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Characterization of a Maize β-Amylase cDNA Clone and Its Expression during Seed Germination[1]

Shue-Mei Wang, Wei-Ling Lue, Shu-Yuann Wu, Hsiu-Wen Huang, and Jychian Chen*

Department of Botany, National Taiwan University, Taipei, Taiwan (S.-M.W., S.-Y.W., H.-W.H.); and the Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan (W.-L.L., J.C.)

A maize (*Zea mays* L.) cDNA clone (pZMB2) encoding β-amylase was isolated from a cDNA library prepared from the aleurone RNA of germinating kernels. The cDNA encodes a predicted product of 488 amino acids with significant similarity to known β-amylases from barley (*Hordeum vulgare*), rye (*Secale cereale*), and rice (*Oryza sativa*). Glycine-rich repeats found in the carboxyl terminus of the endosperm-specific β-amylase of barley and rye are absent from the maize gene product. The N-terminal sequence of the first 20 amino acids of a β-amylase peptide derived from purified protein is identical to the 5th through 24th amino acids of the predicted cDNA product, indicating the absence of a conventional signal peptide in the maize protein. Recombinant inbred mapping data indicate that the cDNA clone is single-copy gene that maps to chromosome 7L at position 83 centimorgans. Northern blot analysis and in vitro translation-immunoprecipitation data indicate that the maize β-amylase is synthesized de novo in the aleurone cells but not in the scutellum during seed germination.

A major carbon source for the growth of germinating seedlings is starch reserves stored in seed organs such as endosperm or cotyledons. Many degradative enzymes, including α- and β-amylases, are engaged in starch breakdown during the germination process. The β-amylases are found only in bacteria and plants, not in animals. Two types of β-amylases found in barley (*Hordeum vulgare*; Shewry et al., 1988), wheat (*Triticum aestivum*; Daussant and Lauriere, 1990), and rye (*Secale cereale*; Daussant et al., 1991) have been described. The endosperm-specific type of β-amylase accumulates only during caryopsis maturation, whereas the ubiquitous type is present in vegetative organs (Daussant and Lauriere, 1990). The complete amino acid sequence of the barley endosperm-specific β-amylase (Kreis et al., 1987; Yoshigi et al., 1994) reveals the presence of four Gly-rich repeats at the carboxyl terminus of the protein. Similarly, the endosperm-specific β-amylase of rye has three Gly-rich repeats (Rorat et al., 1991). The sequence of the ubiquitous β-amylase derived from the cDNA clone of rye (Sadowski et al., 1993) differs from that of the endosperm β-amylase in the absence of the C-terminal Gly-rich repeats. In barley, wheat, and rye, the endosperm-specific β-amylase is synthesized during seed development and deposited in the endosperm as a latent form (Rowsell and Goad, 1962; Duffus and Rosie, 1972; Hara-Nishimura et al., 1986) but is not synthesized de novo during seed germination (Daussant and Corvazier, 1970; Hardie, 1975). In contrast, β-amylase in rice (*Oryza sativa*) (Okamoto and Akazawa, 1980; Wang et al., 1996) is synthesized de novo in aleurone layers during seed germination. It was suggested that rice and maize may not contain a typical endosperm-specific β-amylase such as those found in wheat, rye, and barley (Lauriere et al., 1992).

Previously we purified and characterized from germinating maize (*Zea mays* L.) kernels a 60-kD β-amylase with a pI of 4.2 (Wang et al., 1992). In this paper we report the cloning of the maize β-amylase cDNA. As with the rice β-amylase gene, the maize gene was found to be expressed in the aleurone cells of germinating kernels but not in the scutellum. In contrast to the multigene organization of β-amylases in rice, rye, and barley, maize β-amylase appears to be encoded by a single-copy gene.

MATERIALS AND METHODS

DNA and RNA Analysis

Aleurone layers were isolated from maize kernels (*Zea mays* cv TN351) germinated aseptically on sterilized filters in the dark at 28°C. Total aleurone RNA was isolated by the phenol extraction method and poly(A)$^+$ RNA was purified by oligo(dT)-cellulose column chromatography, as described previously (Sambrook et al., 1989). The cDNA library was constructed in the λ-Zap vector (Stratagene) using the poly(A)$^+$ RNA isolated from the aleurone layer of kernels germinated for 3 d (Sambrook et al., 1989). The λ-DNA was packaged in vitro, plated, and screened with a rice (*Oryza sativa*) β-amylase cDNA probe, ZGSX1 (Wang et al., 1996). Positive clones were purified and the inserted DNA was sequenced with Sequenase (USB) according to the manufacturer's instructions. The DNA sequence was analyzed with the GCG program (version 8.1, Genetics Computer Group, Madison, WI).

