Construction of Chimeric Enzymes out of Maize Endosperm Branching Enzymes I and II:

ACTIVITY AND PROPERTIES

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Branching enzyme I and II isoforms from maize endosperm (mBE I and mBE II, respectively) have quite different properties, and to elucidate the domain(s) that determines the differences, chimeric genes consisting of part mBE I and part mBE II were constructed. When expressed under the control of the T7 promoter in Escherichia coli, several of the chimeric enzymes were inactive. The only fully active chimeric enzyme was mBE II-I BspHI, in which the carboxyl-terminal part of mBE II was exchanged for that of mBE I at a BspHI restriction site and was purified to homogeneity and characterized. Another chimeric enzyme, mBE I-II HindIII, in which the amino-terminal end of mBE II was replaced with that of mBE I, had very little activity and was only partially characterized. The purified mBE II-I BspHI exhibited higher activity than wild-type mBE I and mBE II when assayed by the phosphorylase α stimulation assay. mBE II-I BspHI had substrate specificity (preference for amylopectin rather than amylopeptin) and catalytic capacity similar to mBE I, despite the fact that only the carboxyl terminus was from mBE I, suggesting that the carboxyl terminus may be involved in determining substrate specificity and catalytic capacity. In chain transfer experiments, mBE II-I BspHI transferred more short chains (with a degree of polymerization of around 6) in a fashion similar to mBE II. In contrast, mBE I-II HindIII transferred more long chains (with a degree of polymerization of around 11–12), similar to mBE I, suggesting that the amino terminus of mBEs may play a role in the size of oligosaccharide chain transferred. This study challenges the notion that the catalytic centers for branching enzymes are exclusively located in the central portion of the enzyme; it suggests instead that the amino and carboxyl termini may also be involved in determining substrate preference, catalytic capacity, and chain length transfer.

Starch branching enzyme (BE); 1,4-α-D-glucan:1,4-α-D-glucan 6-α-(1,4-α-D-glucan)-transferase; EC 2.4.1.18) catalyzes the cleavage of an α-1,4-glucosidic bond and the subsequent transfer of α-1,4-glucan to form an α-1,6 branch point. The enzyme plays an important role in starch synthesis (1–3). Multiple forms of BE have been identified in many plants, including maize endosperm (4), pea seed (5), and rice endosperm (6–7). The cDNAs encoding the genes for the BEs have been cloned from various sources, such as maize endosperm (8, 9), pea seed (10), potato tuber (11, 12), and rice endosperm (6, 13). The cDNAs encoding mature mBE I and mBE II have been expressed in Escherichia coli using the T7 promoter (14, 15), allowing the study of the structure-function relationships of mBEs using site-directed mutagenesis and the construction of chimeric enzymes of mBE I and mBE II.

Homology in the primary structures between glycogen branching enzyme and amylolytic enzymes was first reported by Romeo et al. (16). Subsequently, Baba et al. (8) established that BEs contain the four highly conserved regions in the central portion of the enzyme that are present in α-amylases, pullulanase, isoamylase, and cyclodextrin glucanotransferases. Neopullulanase catalyzes the hydrolysis of α-1,4- and α-1,6-glucosidic linkages, as well as transglycosylation to form α-1,4- and α-1,6-glucosidic linkages (17, 18). The introduction of several replacements of the amino residues that constitute the active center of neopullulanase indicated that one active center of the enzyme participated in all four reactions described above (19). This suggested that not only were the structures of BE, α-amylase, pullulanase/isoamylase, and cyclodextrin glucanotransferase similar but also that they shared common catalytic mechanisms (18, 20). Based on these results, the α-amylase enzyme family was defined; it includes BE and other enzymes that catalyze hydrolysis and transglycosylation at α-1,4- and α-1,6-glucosidic linkages (21–25). Structure prediction and hydrophobic cluster analysis of the enzymes mentioned above indicated that they possess a catalytic (β/α)8-barrel (23, 26) like that seen in crystal structure of α-amylases (27–29) and cyclodextrin glucanotransferases (30–31). It is currently considered that four of the β-strands (i.e. the four highly conserved regions in the central portion of the enzymes) make up the catalytic center (23).

Some of the amino acids found to be essential for catalytic activity of the α-amylase family enzymes and also present in the four conserved regions of the maize endosperm branching enzymes were mutated via site-directed mutagenesis in mBE II and were found to be important for branching enzyme activity (32, 33). Thus, the conserved four active site regions found in the α-amylase family enzymes also play a role in branching enzyme catalysis.

