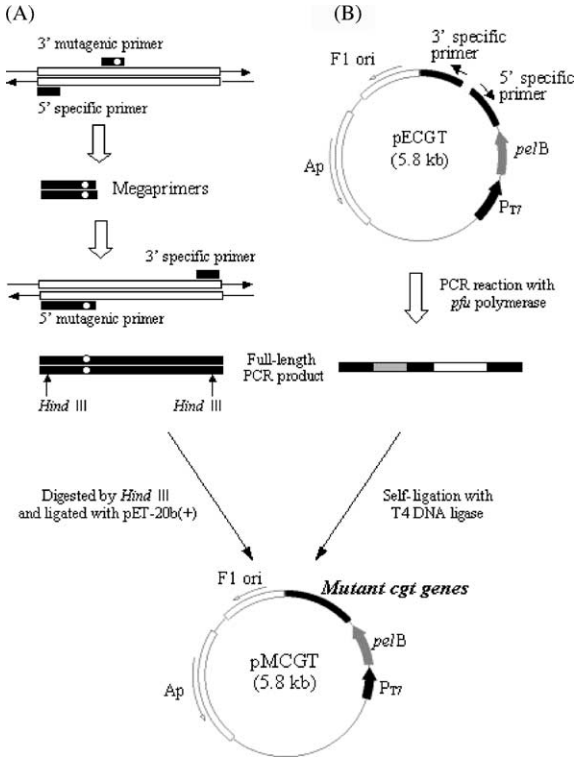

Fig. 3. Site-directed mutagenesis of subsite −3 and −7 in β-CGTase of alkalophilic *B. firmus* var. *alkalophilus* and construction of recombinant plasmids containing mutated gene for secretive production of mutant enzymes. (A) H59Q, Y96M, and 90-PPI-92; (B) Δ(154–160).

mutants were reduced significantly, implying that the catalytic function was effectively modified through site-directed mutagenesis of subsites −3 and −7 in the active site of β-CGTase.

3.3. One-step affinity chromatographic purification of mutant β-CGTases using β-CD polymer

The extracellular mutant β-CGTases were purified by one-step affinity adsorption/desorption chromatography using a β-CD polymer as the adsorption matrix. A nearly homogeneous β-CGTases was obtained after simply one-step purification in SDS-PAGE as shown in Fig. 4.

The purification fold of wild-type β-CGTase was increased 10-fold with a yield of 56.4%, additionally, the mutant β-CGTases were also purified to a similar degree, thereby confirming the effectiveness

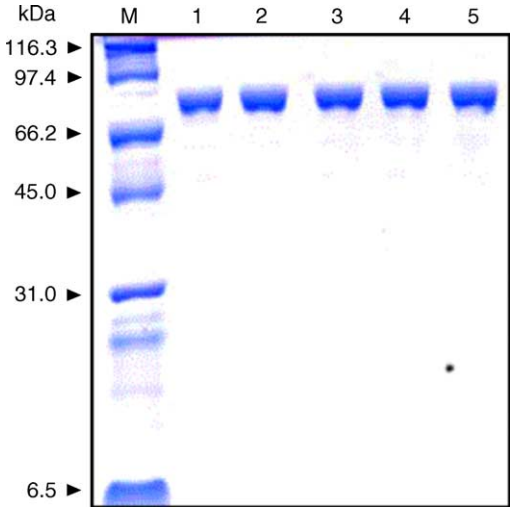

Fig. 4. SDS-PAGE of purified wild-type and mutant β-CGTases. SDS-PAGE was carried out on 10% gels, and protein bands were visualized by staining with Comassie Brilliant Blue R-250. Lane M: M_w protein marker; lane 1: wild β-CGTase; lane 2: H59Q mutant β-CGTase; lane 3: Y96M mutant β-CGTase; lane 4: 90-PPI-92 mutant β-CGTase; lane 5: Δ(154–160) mutant β-CGTase.

of using a β-CD polymer to purify β-type CGTases obtained from various sources, compared to other purification methods, such as, starch adsorption, ion-exchange chromatography, and gel filtration, especially as regards its simplicity and high-purification yield (Table 2).