Southern blot and RFLP analyses were performed as described previously (Eimert et al., 1995). The recombinant inbred lines were kindly supplied by Dr. Ben Burr (Burr et al., 1987). For northern blot analysis, RNA was separated

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* Corresponding author; e-mail mbjchen@ccvax.sinica.edu.tw; fax 8862–782–6085.

Abbreviations: ORF, open reading frame; RFLP, restriction fragment length polymorphism.

on 1% agarose-formaldehyde gels and then transferred to Nytran paper (Schleicher & Schuell) according to methods described previously (Sambrook et al., 1989). DNA probes were labeled with [α-^{32}P]dCTP (Amersham, AA0005, 3000 Ci/mmol) using the random primer method (Sambrook et al., 1989).

Protein Analysis

The peptide sequence of purified maize β-amylase protein (Wang et al., 1992) was obtained by SDS-PAGE, blotting the proteins to PVDF paper, and peptide sequencing with ABI 477A sequencer (Perkin-Elmer). The glycosylation of maize β-amylase was analyzed with the digoxigenin glycan differentiation kit (Boehringer Mannheim). Protein extracts were prepared by grinding tissues in extraction buffer (50 mM Tris, pH 7.0, 3 mM NaCl, 4 mM $CaCl_2$, 0.1% β-mercaptoethanol, 0.2 mM PMSF, and 1 μg/mL leupeptin) and quantified by the Lowry method, as previously described (Wang et al., 1992). To extract the proteins from dried, mature seeds, 1 g of seeds was extracted with 2 mL of buffer containing 1% β-mercaptoethanol with or without 1.2 units of papain (Boehringer Mannheim). Developing seeds were staged according to the size of the embryo, from 1 to 5 mm (representing stages 1–5).

Zymograms of amylolytic activity were obtained by separating protein extracts in 7.5% native polyacrylamide gels, impregnating the gels in 4% starch for 80 min, and then staining with iodine, as described previously (Wang et al., 1992). For western blot analysis, proteins were separated by SDS-PAGE, electroblotted to a nitrocellulose filter, and reacted with rabbit polyclonal antibody raised against purified maize β-amylase. The primary antibody was detected with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA).

To label proteins in vivo, 10 seeds germinated for 5 d were labeled with 200 μCi of [^{35}S]Met (Amersham, 1000 Ci/mmol) for 90 min and extracted with 2 mL of buffer. The extracted proteins were subjected to immunoprecipitation and separated by SDS-PAGE, and fluorography was performed as described previously (Wang and Huang, 1987). In vitro translation of RNA was performed with reticulocyte lysate (Promega) labeled with [^{35}S]Met, as suggested by the manufacturer.

RESULTS AND DISCUSSION

Isolation and Characterization of Maize β-Amylase cDNA Clones

From an aleurone cDNA library we isolated four clones that cross-hybridized with a rice β-amylase cDNA probe. The DNA sequence of these clones indicated that they represented cDNAs of different lengths derived from a single mRNA species. The sequence of the longest cDNA (pZMB2) contained a 1775-bp insert with a single large ORF encoding a predicted product of 488 amino acids (Fig. 1). The cDNA was judged to be near full-length by the presence of a 5′ untranslated region of 47 bp that preceded the ORF and a 3′ untranslated region of 264 bp downstream of the ORF stop codon. Overall, the predicted maize protein shared 84.4% amino acid identity with the rice β-amylase gene. Two β-amylase signature motifs were also found in the maize-predicted protein (Fig. 1).

To confirm the identity of the maize cDNA clone, we performed peptide sequencing of β-amylase protein purified from maize aleurone cells (Wang et al., 1992).

Figure 1. DNA sequence and derived amino acid sequence of the β-amylase cDNA of maize. The sequence from purified maize β-amylase (5–24), β-amylase signature sequences (91–99 and 180–190) identified by MOTIFS of the GCG program and polyadenylation signals are underlined.

