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SEQUENCE CONSERVATION OF THE CATALYTIC REGIONS OF AMYLOLYTIC ENZYMES IN MAIZE BRANCHING ENZYME-I

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We have identified cDNA clones encoding branching enzyme-I (BE-I) from a maize kernel cDNA library. The combined nucleotide sequence of the cDNAs indicates that maize BE-I is initially synthesized as a precursor protein with a putative 64-residue transit peptide at the amino terminus, and that the mature enzyme contains 759 amino acid residues with a calculated molecular mass of 86,236 Da. The four regions, which constitute the catalytic site of amylolytic enzymes, are conserved in the sequences of BE-I and bacterial branching enzymes. This result demonstrates that branching enzyme belongs to a family of the amylolytic enzymes. The BE-I gene is highly expressed in the early stages of kernel development, and the level of the message concentration decreases slowly as kernel maturation proceeds. © 1991 Academic Press, Inc.

In the storage organs of higher plants, starch is synthesized and accumulated in the organelles, amyloplast. Starch is composed of two chemically different molecules, amylose and amylopectin; amylose is an essentially linear polymer with α-1,4-glucosidic bonds, whereas amylopectin is a highly branched molecule containing α-1,4- and α-1,6-linkages. It has long been considered that biosynthesis of starch in the amyloplast is catalyzed by starch synthase, branching enzyme, and possibly phosphorylase (1). However, the synthetic mechanisms of amylose and amylopectin are poorly understood. Therefore, to elucidate the mechanisms at the protein and gene levels, it is essential to isolate and characterize the genes for starch-synthesizing enzymes.

Plant branching enzyme acts on already synthesized and/or elongating amylose chains to form α-1,6-linked branch points. Thus, this enzyme is a key enzyme for amylopectin biosynthesis (5-8). In maize kernels, two types

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of branching enzyme, BE-I and BE-II, have been identified (5-7). Recently, Bhattacharyya et al. (9) reported the cloning of the gene encoding an isoform of branching enzyme in pea. Although the wrinkled-seed character of pea is found to be caused by a transposon-like insertion in the branching enzyme gene (9), the sequence data for the coding region of the gene has not been available yet.

We now describe the amino acid sequence of maize BE-I deduced from the cDNA sequence, and the gene expression during kernel development. This is the first report on the primary structure of branching enzyme in plant.

MATERIALS AND METHODS

Materials. Maize (Zea mays L.) inbred Oh43 was field-grown in 1988. All plants were self- or sib-pollinated, and the ears were harvested 10 to 28 days after pollination, immediately frozen in liquid nitrogen, and stored at -80°C. BE-I was purified according to the previously described procedure (8). The purified enzyme migrated as a protein with a molecular mass of 85 kDa on SDS-PAGE.

Preparation of antibodies. The purified BE-I (50 μg) was mixed with an equal volume of Freund's complete adjuvant, and injected intradermally into New Zealand White rabbits. The injection was repeated twice at intervals of two weeks using Freund's incomplete adjuvant. Antisera were collected two weeks after the final injection. The immunoglobulin fraction was prepared by precipitation with 50% saturated ammonium sulfate, and subjected to immunoaffinity chromatography on a BE-I-conjugated Sepharose 4B column. The antibodies retained on the column were eluted with 0.2 M glycine/HCl, pH 2.2, immediately neutralized with 0.4 M potassium phosphate, pH 8.0, and then dialyzed against phosphate-buffered saline.

Preparation of RNA. Kernels (20 g) were ground with a mortar and pestle in liquid nitrogen, homogenized with a Polytron in 200 ml of 8 M urea containing 3 M LiCl, and allowed to stand overnight at 4°C. After centrifugation at 16,000 x g for 20 min, the precipitate was dissolved in 50 ml of 10 mM Tris/HCl, pH 7.6, containing 0.5% SDS, and treated with phenol/chloroform solution. Total RNA was recovered by ethanol precipitation. Polyadenylated RNA was selected by oligo(dT)-cellulose (Type 7, Pharmacia LKB Biotechnology) column chromatography.