The amino acid sequence of mBE I and mBE II has an identity of 58% (Fig. 1). The identity is higher (67%) in the center portion of the enzymes, which contains several highly conserved regions (regions 1–4), as previously mentioned (8, 15, 21, 23). When amino acid residues with similar functional side chains are taken into consideration, the two enzymes are 75% similar for the total amino acid sequence and 94% similar
It is therefore interesting to focus attention on the relationship between the enzyme specificities and the different structures in the amino terminus and carboxyl terminus. In this paper, we report on the construction and characterization of chimeric enzymes of mBE I and mBE II.

MATERIALS AND METHODS

Media—LB medium (pH 7), consisting of 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter, was used for culture of E. coli. 2 × YT broth (pH 7), consisting of 16 g of tryptone, 10 g of yeast extract, and 5 g of NaCl per liter, was used for the preparation of phage DNA. Ampicillin was used at final concentration of 100 μg/ml.

Bacterial Strains, Plasmid, and Phage—E. coli TG-1 (speA35 thi Δlac-proAB) F (traD36 proAB lacI97ΔlacZΔM15) (42) was used as a host for site-directed mutagenesis and DNA manipulation. E. coli DH5 (supE44 ΔhsdR17 recA1 endA1 gyrA96 thi-1 relA1) (42) and E. coli JM110 (rapL Str*)thr leu thi-1 lacY galK galT araON5 tax dam dcm 787人流UV5-T7 gene 1 (Novagen, Madison, WI) was used to express the gene for wild-type or chimeric BE under control of the T7 promoter (14, 15). Plasmids pET-23b-MBE I (Ap encoding the gene for mature mBE) and pET-23b-mBE II (Ap encoding the gene for mature mBE) were described previously (14, 15). M13mp19 was used for the preparation of single-stranded DNA (34).

Creation of New Restriction Endonuclease Sites—Site-directed mutagenesis (Sculptor in vitro mutagenesis system, Amersham Corp., Amersham, UK) was used for the creation of a new restriction site by the introduction of a silent mutation in the mBE I and II genes. Oligonucleotides were synthesized in an Applied Biosystems model 380A DNA synthesizer (Macromolecular Facility, Department of Biochemistry, Michigan State University, MI). The mutation was confirmed by restriction endonuclease digestion and DNA sequencing. DNA sequencing was done by the dyeoxy chain-terminating method (35); the sequence reaction was started from the M13 linker region with the universal primer or primed by internal annealing of a 17-mer synthetic oligonucleotide.

Construction of mBE I-II BspHI, a Chimeric Gene of mBE I and II—To introduce a BspHI site into the mBE I gene by site-directed mutagenesis (silent mutation), the 1156-bp EcoRI-HindIII fragment from pET-23b-MBE I was cloned into the EcoRI-HindIII sites of phage M13mp19 multiple-cloning sites. Single-stranded DNA was prepared from the phage and used as template for the site-directed mutagenesis reaction. Sequence analysis of the 1156-bp EcoRI-HindIII fragment verified that the desired nucleotide had been changed and that no second-site mutations were present. After preparation of the replicative form of the phage DNA, the plasmid pET-23b-mBE I was exchanged for the mutant fragment, resulting in the construction of pET-23b-mBE I (BspHI). The following additional steps were used to avoid methylation of adenine residues by DNA adenine methylase in the recognition sequence 5′-GATC-3′ (asterisk indicates methylated form) when the BspHI site was used for the construction of the chimeric gene. pET-23b-mBE I (BspHI) was constructed using E. coli TG-1 (rk mk) as a host. E. coli DH5 (rk mk) was transformed with pET-23b-mBE I (BspHI) for plasmid preparation from E. coli DH5. The plasmids obtained were then used for transformation of E. coli JM110 (dam rk) for BspHI sites on the plasmid DNAs prepared from E. coli JM110 were not affected by DNA adenine methylation, pET-23b-mBE I (BspHI) prepared from E. coli JM110 was used for construction of the chimeric gene at the BspHI site.

For the construction of the mBE II II-BspHI gene, the 687-base pair BspHI-carboxyl-terminal fragment of pET-23b-mBE II was exchanged for the 852-base pair BspHI-carboxyl-terminal fragment of pET-23b-mBE I (BspHI). Thus, pET-23b-mBE II-BspHI, encoding the mBE II-BspHI gene, was constructed (Fig. 2). The structure of the plasmid was confirmed by restriction endonuclease mapping.