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1    GCAGAGCAAGCCAGTGAGGTCTCTCAGAGCGTAGCCAGCCAGCTACAATGGCGGGGAACGCGCTAGCCAACTATGTCCAGGTCTACGTCATGCTCCCGCT  100
                                                    M  A  G  N  A  L  A  N  Y  V  Q  V  Y  V  M  L  P  L   18

101  GGATGTCATCACTGTCGACAACACGTTCGAGAAGGAGGACGAGACGAGGGCGCAGCTGAAGAAGCTGACGGAGGCGGGCGCCGACGGGGTCATGATCGAC  200
 19  D  V  I  T  V  D  N  T  F  E  K  E  D  E  T  R  A  Q  L  K  K  L  T  E  A  G  A  D  G  V  M  I  D   51

201  GTCTGGTGGGGGCTGGTGGAAGGGAAGGAGCCGGGAGTCTACGACTGGAGGCGCCTACAGGCAGGTGTTCAAGCTGGTGCAGAGGCCGGGCTGAAGCTGC  300
 52  V  W  W  G  L  V  E  G  K  E  P  G  V  Y  D  W  S  A  Y  R  Q  V  F  K  L  V  Q  E  A  G  L  K  L  Q   85

301  AGGCCATCATGTCGTGCCACCAGTGCGGCCGGCAACGTCGGCGACGTCGTCAACATCCCGATCCCGCAGTGGGTGCGGGACGTCGGCAAAAGCAACCCCGA  400
 86    A  I  M  S  C  H  Q  C  G  G  N  V  G  D  V  V  N  I  P  I  P  Q  W  V  R  D  V  G  K  S  N  P  D  118

401  CATCTTCTACACCAACCGGAGTGGGCTGACGAACATCGAGTATCTCACGCTTGGAGTGGACGACCAGCCTCTCTTCCATGGGAGAACTGCCATTCAGCTG  500
119  I  F  Y  T  N  R  S  G  L  T  N  I  E  Y  L  T  L  G  V  D  D  Q  P  L  F  H  G  R  T  A  I  Q  L  151

501  TATGCTGATTACATGAAGAGCTTCAGGGAGAACATGGCAGACTTCTTGGATGCTGGTGTCGTTGTGGACATTGAGGTGGGACTTGGCCCTGCTGGCGAAA  600
152  Y  A  D  Y  M  K  S  F  R  E  N  M  A  D  F  L  D  A  G  V  V  V  D  I  E  V  G  L  G  P  A  G  E  M  185

601  TGAGGTACCCATCGTATCCCCAGAGTCAGGGATGGGTGTTCCCAGGCGTTGGAGAATTCATATGCTATGATAAGTACCTGCAAGCAGACTTCAAAGCAGC  700
186  R  Y  P  S  Y  P  Q  S  Q  G  W  V  F  P  G  V  G  E  F  I  C  Y  D  K  Y  L  Q  A  D  F  K  A  A  218

701  AGCAGAAGAGGCTGGGCATCCTGAGTGGGATTTGCTTGATGATGCTGGGACGTACAATGACACTCCTGAGAAGACCCAGTTCTTCGCGGATAACGGAACA  800
219  A  E  E  A  G  H  P  E  W  D  L  L  D  D  A  G  T  Y  N  D  T  P  E  K  T  Q  F  F  A  D  N  G  T  251

801  TACCAGACCGACAAGGGGAAGTTCTTCCTCACATGGTACTCAAACAAACTGATCAAGCACGGTGATAAGATCTTGGACGAAGCAAACAAGGTCTTCCTGG  900
252  Y  Q  T  D  K  G  K  F  F  L  T  W  Y  S  N  K  L  I  K  H  G  D  K  I  L  D  E  A  N  K  V  F  L  G  285

901  GATGCAAGGTGCAGCTGGCAATCAAAGTCTCTGGCATACACTGGTGGTACAACGTTCCAAACCATGCAGCTGAGCTCACTGCCGGATACTACAACTTAGA  1000
286    C  K  V  Q  L  A  I  K  V  S  G  I  H  W  W  Y  N  V  P  N  H  A  A  E  L  T  A  G  Y  Y  N  L  D  318

1001 TGACAGAGATGGCTACAGAACCATAGCACACATGCTCACAAGGCATCGTGCTAGCATGAACTTCACTTGTGCTGAGATGAGGGACAGTGAACAGAGTTCA  1100
319  D  R  D  G  Y  R  T  I  A  H  M  L  T  R  H  R  A  S  M  N  F  T  C  A  E  M  R  D  S  E  Q  S  S  351

1101 GAGGCGAAAAGTGCACCTGAGCAACTGGTTCAACAGGTGCTGAGTGCTGGATGGAGAGAGGGCCTAAATTTGGCATGTGAAAATGCACTCAACCGATATG  1200
352  E  A  K  S  A  P  E  E  L  V  Q  Q  V  L  S  A  G  W  R  E  G  L  N  L  A  C  E  N  A  L  N  R  Y  D  385

1201 ATGCAACAGCTTACAACACCATCCTCAGCAATGCAAGACCTCAAGGCATCAACAAGAATGGCCCTCCAGAACACAAGTTGCACGGATTCACCTACCTCCG  1300
386    A  T  A  Y  N  T  I  L  R  N  A  R  P  Q  G  I  N  K  N  G  P  P  E  H  K  L  H  G  F  T  Y  L  R  418