Construction and screening of a maize kernel cDNA library. Polyadenylated RNA from 14-day-old kernels was denatured with 10 mM methylmercury hydroxide for 5 min at room temperature. Double-stranded cDNA was synthesized from the denatured RNA using a commercial kit of Pharmacia LKB Biotechnology. The cDNA was ligated into the EcoRI site of λgt11. After in vitro packaging, 130,000 plaques were screened by affinity-purified anti-BE-I antibodies, as described previously (10). Positive clones were plaque-purified, and the cDNA inserts were subcloned into pUC19 or M13mp19 vector. Nucleotide sequencing was carried out by the dideoxy chain termination method (11), using a Sequenase Version 2.0 (U.S.B. Corp.) or an AmpliTaq sequencing kit (Takara).

Northern blot analysis. Total RNA was denatured with glyoxal, separated by electrophoresis on 1.2% agarose gels, and transferred onto GeneScreen Plus nylon membranes. The blot was probed by the ^{32}P-labeled cDNA fragment

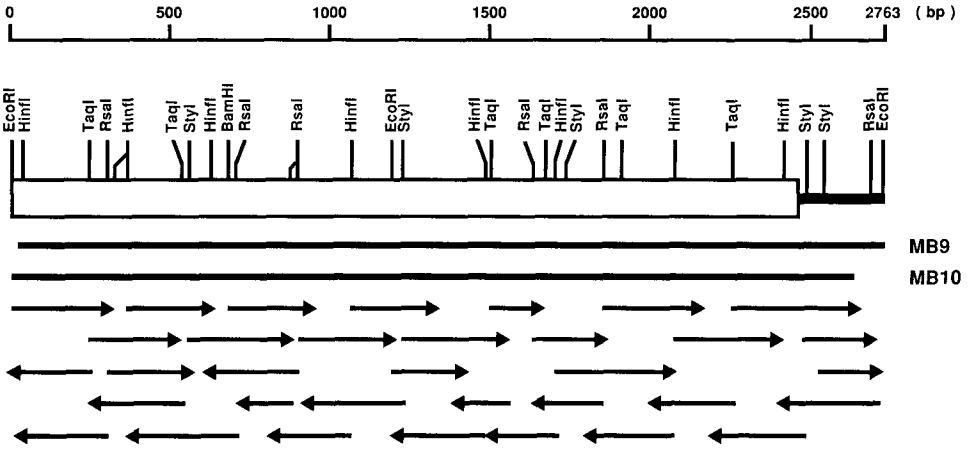

Fig. 1. **Restriction map and sequencing strategy for cDNA inserts encoding maize branching enzyme-I (BE-I).** The scale at the top designates nucleotide position in base pair (bp) from the 5'-end of the MB10 insert. The open box indicates the protein coding region including a putative transit peptide. The arrows indicate the direction and extent of nucleotide sequence determined from each site.

of MB9 (Fig. 1). Stringencies employed for hybridization and washing were the same as those described previously (12).

Protein analysis. Purified BE-I (0.1 mg of protein) was lyophilized, dissolved in 70% formic acid (0.2 ml) containing 0.1 mg CNBr, and incubated at room temperature for 2 days in the dark. The mixture was applied on a LiChrosphere RP-8 reverse phase column (Cica-Merck) previously equilibrated with 0.1% trifluoloacetic acid. Peptides were eluted with a linear gradient (0-60%) of acetonitrile in the same solution, collected manually, and lyophilized. The original BE-I and the two CNBr fragments were sequenced by automated Edman degradation using an Applied Biosystems Model 477A protein sequencer equipped with a Model 120A on-line PTH analyzer.