3.4. CD product specificity in mutant β-CGTases from B. firmus var. alkalophilus

The profile of the α-, β-, and γ-CD and maltooligosaccharides was analyzed after reaction with soluble starch as the substrate to investigate the effect of the site-directed mutagenesis of subsites −3 and −7 in the active site on the CD product specificities. The distribution of CDs did not change appreciably as shown in Fig. 5. β-CD was mostly produced without any significant accumulation of α-CD similar with wild-type, however, the maltooligosaccharide accumulation was significantly reduced after the mutagenesis.

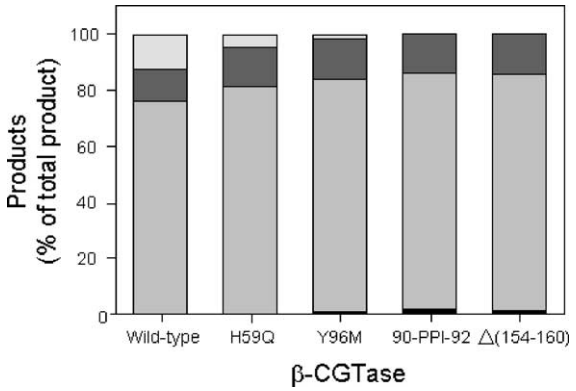

Fig. 5. Product specificity of wild-type and mutant β-CGTases from *B. firmus* var. *alkalophilus*. The wild-type and mutant β-CGTases (0.1 U/ml) were reacted with 5.0% (w/v) of soluble starch at 50 °C for 24 h. (■) α-CD; (▒) β-CD; (▓) γ-CD; (□) maltooligosaccharides.

It has been reported that CD product specificity of the CGTase can be modified after mutagenesis of the sugar-binding subsites −3 and −7 in the CGTase, such as an increment of α-CD using Y89D mutant from *B. circulans* strain 251 [15], enhanced γ-CD production through the modification of Tyr 195 in the *B. circulans* strain 8 [5]. Conversely with other works, the current result shows that the mutagenesis of subsites −3 and

Table 2
Affinity purification of wild-type and mutant β-CGTases from *B. firmus* var. *alkalophilus*

β-CGTase	Activity of supernatant		Activity of purified protein		Yield (%)	Purification fold
	Specific (U/mg)	Total (U)	Specific (U/mg)	Total (U)		
Wild-type	23	7560	226	4262	56	10
H59Q	14	5050	142	3061	61	10
Y96M	29	9620	332	5362	56	11
90-PPI-92	11	3150	102	1634	52	10
Δ(154–160)	30	9120	342	5062	56	11

One-step affinity chromatography using β-CD polymer as adsorbent. Yield* = (total activity of purified fraction) × 100/(total activity of supernatant). One liter supernatant was used for protein purification.

Table 3
Specific activities for wild-type and mutant β-CGTases from *B. firmus* var. alkalophilus

β-CGTase	Specific enzyme activity (U/mg of protein)		
	Cyclization activity	Coupling activity	Hydrolyzing activity
Wild-type	226 ± 4	39 ± 2.1	1940 ± 22
H59Q	142 ± 6	27 ± 1.4	1590 ± 31
Y96M	332 ± 7	39 ± 1.8	2050 ± 26
90-PPI-92	102 ± 3	26 ± 1.5	1320 ± 20
Δ(154–160)	342 ± 6	30 ± 2.4	1975 ± 29

−7 in the β-CGTase from *B. firmus* var. *alkalophilus* do not influenced on the CD product specificity, but rather influences on the hydrolytic activity of CGTase producing maltooligosaccharide reducibly.