1301 AGTATCTGATGAACTGTTCCAGGAACAGAACTACACCACTTTCAAAACTTTTGTCAGGAGAATGCATGCTAACCTGGATTATAATCCAAATGTCGATCCA  1400
419  V  S  D  E  L  F  Q  E  Q  N  Y  T  T  F  K  T  F  V  R  R  M  H  A  N  L  D  Y  N  P  N  V  D  P  451

1401 GTTGCACCATTGGAAAGATCAAAGGCAGAGATACCAATTGAAGAAATCCTAGAAGTAGCACAGCCAAAATTGGAGCCATTTCCCTTCGACAAGGACACCG  1500
452  V  A  P  L  E  R  S  K  A  E  I  P  I  E  E  I  L  E  V  A  Q  P  K  L  E  P  F  P  F  D  K  D  T  D  485

1501 ACCTACCAGTTTAAACACTCCAATGATGCAAACTACAGGAAGAATCACGAGACACTTAAAAAATCAAGACAAATAGTAAGCCTATTGTCGAGCAGTAGTC  1600
486    L  P  V

1601 TATTAACTAAATAAAATGCTGTGTGGAGCATATTATAATCGCATATTGCCCTAATAATATTGTGTTGTTAAACCTGAGGCTTGATGGTTATCTGATGAGT  1700
1701 TGTAATAATAGCCTTGGTCTTCCTGTTGTATGTATGTATTTGAGCTAAGGAATAAAAATTGTGGCTTTGTGGGTT(A)9
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N-terminal blockage prevented protein sequence determination from freshly purified β-amylase. However, after prolonged storage of the purified protein at −20°C, a slightly smaller protein was formed that was not blocked and that yielded a 20-amino-acid, N-terminal peptide sequence (ALANYVQVYVMLPLDVITVD), which corresponded precisely to amino acids 5 through 24 of the large ORF found in the cDNA. This result confirms that the cDNA we isolated encodes the maize β-amylase protein purified from aleurone cells. Although the degraded product has 4 amino acids removed from the N terminus, the colinearity with the N-terminal region of the cDNA indicates that the maize gene lacks a conventional signal peptide. In this respect, the maize protein resembles other β-amylase proteins found in Arabidopsis (Monroe and Preiss, 1991), soybean, and sweet potato (Mikami et al., 1986), which also lack conventional signal peptides. It had been shown that under in vitro experimental conditions both the aleurone layer and the scutellum secrete β-amylase during maize seed germination, the secretion being stimulated by Ca^{2+} (Lauriere et al., 1992). Given that no β-amylase mRNA or protein was detected in the scutellum (see later sections), we thought that these previous results may be due to the leakage of β-amylase from aleurone cells during in vitro treatments. Alternatively, if this secretion process does exist in vivo, it may not depend on an N-terminal hydrophobic signal peptide, as reported for chicken ovalbumin (Gagnon et al., 1978) and human plasminogen activator inhibitor-2 (Ye et al., 1988).

The molecular mass of maize β-amylase protein that was predicted from the cDNA sequence was 54 kD, which is smaller than the apparent molecular mass (60 kD) that was determined by SDS-PAGE (Fig. 2). To determine whether any posttranslational modifications were present on maize β-amylase, we performed several immunological lectin-binding assays (including *Maackia amurensis* agglutinin, *Galanthus nivalis* agglutinin, *Sambucus nigra* agglutinin, and *Datura stramonium* agglutinin; data not shown) but were unable to detect carbohydrate modifications. To further examine the possible posttranslational modifications, we compared the in vitro translated protein with the in vivo labeled β-amylase by immunoprecipitation and SDS-PAGE analysis. The results indicated that both the in vitro and the in vivo proteins have similar mobilities, approximately 60 kD on SDS-gels (Fig. 2). Thus, it appears that there is no major posttranslational modification of the maize β-amylase protein. The difference in predicted and apparent molecular mass may be due simply to anomalous migration, as has been observed for other proteins on SDS-gels (Hayes and Mantle, 1986). It has been suggested that anomalous migration may be due to some proteins not adopting a random-coil configuration after denaturation in the presence of SDS (Hayes and Mantle, 1986).