RESULTS AND DISCUSSION

When a maize kernel cDNA library was screened by affinity-purified anti-BE-I antibodies, four positive clones, termed MB9, MB10, MB18, and MB19, were obtained. Restriction mapping and partial nucleotide sequencing of these cDNA inserts indicated that they were all related to one another. Of the four clones, the cDNA inserts of MB9 and MB10 were completely sequenced (Fig. 1). The combined nucleotide sequence of the overlapping cDNAs was 2,763 base pairs (bp) in length and contained an open reading frame of 2,469 nucleotides (Fig. 2). The deduced amino acid sequence at residues 1-6 matched the amino-terminal sequence of BE-I (Ala-Thr-Val-Gln-Glu-Asp-) determined by protein analysis. The sequences of two CNBr fragments from BE-I were also found at residues 10-29 and 534-546 (underlines in Fig. 2). These

results clearly demonstrate the identities of these inserts as the BE-I cDNA clone. Since the nucleotide sequence lacked an ATG initiation codon, rescreenings of both the maize kernel cDNA and genomic libraries were carried out using the cDNA fragment of MB9 as a probe. Positive clones were practically isolated, but failed to complete the open reading frame. At any rate, the mature BE-I contains 759 amino acid residues with a calculated molecular mass of 86,236 Da. This value agrees well with that obtained experimentally by SDS-PAGE (8).

The amino acid sequence of BE-I at residues -63 to -1 preceding the amino-terminal amino acid (Ala^{1}) is significantly different from the signal sequences of secretory proteins in length and amino acid composition (Fig. 2). It is widely known that precursors of nuclear-encoded chloroplastic proteins have transit peptides at their amino termini (13). The function of the transit peptide is believed to be the transport and/or import of the precursors into the organella. The 63-residue sequence as the amino-terminal extension of BE-I is rich in basic (21% as Arg, Lys, and His) and hydroxylic (19% as Ser and Thr) amino acids, and contains only an acidic amino acid (Asp^{-40}, see Fig. 2). This feature is consistent with the characteristic of the transit peptide of potato phosphorylase precursor which is routed into the amyloplast (14). Thus, the amino-terminal extension of BE-I most likely acts as a transit peptide for the amyloplast.

We have recently identified a cDNA clone, termed RB13, encoding rice BE-I (the details will be reported elsewhere). The insert of RB13 contains an almost full-length cDNA which includes an initiator ATG codon. Comparison of the amino-terminal extensions between maize and rice BE-I shows an extensive sequence similarity (55% identity); especially, the 7 amino acids of the amino-terminal 9-residue sequence (at residues -63 to -55 in maize BE-I, see Fig. 2) are exactly identical between maize and rice. This suggests that the maize sequence lacks only the initiator methionine from the full protein coding region. In fact, Northern blot analysis yields a 2.8-kb signal as the transcript size corresponding to the overlapping cDNA inserts of MB9 and MB10, as described below. Therefore, it is reasonable to consider that maize BE-I is initially synthesized as a 823-residue precursor protein, including a 64-residue transit peptide.

Fig. 2. **Nucleotide sequence of maize branching enzyme-I (BE-I) cDNA and its deduced amino acid sequence.** The deduced amino acid sequence is shown by the standard one-letter code below the nucleotide sequence numbered in the 5' to 3' direction. The amino-terminal sequences of BE-I and its two CNBr fragments determined by protein analysis are underlined. The amino acids are numbered from the amino terminus of the mature enzyme, and the residues on the amino-terminal side of residue 1 (Ala) are indicated by negative numbers. A putative polyadenylation signal is broken-underlined.

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                                                                    +1   G CTG TGC CTC      10
                                                                   -63     L  C  L       -61

GTG TCG CCC TCT TCC TCG CCG ACT CCG CTT CCG CCG CCG CGG CGC TCT CGC TCG CAT GCT GAT CGG GCG GCA CCG CCG GGG ATC GCG GGT     100
 V  S  P  S  S  S  P  T  P  L  P  P  P  R  R  S  R  S  H  A  D  R  A  A  P  P  G  I  A  G      -31

GGC GGC AAT GTG CGC CTG AGT GTG TTG TCT GTC CAG TGC AAG GCT CGC CGG TCA GGG GTG CGG AAG GTC AAG AGC AAA TTC GCC ACT GCA     190
 G  G  N  V  R  L  S  V  L  S  V  Q  C  K  A  R  R  S  G  V  R  K  V  K  S  K  F  A  T  A       -1