Several other chimeric enzymes, mBE I-I-Nol I, mBE I-I-Nol II, mBE I-II-HindIII, mBE I-II-BspHI, mBE II-I, and mBE II-II, were also constructed (Fig. 2). To construct these other chimeric enzymes, two silent mutations were introduced by site-directed mutagenesis to produce HindIII and Nol endonuclease restriction sites in mBE I and II, respectively. The procedures used were similar to those described previously.

Preparation and Purification of Wild-type mBE I, mBE II, and Chimeric BEs—E. coli BL21(DE3) carrying a recombinant plasmid encoding the chimeric enzymes I (8) and II (15). The probable secondary structures (23) and endonuclease restriction sites used in the construction of the chimeric enzymes (introduced sites are shown in parentheses) are indicated. The double underlined regions (regions 1–4), which constitute the putative catalytic sites of amylolytic enzymes (27, 28), are conserved along all of the known branching enzyme amino acid sequences. for the center portion. In contrast, the amino acid sequences and the numbers of amino acid residues are quite different for the amino-terminal and carboxyl-terminal sides of the center portion (Figs. 1 and 2).

FIG. 1.  Alignment of the deduced primary structures of maize branching enzymes I (8) and II (15). The probable secondary structures (23) and endonuclease restriction sites used in the construction of the chimeric enzymes (introduced sites are shown in parentheses) are indicated. The double underlined regions (regions 1–4), which constitute the putative catalytic sites of amylolytic enzymes (27, 28), are conserved along all of the known branching enzyme amino acid sequences.
ing the gene for wild-type BE I, wild-type BE II, or chimeric BEs was grown overnight in LB medium containing 100 \( \mu \)g/ml ampicillin. The preculture was then diluted 1:20 (v/v) in fresh LB medium containing 100 \( \mu \)g/ml ampicillin, and the cells grown at 37 °C to mid-log phase (\( A_{600 \text{nm}} \) = 0.6). At this point, expression of the BE gene was induced by addition of isopropyl-\( \beta \)-thio-galactopyranoside (final concentration, 0.5 \( \mu \)M), and were cultures transferred to 25 °C for 12 h. Cells were then harvested by centrifugation (10,000 \( \times \) g for 10 min), and the pellet was resuspended and lysed by sonication in 50 mM Tris acetate buffer (pH 7.5) containing 10 mM EDTA and 5 mM dithiothreitol. The lysed suspension was then centrifuged at 30,000 \( \times \) g for 15 min, and the resulting supernatant (cell extract) was used as crude enzyme for preliminary assays of BEs. Purification of wild-type BEs and the chimeric enzymes mBE II-I BspHI and mBE I-II HindIII was carried out according to Guan et al. (14, 15), with the exception that mBE I-II HindIII was expressed in AC71 (glgB+), a glycogen BE-deficient strain of E. coli (3).

**Assay of BE Activity**—BE activity was measured by three different assays as described by Guan and Preiss (36).

**Assay a**—The phosphorylase \( a \) stimulation assay is based on the stimulation by BE of the synthesis of 2,4-O-linked glucose from 2,4-O-glucan-1-P catalyzed by rabbit phosphorylase \( a \) (37). Reaction mixtures contained, in a final volume of 200 \( \mu \)l, 100 mM citrate (pH 7), 10 mM AMP, 0.4 mg phosphorylase \( a \), and 50 mM \( \text{Na}^{+} \)-2-[\( \beta \)-D-glucopyranosyl]-Gl-1-P (50 \( \mu \)mol). One unit of enzyme activity is defined as 1 \( \mu \)mol of glucose incorporated into 2,4-O-glucan per min at 30 °C.

**Assay b**—The branching linkage assay determines the number of branching linkages introduced by BE into the substrate, reduced amylose (38). Substrates were prepared by the reduction of enzymatically synthesized amylose (AS-320, AS-110, and AS-70, Nakano Vinegar Co., Aichi, Japan; these amylose varieties had average degrees of polymerization of 1815, 722, and 438, respectively) as described by Takeda et al. (38). Reaction mixtures contained, in a final volume of 100 \( \mu \)l, 25 mM 4-morpholinepropanesulfonic acid (pH 7.5) and an appropriate amount of substrate. The reaction was initiated by the addition of an appropriate amount of enzyme. One unit of enzyme activity is defined as 1 \( \mu \)mol of branching linkages formed per min at 30 °C.