3.5. Comparison of cyclization, couplings and hydrolysis activities of mutant β-CGTases

Table 3 compares activities of three catalytic reactions, cyclization, coupling, and hydrolysis reaction of the mutant β-CGTases. The Y96M mutant β-CGTase exhibited 1.5 times higher cyclization activity compared to wild-type, however, its hydrolysis and coupling activities were remained constant without any significant changes. This implies that Y96 residue at subsite −3 is playing an important role in the cyclization reaction of CGTase. Meanwhile, the H59Q and 90-PPI-92 mutant β-CGTases did not influence on the cyclization activity, yet decreased hydrolysis and coupling activities significantly. This indicates that these residues is involved in the coupling reaction and hydrolysis reaction of maltooligosaccharides or CDs.

Meanwhile, the Δ(154–160) β-CGTase mutated at subsite −7 exhibited the highest cyclization activity compared to the wild-type and mutant strains at subsite −3, and its coupling activity was significantly reduced. This implies that the amino acid residues between 154 and 160 located at subsite −7 are the determinative sites for cyclization and coupling reactions of β-CGTase from *B. firmus* var. *alkalophilus*.

3.6. Utilization of mutant CGTases for CD production

The mutant β-CGTases were used for CD production reaction using soluble starch as the substrate and the produced CDs and maltooligosaccharides were analyzed as shown in Fig. 6. α-, β-, and γ-CD CD product specificity was not changed recognizably after site-directed mutagenesis of subsites −3 and −7 in active site of β-CGTases from *B. firmus* var. *alkalophilus*. The β-CD content steadily increased occupying around 85–87%, the γ-CD occupying 14%, and a trace amount of maltooligosaccharides.

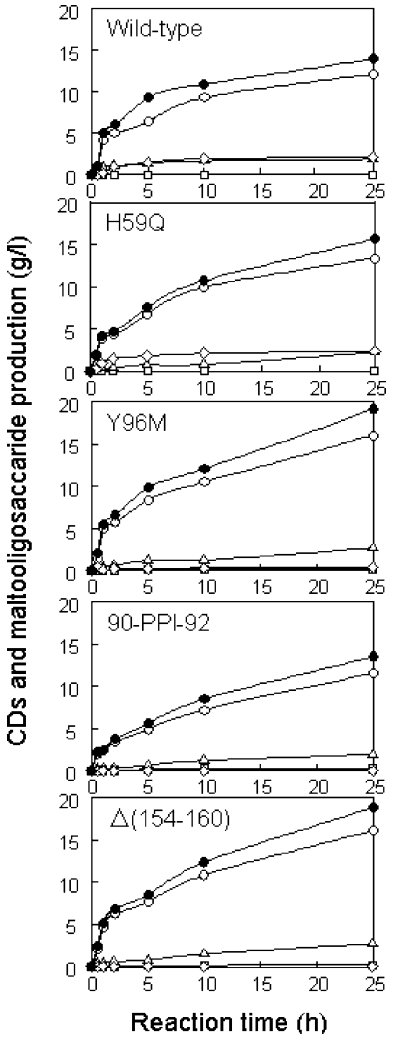

Fig. 6. Conversion yield of starch to CDs and maltooligosaccharides by wild-type and mutant β-CGTases from *B. firmus* var. *alkalophilus*. The wild-type and mutant β-CGTases (0.1 U/ml) were reacted with 5.0% (w/v) of soluble starch at 50 °C for 24 h. (□) α-CD; (○) β-CD; (△) γ-CD; (●) total CDs; (◇) maltooligosaccharides.

Meanwhile, the CD production proceeded more rapidly at Y96M mutated at subsite −3 and Δ(154–160) mutated at subsite −7 from the initial stage, and increased 1.4 times higher compare to the wild-type β-CGTase due to the combination of effects, the activated cyclization reaction and decreased coupling and/or hydrolyzing reaction as be previously observed.

3.7. Variation of pH-dependent activity and thermostability of mutant β-CGTases

The optimal pH of β-CGTases shifted slightly to the alkaline side after the mutagenesis, the most particularly at Y96M as shown in Fig. 7. The pH stability of Δ(154–160) decreased drastically in the acidic pH region. The thermal stability tended to decreased after mutagenesis as shown in Fig. 7, and unstable at over than 60 °C.