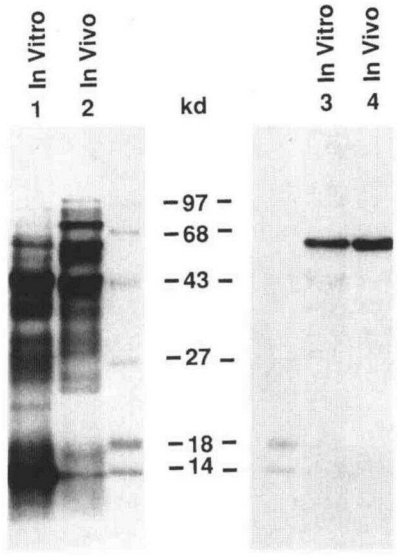

Figure 2. No apparent posttranslational modification of β-amylase. Total RNA (6 μg) isolated from aleurone layers of seeds germinated for 5 d was translated in vitro with reticulocyte lysate labeled with [^{35}S]Met and 25,000 cpm was loaded in the SDS-PAGE (lane 1). Ten seeds germinated for 5 d were labeled in vivo with [^{35}S]Met for 90 min and the extracted proteins (25,000 cpm) were separated by SDS-PAGE (lane 2). The in vitro translated products and in vivo labeled proteins (370,000 cpm) were immunoprecipitated with rabbit polyclonal antibody raised against purified maize β-amylase (lanes 3 and 4).

Maize β-Amylase Is Present as a Single-Copy Gene

It has been shown that the β-amylase gene in barley, rye, and rice exists as a member of a multigene family (Kreis et al., 1987; Rorat et al., 1991). To analyze whether the maize gene also represents a member of a multigene family, we performed Southern blot analysis on two maize inbred lines (TA80-1410 and TA80-2598) and a series of recombinant inbred lines for gene mapping. Hybridization and washing were done under low-stringency conditions that would permit the detection of related but nonidentical sequences. Genomic DNA of TA80-1410 and TA80-2598 were digested with several restriction enzymes and probed with a radioactively labeled pZMB2 cDNA insert. In TA80-1410, both *Hin*dIII and *Xho*I digests showed a single band of hybridization, and two *Bam*HI bands were detected (Fig. 3A). Similarly, in TA80-2598, both *Bam*HI and *Xho*I digests showed a single band of hybridization, and two *Hin*dIII bands were detected (Fig. 3B). This result can be explained in two ways: either there is a single gene with an internal *Bam*HI site in TA80-1410 and an internal *Hin*dIII site in TA80-2598 or there are two very similar genes. The RFLP analysis described below supports the former possibility.

For the RFLP analysis, maize genomic DNA of two inbred lines, CM37 and T232, which were digested with *Sst*I and probed with a radioactively labeled pZMB2 cDNA insert, showed two RFLP bands in each line (Fig. 3B, 3.4 and 2.4 kb in CM37 line; 3.7 and 2.1 kb in T232 line). Since an *Sst*I site is present in the cDNA clone, these two bands are derived from a single genomic DNA fragment. In the RFLP analysis with recombinant inbreds derived from the two parental inbreds, we found that the two bands of parental lines co-segregated. The data indicate that the maize β-amylase cDNA clone hybridized to a single locus at position 83 centimorgans on chromosome 7L. No cross-hybridization to other loci was detected. A single-gene