GCT ACT GTG CAA GAA GAT AAA ACT ATG GCA ACT GCC AAA GGC GAT GTC GAC CAT CTC CCC ATA TAC GAC CTG GAC CCC AAG CTG GAG ATA     280
 A  T  V  Q  E  D  K  T  M  A  T  A  K  G  D  V  D  H  L  P  I  Y  D  L  D  P  K  L  E  I       30

TTC AAG GAC CAT TTC AGG TAC CGG ATG AAA AGA TTC CTA GAG CAG AAA GGA TCA ATT GAA GAA AAT GAG GGA AGT CTT GAA TCT TTT TCT     370
 F  K  D  H  F  R  Y  R  M  K  R  F  L  E  Q  K  G  S  I  E  E  N  E  G  S  L  E  S  F  S       60

AAA GGC TAT TTG AAA TTT GGG ATT AAT ACA AAT GAG GAT GGA ACT GTA TAT CGT GAA TGG GCA CCT GCT GCG CAG GAG GCA GAG CTT ATT     460
 K  G  Y  L  K  F  G  I  N  T  N  E  D  G  T  V  Y  R  E  W  A  P  A  A  Q  E  A  E  L  I       90

GGT GAC TTC AAT GAC TGG AAT GGT GCA AAC CAT AAG ATG GAG AAG GAT AAA TTT GGT GTT TGG TCG ATC AAA ATT GAC CAT GTC AAA GGG     550
 G  D  F  N  D  W  N  G  A  N  H  K  M  E  K  D  K  F  G  V  W  S  I  K  I  D  H  V  K  G      120

AAA CCT GCC ATC CCT CAC AAT TCC AAG GTT AAA TTT CGC TTT CTA CAT GGT GGA GTA TGG GTT GAT CGT ATT CCA GCA TTG ATT CGT TAT     640
 K  P  A  I  P  H  N  S  K  V  K  F  R  F  L  H  G  G  V  W  V  D  R  I  P  A  L  I  R  Y      150

GCG ACT GTT GAT GCC TCT AAA TTT GGA GCT CCC TAT GAT GGT GTT CAT TGG GAT CCT CCT GCT TCT GAA AGG TAC ACA TTT AAG CAT CCT     730
 A  T  V  D  A  S  K  F  G  A  P  Y  D  G  V  H  W  D  P  P  A  S  E  R  Y  T  F  K  H  P      180

CGG CCT TCA AAG CCT GCT GCT CCA CGT ATC TAT GAA GCC CAT GTA GGT ATG AGT GGT GAA AAG CCA GCA GTA AGC ACA TAT AGG GAA TTT     820
 R  P  S  K  P  A  A  P  R  I  Y  E  A  H  V  G  M  S  G  E  K  P  A  V  S  T  Y  R  E  F      210

GCA GAC AAT GTG TTG CCA CGC ATA CGA GCA AAT AAC TAC AAC ACA GTT CAG TTG ATG GCA GTT ATG GAG CAT TCG TAC TAT GCT TCT TTC     910
 A  D  N  V  L  P  R  I  R  A  N  N  Y  N  T  V  Q  L  M  A  V  M  E  H  S  Y  Y  A  S  F      240

GGG TAC CAT GTG ACA AAT TTC TTT GCG GTT AGC AGC AGA TCA GGC ACA CCA GAG GAC CTC AAA TAT CTT GTT GAT AAG GCA CAC AGT TTG    1000
 G  Y  H  V  T  N  F  F  A  V  S  S  R  S  G  T  P  E  D  L  K  Y  L  V  D  K  A  H  S  L      270

GGT TTG CGA GTT CTG ATG GAT GTT GTC CAT AGC CAT GCA AGT AAT AAT GTC ACA GAT GGT TTA AAT GGC TAT GAT GTT GGA CAA AGC ACC    1090
 G  L  R  V  L  M  D  V  V  H  S  H  A  S  N  N  V  T  D  G  L  N  G  Y  D  V  G  Q  S  T      300