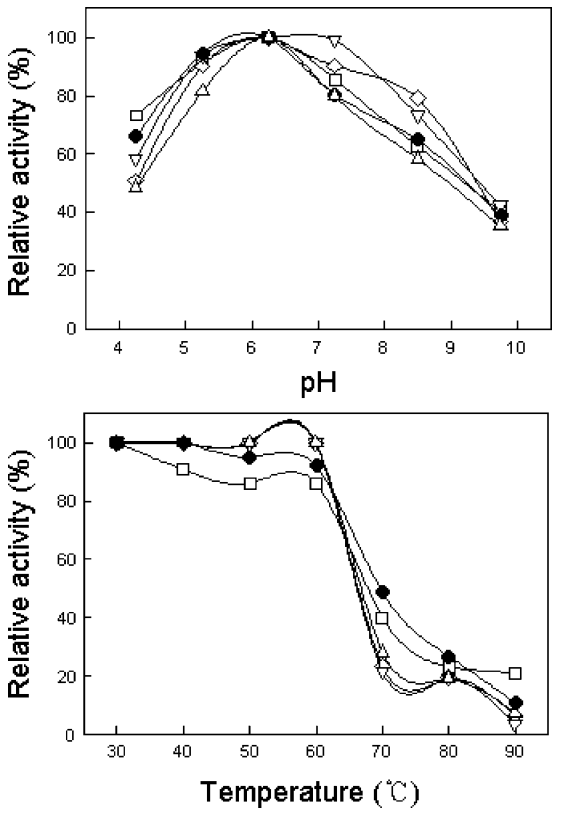

Fig. 7. Comparison of pH-dependent CGTase activity (A) and thermostability (B) of the wild-type and mutant β-CGTases. The pH-dependent cyclization activities were measured after reaction at 50 °C for 30 min using buffer solutions, and thermostability was measured after reaction at the indicated temperature for 10 min. (●) wild-type β-CGTase; (◇) H59Q β-CGTase; (▽) Y96M β-CGTase; (□) 90-PPI-92 β-CGTase; (△) Δ(154–160) β-CGTase.

Acknowledgements

This work was supported by a program of the 21C Frontier Microbial Genomics and Application Center (MG 02-0101-002-2-1-0), the Ministry of Science and Technology, the Republic of Korea, and by the Brain Korea 21 Project in 2003.

References

[1] H. Bender, Carbohydr. Res. 78 (1980) 147.
[2] G. Schmid, Trends Biotechnol. 7 (1988) 244.
[3] S. Janecek, FEBS Lett. 353 (1994) 119.
[4] C.L. Lawson, R. van Montfort, B. Strokopytov, H.J. Rozeboom, K.H. Kalk, G.E. de Vries, D. Penniga, L. Dijkhuizen, B.W. Dijkstra, J. Mol. Biol. 236 (1994) 590.
[5] G. Parsiegla, A.K. Schmidt, G.E. Schulz, Eur. J. Biochem. 255 (1998) 710.
[6] J. Hellman, M. Wahberg, M. Karp, T. Korpela, P. Mantsala, Biotechnol. Appl. Biochem. 12 (1990) 387.
[7] R.D. Wind, R.M. Buitelaar, L. Dijkuhizen, Eur. J. Biochem. 253 (1998) 598.
[8] H. Bender, Carbohydr. Res. 206 (1990) 257.
[9] B. Strokopytov, R.M.A. Knegtel, D. Penniga, H.J. Rozeboom, K.H. Kalk, L. Dijkhuizen, B.W. Dijkstra, Biochemistry 35 (1996) 4241.
[10] J.C.M. Uitdehaag, G.J.W.M. van Alebeek, B.A. van der Veen, L. Dijkhuizen, B.W. Dijkstra, Biochemistry 39 (2000) 7772.
[11] B.A. van der Veen, H. Leemhuis, S. Kralj, J.C.M. Uitdehaag, B.W. Dijkstra, L. Dijkhuizen, J. Biol. Chem. 276 (2001) 44557.
[12] J.C.M. Uitdehaag, K.H. Kalk, B.A. van der Veen, L. Dijkhuizen, B.W. Dijkstra, J. Biol. Chem. 274 (1999) 34868.
[13] B.A. van der Veen, J.C.M. Uitdehaag, B.W. Dijkstra, L. Dijkhuizen, Biochim. Biophys. Acta 1543 (2000) 336.
[14] H. Leemhuis, J.C.M. Uitdehaag, H.J. Rozeboom, B.W. Dijkstra, L. Dijkhuizen, J. Biol. Chem. 277 (2002) 1113.
[15] B.A. van der Veen, J.C.M. Uitdehaag, D. Penninga, G.J.W.M. van Alebeek, L.M. Smith, B.W. Dijkstra, L. Dijkhuizen, J. Mol. Biol. 296 (2000) 1027.
[16] B.A. van der Veen, J.C.M. Uitdehaag, B.W. Dijkstra, L. Dijkhuizen, Eur. J. Biochem. 267 (2000) 3432.
[17] E.J. Do, J.B. Park, Y.H. Lee, Kor. J. Appl. Microbiol. Biotechnol. 21 (1993) 119.
[18] H.D. Shin, C. Kim, Y.H. Lee, Kor. J. Appl. Microbiol. Biotechnol. 26 (1998) 323.
[19] T.H. Park, H.D. Shin, Y.H. Lee, J. Microbiol. Biotechnol. 9 (1999) 811.