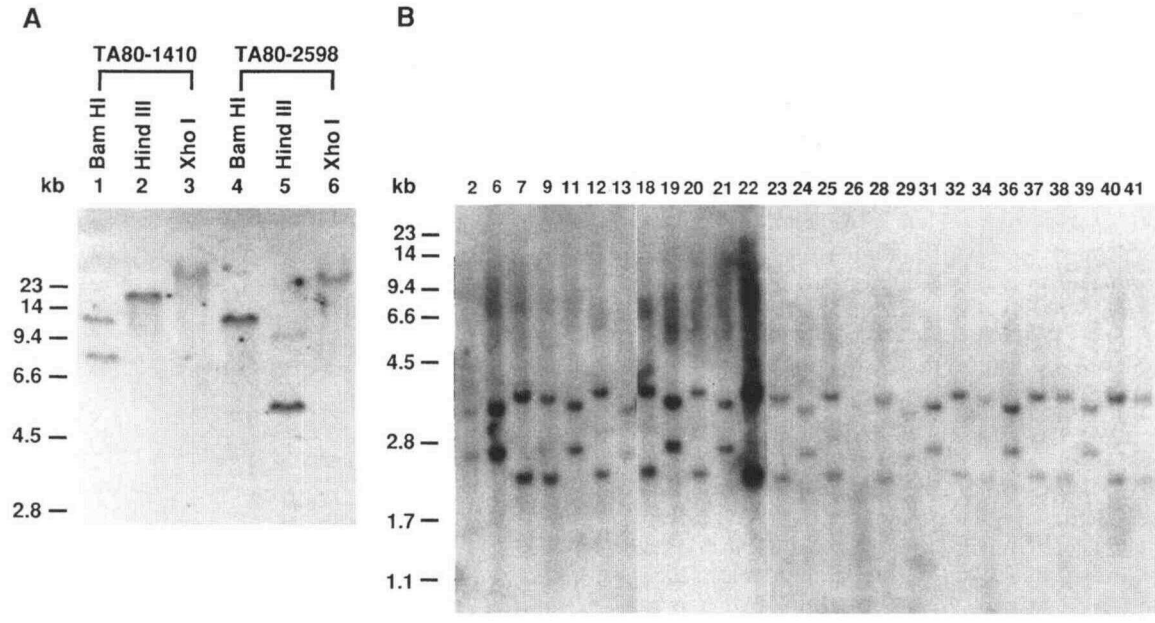

Figure 3. Southern blot and RFLP analyses of the maize β-amylase gene. A, Genomic DNA (3 μg) of maize inbred lines was digested with the restriction enzymes indicated and probed with the cDNA clone pZMB2. B, For RFLP analysis of the recombinant inbred lines derived from CM37 and T232, DNAs were digested with *Sst*I and probed with pZMB2. The number on the top of the gel indicates the individual number of the recombinant inbred lines. The recombinant lines have either 3.4- and 2.4-kb hybridizing fragments (the same as the CM37 parental line) or 3.7- and 2.1-kb hybridizing fragments (the same as the T232 parental line).

organization of β-amylase in maize is also consistent with evidence presented by Lauriere et al. (1992). Our results are inconsistent with the mapping data of Chao and Scandalios (1969), which placed the gene at position 5S-17 centimorgans based on the eletrophoretic mobility polymorphisms on zymograms. This discrepancy may be due to either misidentification of the band representing β-amylase activity or the presence of a different β-amylase isozyme encoded by a highly divergent gene.

Expression of Maize β-Amylase during Seed Germination

It has been shown that the barley β-amylase is synthesized during seed development as a bound-form precursor and is released to its free form by protease during seed germination (Sopanen and Lauriere, 1989). We used native gel and western blot analyses to examine the expression of β-amylase during maize seed development and germination and found activity at both times. However, β-amylase activity (data not shown, fig. 1 of Lauriere et al., 1992) and proteins (examined by western blot analysis, Fig. 4) could not be detected in the mature, dry seeds. β-Amylase activity was found in the aleurone layer but not in the scutellum during seed germination by assaying amylolytic activity of the native gel (Fig. 5A). The β-amylase activity was observed in the aleurone layers of germinating seeds after d 2, reached its peak at d 5 to 6, and was maintained at a high level through d 7 to 10 (Fig. 5A).

To demonstrate that β-amylase is synthesized in germinating seeds, in vitro translation-immunoprecipitation and northern blot analysis were performed. Total RNA isolated from the aleurone layer and the scutellum of germinating maize seeds were in vitro translated and then immunoprecipitated with rabbit polyclonal antibody against purified maize β-amylase. The β-amylase protein can be detected in the in vitro translated products using RNA isolated from the aleurone layer but not from the scutellum (Fig. 5C). Furthermore, northern blot analysis showed that during seed germination β-amylase mRNA was detected in total RNA isolated from the aleurone layer but not from the scutellum (Fig. 6), which is consistent with the distribution of the protein (Fig. 5). However, the β-amylase mRNA in the aleurone layers appeared at d 1, increased with time

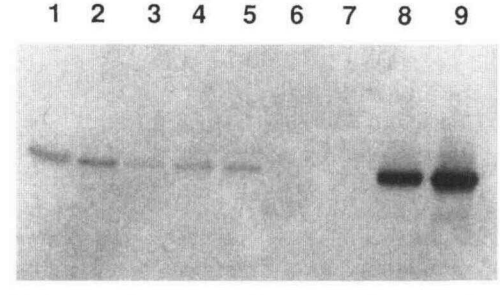

Figure 4. Western blot analysis of β-amylase in developing kernels, dry kernels, and germinating seeds. Proteins (30 μg) were extracted, separated by SDS-PAGE, and electrotransferred to a nitrocellulose filter. The β-amylase was detected with rabbit antibody against purified maize β-amylase protein. Lanes 1 to 5, Proteins isolated from developing kernels at stages 1 to 5; lane 6, protein extracted from dried, mature kernels; and lanes 7, 8, and 9, proteins isolated from germinating seeds for 1, 3, and 5 d, respectively.