**Assay c**—The iodine stain assay is based on monitoring the decrease in absorbance of the glucan-iodine complex resulting from the branching of the substrate, amylose or amylopectin (potato type III and corn, respectively; Sigma) (4, 36). Reaction mixtures contained, in a final volume of 200 \( \mu \)l, 50 mM citrate (pH 7) and 0.1 mg of substrate. The reaction was initiated by the addition of an appropriate amount of enzyme. One unit of enzyme activity is defined as the decrease in absorbance of 1.0 per min at 30 °C.

**Protein Assay**—Protein concentration was measured by the BCA protein assay reagent (39), using bovine serum albumin as the standard.

**SDS-PAGE and Western Blotting**—SDS-PAGE was performed on 8% polyacrylamide gels according to the method of Laemmli (40). Western blotting (immunoblotting) was carried out according to the method of Burnette (41). The primary rabbit antibodies, anti-mBE I and anti-mBE II, were diluted 1:200 and 1:2000, respectively, in 25 mM KH\(_2\)PO\(_4\) (pH 7.2) containing 150 mM NaCl and 3% gelatin. The antigen-antibody complex was detected using anti-rabbit IgG conjugated with alkaline phosphatase (United States Biochemicals; diluted 1:10,000) with a chromogenic substrate (Boehringer Mannheim GmbH). Molecular weights were determined using Perfect Protein Western markers (Novagen) as standards (M, 15,000, 25,000, 35,000, 50,000, 75,000, 100,000, and 150,000).

**Preparation of Debranched \( \alpha \)-Glucan for Chain Length Distribution Analysis** by High Performance Anion Exchange Chromatography—Reduced amylose (1 mg of AS-320; degree of polymerization, 1815) was incubated in 25 mM 4-morpholinepropanesulfonic acid, pH 7.5 (200 \( \mu \)l) at 30 °C with BE (1.5 milliunits of mBE I, mBE II, mBE II-I BspHI, and mBE I-II HindIII by assay b). After 80, 160, and 320 min, the reaction was terminated by heating in a boiling water bath for 2 min, and M acetate buffer (pH 3.5, 20 \( \mu \)l) and isoamylase (5 \( \mu \)l of 590 units \( \mu \)l\(^{-1} \)) were added after the solution had cooled to room temperature. After 1 h of incubation at 45 °C, the solution was heated in a boiling water bath for 2 min. High performance anion exchange chromatography was performed with a Dionex BioLC system as described previously (3).

**Other Procedures**—Plasmid or M13 replicative form DNA was prepared by either the rapid alkaline extraction method (42) or QIAGEN Plasmid Maxi Kit (QIAGEN Inc., Chatsworth, CA). Treatment of DNA with restriction enzymes and ligation of DNA were done as recommended by the manufacturer. QIAquick Gel Extraction Kit (QIAGEN Inc.) was used for recovery of DNA from agarose. Transformation of E. coli with plasmid DNA and M13 single-stranded template DNA preparation were done as described elsewhere (42).
RESULTS AND DISCUSSION

Activity of Branching Enzyme—Branching enzyme activity for wild-type mBE I and II and chimeric enzymes (Table I) and mBE I and II with silent mutations (BspHI and HindIII for mBE I and NcoI for mBE II) was determined in E. coli BL21(DE3) cell extracts. The silent mutations did not affect enzyme activity (results not shown). mBE II-I BspHI (17 units·mg⁻¹ of protein) exhibited higher activity than wild-type mBE I (5.2 units·mg⁻¹ of protein) and mBE II (11 units·mg⁻¹ of protein) (Table I). The remaining chimeric enzymes were inactive, with the exception of mBE I-II HindIII and mBE II-I HindIII, which had a very small amount of activity (0.7 and 0.6 units·mg⁻¹ protein, respectively) (Table I). To avoid destruction of the secondary structures of the chimeric enzymes, we chose all three endonuclease restriction sites (HindIII, NcoI, and BspHI) that were used for the construction of the chimeric enzyme genes, located on the highly homologous regions on the primary structures of mBE I and II. Furthermore, these sites were not in the areas forming the probable secondary structures on the α-helix or β-strand. It is therefore likely that despite the sequence similarities of mBE I and II, with the exception of mBE II-I BspHI, the chimeric enzymes did not fold in a way that produced wholly functional three-dimensional arrangements.