CAA GAG TCC TAT TTT CAT GCG GGA GAT AGA GGT TAT CAT AAA CTT TGG GAT AGT CGG CTG TTC AAC TAT GCT AAC TGG GAG GTA TTA AGG    1180
 Q  E  S  Y  F  H  A  G  D  R  G  Y  H  K  L  W  D  S  R  L  F  N  Y  A  N  W  E  V  L  R      330

TTT CTT CTT TCT AAC CTG AGA TAT TGG TTG GAT GAA TTC ATG TTT GAT GGC TTC CGA TTT GAT GGA GTT ACA TCA ATG CTG TAT CAT CAC    1270
 F  L  L  S  N  L  R  Y  W  L  D  E  F  M  F  D  G  F  R  F  D  G  V  T  S  M  L  Y  H  H      360

CAT GGT ATC AAT GTG GGG TTT ACT GGA AAC TAC CAG GAA TAT TTC AGT TTG GAC ACA GCT GTG GAT GCA GTT GTT TAC ATG ATG CTT GCA    1360
 H  G  I  N  V  G  F  T  G  N  Y  Q  E  Y  F  S  L  D  T  A  V  D  A  V  V  Y  M  M  L  A      390

AAC CAT TTA ATG CAC AAA CTC TTG CCA GAA GCA ACT GTT GTT GCT GAA GAT GTT TCA GGC ATG CCG GTC CTT TGC CGG CCA GTT GAT GAA    1450
 N  H  L  M  H  K  L  L  P  E  A  T  V  V  A  E  D  V  S  G  M  P  V  L  C  R  P  V  D  E      420

GGT GGG GTT GGG TTT GAC TAT CGC CTG GCA ATG GCT ATC CCT GAT AGA TGG ATT GAC TAC CTG AAG AAT AAA GAT GAC TCT GAG TGG TCG    1540
 G  G  V  G  F  D  Y  R  L  A  M  A  I  P  D  R  W  I  D  Y  L  K  N  K  D  D  S  E  W  S      450

ATG GGT GAA ATA GCG CAT ACT TTG ACT AAC AGG AGA TAT ACT GAA AAA TGC ATC GCA TAT GCT GAG AGC CAT GAT CAG TCT ATT GTT GGC    1630
 M  G  E  I  A  H  T  L  T  N  R  R  Y  T  E  K  C  I  A  Y  A  E  S  H  D  Q  S  I  V  G      480

GAC AAA ACT ATT GCA TTT CTC CTG ATG GAC AAG GAA ATG TAC ACT GGC ATG TCA GAC TTG CAG CCT GCT TCA CCT ACA ATT GAT CGA GGG    1720
 D  K  T  I  A  F  L  L  M  D  K  E  M  Y  T  G  M  S  D  L  Q  P  A  S  P  T  I  D  R  G      510

ATT GCA CTC CAA AAG ATG ATT CAC TTC ATC ACA ATG GCC CTT GGA GGT GAT GGC TAC TTG AAT TTT ATG GGA AAT GAG TTT GGT CAC CCA    1810
 I  A  L  Q  K  M  I  H  F  I  T  M  A  L  G  G  D  G  Y  L  N  F  M  G  N  E  F  G  H  P      540

GAA TGG ATT GAC TTT CCA AGA GAA GGG AAC AAC TGG AGC TAT GAT AAA TGC AGA CGA CAG TGG AGC CTT GTG GAC ACT GAT CAC TTG CGG    1900
 E  W  I  D  F  P  R  E  G  N  N  W  S  Y  D  K  C  R  R  Q  W  S  L  V  D  T  D  H  L  R      570

TAC AAG TAC ATG AAT GCG TTT GAC CAA GCG ATG AAT GCG CTC GAT GAG AGA TTT TCC TTC CTT TCG TCG TCA AAG CAG ATC GTC AGC GAC    1990
 Y  K  Y  M  N  A  F  D  Q  A  M  N  A  L  D  E  R  F  S  F  L  S  S  S  K  Q  I  V  S  D      600