[20] H.D. Shin, T.H. Park, Y.H. Lee, Biotechnol. Lett. 22 (2000) 115.
[21] K.W. Lee, H.D. Shin, Y.H. Lee, J. Microbiol. Biotechnol. 12 (2002) 753.
[22] H.G. Griffin, A.M. Griffin, PCR Technology–Current Innovations, CRC Press, 1994, p. 5.
[23] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Habor Laboratory, Cold Spring Habor, 1989.
[24] K.P. Lee, S.H. Choi, E.N. Ryo, J.J. Ryo, J.H. Park, Y.S. Kim, M.H. Hyun, Anal. Sci. 18 (2002) 31.
[25] T. Kaneko, T. Kato, N. Nakamura, K. Horikoshi, Jpn. Soc. Starch Sci. 34 (1988) 45.
[26] F. Binder, O. Huber, A. Böck, Gene 47 (1986) 269.
[27] S. Sakai, M. Kubota, K. Yamamoto, T. Nakada, K. Torigoe, O. Ando, T. Sugimoto, J. Jpn. Soc. Starch Sci. 34 (1987) 140.
[28] R.D. Wind, W. Libel, R.M. Buitelaar, D. Penninga, A. Spreinat, L. Dijkhuizen, H. Bahl, Appl. Environ. Mirobiol. 61 (1994) 1257.
[29] D. Hill, R. Aldape, J. Rozzell, Nucleic Acids Res. 18 (1990) 199.
[30] P. Itkor, N. Tsukagoshi, S. Udaka, Biochem. Biophys. Res. Commun. 166 (1990) 630.
[31] T. Hammamoto, T. Kaneko, K. Horikoshi, Agric. Biol. Chem. 51 (1987) 2019.
[32] K. Kimura, S. Kataoka, Y. Ishii, T. Takano, K. Yamane, J. Bacteriol. 169 (1987) 4399.
[33] N. Kitamoto, T. Kimura, Y. Kito, K. Ohmiya, J. Ferment. Bioeng. 74 (1992) 345.
[34] G. Schmid, A. Englbrecht, D. Schmid, in: O. Huber, J. Szejtli (Eds.), Proceedings of the 4th International Symposium on Cyclodextrins, Kluwer Academic Publishers, Dordrecht, 1988, p. 81.
[35] K.A. Sin, A. Nakamura, K. Kobayashi, H. Masaki, T. Uozumi, Appl. Microbiol. Biotechnol. 35 (1991) 600.
[36] A. Englbrecht, G. Harrer, M. Lebert, G. Schmid, in: O. Huber, J. Szejtli (Eds.), Proceedings of the 4th International Symposium on Cyclodextrins, Kluwer Academic Publishers, Drodecht, 1988, p. 87.