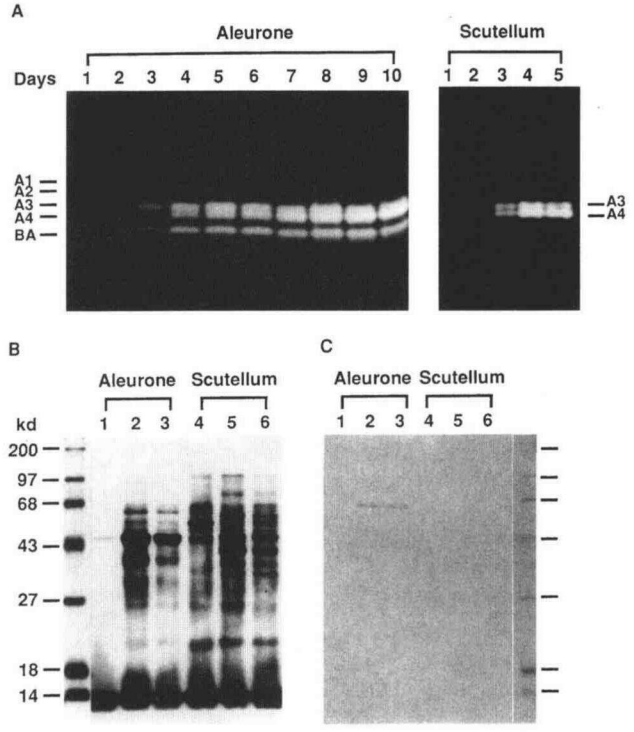

Figure 5. β-Amylase activity and RNA message in aleurone and scutellum of germinating seeds. A, Zymogram of the amylolytic activities in the aleurone and scutellum from germinating seeds. Proteins (4 μg) were extracted from aleurone and scutellum tissue isolated from seeds germinated for the indicated number of days and separated by a native gel. The amylolytic activity was detected by impregnating the gel in 4% starch solution for 80 min and then staining with I_2-KI solution. The designation of bands is according to the method of Wang et al. (1992). B, In vitro translation of RNA isolated from aleurone and scutellum of germinating seeds. Total RNA (6 μg) was isolated from aleurone and scutellum of germinating seeds and was translated in vitro with reticulocyte lysate. The in vitro translated products were separated by SDS-PAGE and then fluorographed. Lanes 1, 2, and 3, Samples of aleurone tissue from germinating seeds of 1, 3, and 5 d, respectively. Lanes 4, 5, and 6, Samples of scutellum tissue from germinating seeds of 1, 3, and 5 d, respectively. C, Immunoprecipitation of in vitro translated products. In vitro translated products (the same as in B) were immunoprecipitated with rabbit antibody against maize β-amylase. The immunoprecipitated products were separated by SDS-PAGE and then fluorographed. The order of samples is the same as in B.

during seed germination, reached its peak at d 3 to 5, and decreased thereafter (Figs. 5C, lanes 2 and 3, and 6, lanes 1–4). The relatively high-level enzyme activity (Fig. 5A, d 7–10) but low mRNA level (Fig. 6, lane 4) during the later stage of seed germination suggests that the β-amylase protein is relatively stable and accumulates at that stage.

Our results demonstrate that the β-amylase present in germinating maize seeds is synthesized de novo, which is clearly different from the barley β-amylase that is synthesized during seed development as a latent precursor, and processed to the free form during seed germination (Shewry et al., 1988). The presence of β-amylase activity in the aleurone tissue of germinating seeds is similar to the observations of Lauriere et al. (1992); however, they reported finding a minute amount of β-amylase activity in the scutellum tissue. This discrepancy may have been due to contamination by aleurone tissue in the dissected scutellum tissue.

Phylogenetic Analysis of β-Amylase Genes

The comparison of derived maize β-amylase protein sequence from the cDNA clone with other known β-amylase sequences available in the GenBank database showed a high degree of homology. However, there are no carboxyl-terminal Gly-rich repeats in the β-amylase of maize, rice, and several dicotyledons or in the ubiquitous type of β-amylase from rye, whereas these are present in the endosperm type β-amylase of barley and rye (Rorat et al., 1991). Barley and rye are members of the Triticeae, a tribe of the Festucoideae subfamily (Lauriere et al., 1992). For the Triticeae type, the endosperm type β-amylase accumulates in seeds in both free and starch granule-bound forms, and upon germination the β-amylase undergoes proteolytic modification. However, some other members of the Gramineae family, e.g. maize and rice, contain the ubiquitous type of β-amylase but not the endosperm type β-amylase. We analyzed the evolutionary distance and phylogeny of β-amylases from different species by the DISTANCE and GROWTREE programs of the GCG sequence analysis package (Genetics Computer Group), which showed that the β-amylases of dicotyledonous species and monocotyledonous species separated into two clusters. In addition, within the monocotyledonous species the endosperm type β-amylases branched from the ubiquitous type β-amylases, suggesting a distinct evolution route of the endosperm β-amylase from the ubiquitous β-amylases (Fig. 7). The