ATG AAC GAT GAG GAA AAG GTT ATT GTC TTT GAA CGT GGA GAT TTA GTT TTT GTT TTC AAT TTC CAT CCC AAG AAA ACT TAC GAG GGC TAC    2080
 M  N  D  E  E  K  V  I  V  F  E  R  G  D  L  V  F  V  F  N  F  H  P  K  K  T  Y  E  G  Y      630

AAA GTG GGA TGC GAT TTG CCT GGG AAA TAC AGA GTA GCC CTG GAC TCT GAT GCT CTG GTC TTC GGT GGA CAT GGA AGA GTT GGC CAC GAC    2170
 K  V  G  C  D  L  P  G  K  Y  R  V  A  L  D  S  D  A  L  V  F  G  G  H  G  R  V  G  H  D      660

GTG GAT CAC TTC ACG TCG CCT GAA GGG GTG CCA GGG GTG CCC GAA ACG AAC TTC AAC AAC CGG CCG AAC TCG TTC AAA GTC CTT TCT CCG    2260
 V  D  H  F  T  S  P  E  G  V  P  G  V  P  E  T  N  F  N  N  R  P  N  S  F  K  V  L  S  P      690

CCC CGC ACC TGT GTG GCT TAT TAC CGT GTA GAC GAA GCA GGG GCT GGA CGA CGT CTT CAC GCG AAA GCA GAG ACA GGA AAG ACG TCT CCA    2350
 P  R  T  C  V  A  Y  Y  R  V  D  E  A  G  A  G  R  R  L  H  A  K  A  E  T  G  K  T  S  P      720

GCA GAG AGC ATC GAC GTC AAA GCT TCC AGA GCT AGT AGC AAA GAA GAC AAG GAG GCA ACG GCT GGT GGC AAG AAG GGA TGG AAG TTT GCG    2440
 A  E  S  I  D  V  K  A  S  R  A  S  S  K  E  D  K  E  A  T  A  G  G  K  K  G  W  K  F  A      750

CGG CAG CCA TCC GAT CAA GAT ACC AAA TGA     AGCCACGAGT CCTTGGTGAG GACTGGACTG GCTGCCGGCG CCCTGTTAGT AGTCCTGCTC TACTGGACTA    2540
 R  Q  P  S  D  Q  D  T  K  *                                                                    759

GCCGCCGCTG GCGCCCTTGG AACGGTCCTT TCCTGTAGCT TGCAGGCGAC TGGTGTCTCA TCACCGAGCA GGCAGGCACT GCTTGTATAG CTTTTCTAGA ATAATAATCA    2650

GGGATGGATG GATGGTGTGT ATTGGCTATC TGGCTAGACG TGCATGTGCC CAGTTTGTAT GTACAGGAGC AGTTCCCGTC CAGAATAAAA AAAAACTTGT TGGGGGGTTT    2760

TTC                                                                                              2763
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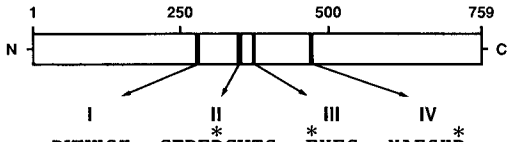

Branching enzyme-I (maize)	...DVVHSH. .GFRFDGVTS...EYFS...YAESHD...
Branching enzyme (*E. coli*)	...DWVPGH...ALRVDAVAS...EFGG...LPLSHD...
Branching enzyme (*Synechococcus* sp.)	. .DWVPGH . GIRVDAVAS...EYGG...LALSHD...
α-Amylase (*A. oryzae*)	.. DVVANH. .GLRIDTVKH...EVLD...FVENHD...
α-Amylase (*B. subtilis*)	...DAVINH.. GFRFDAAKH...EILQ. .WVESHD...
α-Amylase (barley)	.DIVINH...DGRLDWGPH...EVWD...FVDNAD...
α-Amylase (human)	...DAVINH...GFRIDASKH...EVID...FVDNHD...
G4-forming amylase (*P. saccharophila*)	...DVVPNH...GFRFDFVRG...ELWK.. FVDNHD...
CGTase (*K. pneumoniae*)	...DYAPNH...AIRIDAIKH...EWFG. FMDNHD...
Isoamylase (*P. amyloderamosa*)	...DVVYNH...GFRFDLASV...EWSV...FIDVHD...
Pullulanase (*K. aerogenes*)	. DVVYNH ..GFRFDLMGY...EGWD...YVSKHD...