Cell extracts were analyzed by SDS-PAGE followed by immunoblotting. The presence of immunoreactive proteins with molecular weights the same as those calculated from the amino acid sequences indicated the expression of mBE I and II and chimeric enzymes. An immunoblut of purified mBE I, mBE II, and mBE II-I BspHI is shown in Fig. 3.

Purification and Characterization of Chimeric Enzymes mBE II-I BspHI and mBE II-I HindIII—mBE II-I BspHI was purified to homogeneity, as indicated by a single band in SDS-PAGE, from E. coli BL21(DE3) cell extract as described previously (Ref. 15; Table II). mBE I-II HindIII, which had very low activity, was partially purified from E. coli AC71 (glgB⁻) cell extract according to the method of Guan et al. (Ref. 15; Table III). Three bands of approximately equal intensity were observed when the sample was subject to SDS-PAGE (not shown).

One of the protein bands had a molecular weight and immunoreactivity consistent with mBE I-II HindIII. Due to the low activity mBE I-II HindIII, a thorough characterization could not be carried out, although useful information was gained on chain length transfer.

The optimum temperature for catalysis by mBE II-I BspHI was 25 °C, intermediate between the optimum temperatures of mBE I (30 °C) and mBE II (20 °C). Although the change of the carboxyl terminus had some effect on the characteristics of the enzyme, there does not appear to be a specific relationship between the carboxyl terminus of mBES and the optimum temperature for catalysis.

The specific activity of mBE II-I BspHI measured using assay a, the phosphorylation a stimulation assay, was 3880 units·mg⁻¹ of protein, which is 3–4 times higher than wild-type mBE I (1196 units·mg⁻¹ of protein) and mBE II (1017 units·mg⁻¹ of protein) (Ref. 36; Table IV). The partially purified mBE II-I HindIII extract had a specific activity of 3.3 units·mg⁻¹ of protein, over 2 orders of magnitude lower than wild-type mBE. Using assay b, the branching linkage assay, the specific activity of mBE II-I BspHI with different reduced amylases as substrates (AS-320, AS-110, and AS-70) at a concentration of 100 μM was 1.3, 0.89, and 0.46 units·mg⁻¹ of protein, respectively (Table IV). This value is similar to that of mBE I, at 2.1, 1.3, and 0.32 units·mg⁻¹ of protein, respectively, but approximately 1 order of magnitude higher than that obtained from mBE II (0.4, 0.2, and 0.028 units·mg⁻¹ of protein, respectively; Table IV). These results confirm that mBE I has a

TABLE I
Specific activity of maize endosperm branching enzyme isoforms and chimeric enzymes in E. coli pET-23d-BL21(DE3) cell extracts

| Enzyme activity | Branching enzyme isoform | Activity | Specific activity |
|-----------------|--------------------------|----------|-----------------|
|                 | BL21(DE3) with pET-23d only | 0.32     | 0.32 units·mg⁻¹ of protein |
|                 | mBE I                    | 5.5      | 5.5 units·mg⁻¹ of protein |
|                 | mBE II                   | 11       | 11 units·mg⁻¹ of protein |
|                 | mBE I-II BspHI           | 0        | 0 units·mg⁻¹ of protein |
|                 | mBE II-I HindIII         | 0.7      | 0.7 units·mg⁻¹ of protein |
|                 | mBE II-I HindIII         | 0.6      | 0.6 units·mg⁻¹ of protein |
|                 | mBE I-II NcoI            | 0        | 0 units·mg⁻¹ of protein |
|                 | mBE II-II NcoI           | 0        | 0 units·mg⁻¹ of protein |
|                 | mBE II-I                 | 0        | 0 units·mg⁻¹ of protein |
|                 | mBE II-II                | 0        | 0 units·mg⁻¹ of protein |

Fig. 3. Western blot analysis of pure mBE I, mBE II, and mBE II-I BspHI. Anti-mBE I contained mBE I (0.5 μg) (lane 1), mBE II (0.5 μg) (lane 2), and mBE II-I BspHI (0.5 μg) (lane 3). Anti-mBE II contained mBE I (0.1 μg) (lane 1), mBE II (0.1 μg) (lane 2), and mBE II-I BspHI (0.1 μg) (lane 3). The lane labeled Std. contained molecular weight standards (Novagen). The primary antibodies used were anti-mBE I (1:200) and anti-mBE II (1:2000). Goat anti-rabbit immunoglobulin conjugated with alkaline phosphatase (1:10,000) was used as the secondary antibody.