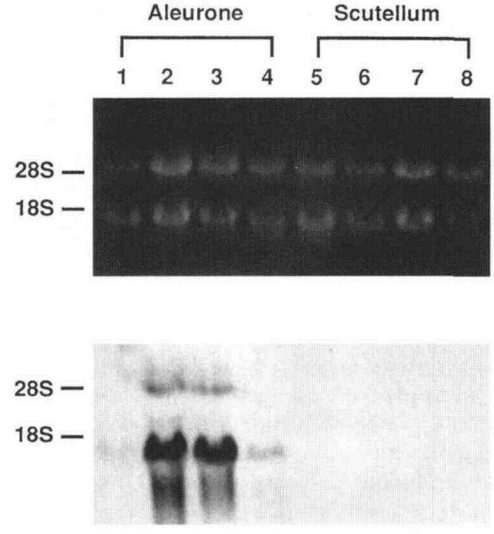

Figure 6. Northern blot analysis of RNA isolated from the aleurone layer and scutellum of germinating seeds. Total RNA (40 μg) was separated by 1% formaldehyde agarose gel and probed with pZMB2. Top, RNA stained with ethidium bromide before blotting. Bottom, Hybridization results. RNA in lanes 1 and 5 was isolated from maize seeds germinated for 1 d; lanes 2 and 6 were from d 3; lanes 3 and 7 were from d 5; and lanes 4 and 8 were from d 7.

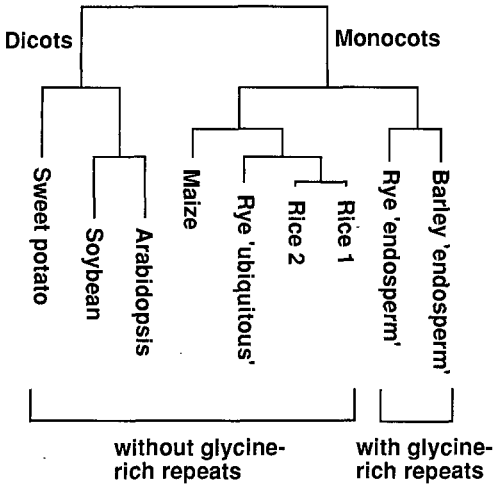

Figure 7. Phylogenetic dendrogram of the β-amylase protein sequences from different species. β-amylase protein sequences of sweet potato (accession no. D01022), soybean (accession no. M92090), Arabidopsis (accession no. M73467), barley (accession no. D21349), rye (accession nos. Z11772 and X56785), rice (accession nos. L10345 and L10346), and maize (accession no. Z25871) found in the GenBank database were aligned by the PILEUP program of the GCG DNA sequence analysis package (Genetics Computer Group). The evolutionary distances and the phylogenetic dendrogram were analyzed by the DISTANCE and GROWTREE programs. The two types of β-amylase of rye were labeled as rye "endosperm" and rye "ubiquitous." The two rice β-amylase sequences were labeled as rice 1 and rice 2.

different expression pattern and posttranslational processing between the endosperm and ubiquitous type β-amylases imply that the divergence in regulatory mechanisms of these two types is accompanied by changes of the gene structure.

CONCLUSION

We have isolated and sequenced cDNA clones encoding maize β-amylase, which was previously reported to be a secretory protein (Lauriere et al., 1992). However, our protein sequence analysis indicated that maize β-amylase does not contain a canonical N-terminal signal peptide sequence. It would be interesting to analyze the mechanism of the possible secretion process.

In maize and rice (Wang et al., 1996), β-amylase is synthesized de novo during seed germination and accumulates because of a slow turnover rate. On the contrary, the β-amylase present in the seeds of barley, rye, and wheat is synthesized during seed development and is processed during seed germination (Rowsell and Goad, 1962; Duffus and Rosie, 1972; Hara-Nishimura et al., 1986). Southern blot and RFLP analyses indicated that there is only a single gene in the maize genome encoding the β-amylase. Because there is no endosperm type β-amylase in maize, it will be of interest to investigate further the mechanism of divergence in the regulation of ubiquitous β-amylase gene expression from that of endosperm β-amylase genes.

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The GenBank accession number for the sequence reported in this article (the maize β-amylase cDNA sequence) is Z25871.

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