Fig. 3. **Comparison of the deduced amino acid sequence of maize branching enzyme-I (BE-I) with those of bacterial branching enzymes and typical amylolytic enzymes.** The sequences of maize BE-I at residues 277-282, 347-355, 373-376, and 470-475 (see Fig. 2) are compared with those of the four conserved regions (Regions I, II, III, and IV) of typical amylolytic enzymes, as described by Nakajima et al. (19): α-amylases from Aspergillus oryzae (20), Bacillus subtilis (21), barley (22), and human (23), Pseudomonas saccharophila maltotetraohydrolase (G4-forming amylase, ref. 24), Klebsiella pneumoniae cyclodextrin-glycosyltransferase (CGTase, ref. 25), Pseudomonas amyloderamosa isoamylase (26), and Klebsiella aerogenes pullulanase (27). The sequences of branching enzymes from Escherichia coli (15) and Synechococcus sp. (16) are also aligned. The possible catalytic residues for cleavage of α-1,4-linkages (17, 18) are indicated by asterisks.

The genes for two bacterial branching enzymes have been cloned and sequenced (15, 16). A significant similarity was found in the amino acid sequences between these two proteins (46% identity, see ref. 16). When the sequence of maize BE-I was compared with those of the bacterial enzymes, the similarity was low (approximately 23% identities for both bacterial enzymes). Also, the sequence similarity of BE-I with various amylolytic enzymes was quite limited. However, the four regions (Regions I, II, III, and IV), which constitute the catalytic site of the amylolytic enzymes, including isoamylase and pullulanase that split α-1,6-glucosidic bonds in the branched glucans, are conserved in the sequence of maize BE-I as well as of bacterial branching enzymes (Fig. 3). Interestingly, plant and bacterial branching enzymes all conserve the possible catalytic residues that are required for cleavage of α-1,4-glucosidic bond, including aspartate, glutamate, and aspartate (17, 18) in Regions II, III, and IV, respectively (residues 351, 373, and 475 in the BE-I sequence, see Figs. 2 and 3). These data demonstrate that branching enzyme belongs to a family of the amylolytic enzymes.

The developmental pattern of BE-I gene expression was examined using total RNAs from kernels at 10 to 28 days after pollination (Fig. 4). The

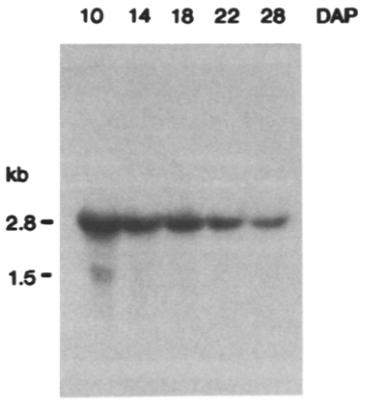

Fig. 4. **Expression of the maize branching enzyme-I (BE-I) gene during kernel development.** Total RNA was isolated from kernels at 10 to 28 days after pollination (DAP). A Northern blot was prepared, using 20 μg each of these RNAs, and probed by the ^{32}P-labeled cDNA insert of MB9 (nucleotides 19-2763, see Figs. 1 and 2), as described in MATERIALS AND METHODS.

2.8-kb BE-I message was found abundantly at the 10th day of kernel development. The level of the message concentration decreased slowly as kernel maturation proceeded. A transcript with an approximate size of 1.5 kb, which was hybridized with the BE-I cDNA, was also detectable (Fig. 4). The 1.5-kb mRNA concentration was more abundant in the early stages than in the later stages of kernel development. However, it is not clear at present what is encoded by the 1.5-kb message.