TABLE II
Purification of maize endosperm chimeric branching enzyme II-I BspHI expressed in E. coli BL21(DE3)

| Purification step | Volume | Protein | Activity | Specific activity | Yield |
|------------------|--------|---------|----------|------------------|-------|
|                   | ml     | mg      | units    | units·mg⁻¹ protein | %     |
| Homogenate        | 170    | 1990    | 36440    | 18.3             | 100   |
| Supernatant       | 161    | 1470    | 38010    | 24.5             | 99    |
| Amino-octyl agarose | 70     | 407     | 39940    | 76.0             | 85    |
| Amino-octyl agarose | 12     | 3.2     | 4000     | 1250             | 11    |
| Mono Q H/R 5/5    | 0.48   | 0.58    | 2239     | 3880             | 6     |
higher rate in branching amylose than mBE II (36), and indicate that mBE II-I BspHI has a substrate specificity similar to mBE I for amylose. The mBE I-II HindIII chimeric enzyme preparation had insufficient activity for use in assay b. Using assay c, the iodine stain assay, mBE I and mBE II-I BspHI had higher activity with amylose (90 and 69 units·mg⁻¹ of protein, respectively) than with amylopectin (2.3 units·mg⁻¹ of protein), giving a high amylose to amylopectin activity ratio (Table IV). In contrast, mBE II was more active with amylopectin (97 units·mg⁻¹ of protein) than with amylose (6.4 units·mg⁻¹ of protein), giving a lower amylose to amylopectin activity ratio (Table IV). There was insufficient mBE I-II HindIII activity to use for assay c. The ratios of mBE I and mBE II activity with amylose as a substrate to that with amylopectin as a substrate are similar to those previously reported (36). This result provides further evidence that mBE I and mBE II-I BspHI have similar substrate specificity for amylose, as well as for amylopectin. Because the carboxyl terminus of mBE II-I BspHI is from mBE I, it is possible that in addition to the four highly conserved regions in the central portion of mBEs, the carboxyl terminus may also be involved in substrate binding.

Using assay b with unbranched amylose (AS-320) as a substrate, the $K_m$ values obtained for mBE I, II, and II-I BspHI were quite similar, although the $V_{max}$ for mBE I and II-I BspHI (3.3 ± 0.4 and 2.7 ± 0.3 units·mg⁻¹ of protein, respectively) were higher than the value obtained for mBE II (0.62 ± 0.02 units·mg⁻¹ of protein; Table V). This indicates that the catalytic capacity of mBE II-I BspHI is similar to that of mBE I, suggesting that the carboxyl terminus of BEs may also play a role in determining the catalytic efficiency of the enzymes.

Chain length distribution of debranched products was analyzed by high performance anion exchange chromatography following the incubation of reduced amylose (AS-320) with mBE I, mBE II, or mBE II-I BspHI for 80, 160, and 320 min at 30 °C, with a subsequent incubation with iso-amylase for 1 h at 45 °C, suggesting that the carboxyl terminus of BEs may also play a role in determining the catalytic efficiency of the enzymes.
sitting of 7 glucose units and few chains longer than 30 glucose units (Fig. 4). The transfer of chains of 36 glucose units or longer was not detected under the conditions employed. For mBE II the ratio of short to long chains transferred did not change markedly over the time monitored (Fig. 4). mBE I-II BspHI transferred chains in a fashion similar to mBE II, since very few chains over 30 glucose units were detected, and there was no shift over time toward transferring shorter chains. Six glucose units was the most common length transferred. mBE I-II as for mBE II (Fig. 4); more mid-sized (degree of polymerization) chains. Six glucose units was the most common length transferred initially, but with time, many more chains with a degree of polymerization of 11–12 were transferred (Fig. 4). Thus, the amino termini of mBEs appear to influence the length of the α-D-glucan chains transferred.

The construction and characterization of a chimeric enzyme, mBE II-I BspHI, which consisted of the carboxyl terminus of mBE I and the amino terminus and central portion of mBE II (Fig. 2), which had substrate specificity and catalytic capacity, and chain transfer of the isoforms may be due to conformational changes in the central region caused by the carboxyl-terminal and amino-terminal regions or that one or more amino acid residues essential for activity are present in the terminal region(s) of mBEs.

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