In this paper, we describe the sequence conservation of the catalytic site of amylolytic enzymes in maize BE-I. This fact will be an important clue for studies of the structure and function of the enzyme. In addition, experiments are underway to define the roles served by the transit peptide of the BE-I precursor.

REFERENCES

1. Preiss, J., and Levi, C. (1980) In the Biochemistry of Plants (Preiss, J., ed), pp. 371-423. Academic Press, New York.
2. Drummond, G. S., Smith, E. E., and Whelan, W. J. (1972) Eur. J. Biochem. 26, 168-176.
3. Borovsky, D., Smith, E. E., and Whelan, W. J. (1975) Eur. J. Biochem. 59, 615-625.
4. Borovsky, D., Smith, E. E., Whelan, W. J., French, D., and Kikumoto, S. (1979) Arch. Biochem. Biophys. 198, 627-631.
5. Boyer, C. D., and Preiss, J. (1978) Carbohydr. Res. 61, 321-334.
6. Boyer, C. D., and Preiss, J. (1978) Biochem. Biophys. Res. Commun. 80, 169-175.
7. Singh, B. K., and Preiss, J. (1985) Plant Physiol. 79, 34-40.
8. Baba, T., Arai, Y., Ono, T., Munakata, A., Yamaguchi, H., and Itoh, T. (1982) Carbohydr. Res. 107, 215-230.

9. Bhattacharyya, M. K., Smith, A. M., Ellis, T. H. N., Hedley, C., and Martin, C. (1990) Cell 60, 115-122.
10. Huynh, T. V., Young, R. A., and Davis, R. W. (1985) In DNA Cloning (Glover, D. M., ed) vol. 1, pp. 49-78, IRL Press, Oxford.
11. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467.
12. Baba, T., Kashiwabara, S., Watanabe, K., Itoh, H., Michikawa, Y., Kimura, K., Takada, M., Fukamizu, A., and Arai, Y. (1989) J. Biol. Chem. 264, 11920-11927.
13. Schmidt, G. W., and Mishkind, M. L. (1986) Ann. Rev. Biochem. 55, 879-912.
14. Nakano, K., Mori, H., and Fukui, T. (1989) J. Biochem. 106, 691-695.
15. Baecker, P. A., Greenberg, E., and Preiss, J. (1986) J. Biol. Chem. 261, 8738-8743.
16. Kiel, J. A. K. W., Boels, J. M., Beldman, G., and Venema, G. (1990) Gene 89, 77-84.
17. Matsuura, Y., Kusunoki, M., Harada, W., and Kakudo, M. (1984) J. Biochem. 95, 697-702.
18. Buisson, G., Duee, E., Haser, R., and Payan, F. (1987) EMBO J. 6, 3909-3916.
19. Nakajima, R., Imanaka, T., and Aiba, S. (1986) Appl. Microbiol. Biotech. 23, 355-360.
20. Toda, H., Kondo, K., and Narita, K. (1982) Proc. Japan Acad. 58, 208-212.
21. Yang, M., Galizzi, A., and Henner, D. (1983) Nucl. Acids Res. 11, 237-249.
22. Rogers, J. C., and Milliman, C. (1983) J. Biol. Chem. 258, 8169-8174.
23. Nakamura, Y., Ogawa, M., Nishide, T., Emi, M., Kosaki, G., Himeno, S., and Matsubara, K. (1984) Gene 28, 263-270.
24. Zhou, J., Baba, T., Takano, T., Kobayashi, S., and Arai, Y. (1989) FEBS Lett. 255, 37-41.
25. Binder, F., Huber, O., and Bock, A. (1986) Gene 47, 269-277.
26. Amemura, A., Chakraborty, R., Fujita, M., Noumi, T., and Futai, M. (1988) J. Biol. Chem. 263, 9271-9275.
27. Katsuragi, N., Takizawa, N., and Murooka, Y. (1987) J. Bacteriol. 169, 2301-2306.