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Organ-Specific and Hormone-Dependent Expression of Genes for Serine Carboxypeptidases during Development and Following Germination of Rice Grains

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Several cDNA clones encoding either serine carboxypeptidases or related proteins of *Oryza sativa* L. were identified, and the abundance of the corresponding mRNA in immature and germinated grains was examined. The deduced amino acid sequence of each cDNA included key sequences, such as a pentapeptide (G-X-S-X-G/A) that is conserved among many serine carboxypeptidases, and the putative protein products were classified as two general and one novel type of cereal serine carboxypeptidases. Two general types exhibited considerable homology to type I and type III carboxypeptidases of cereal plants. The novel type encoded a serine carboxypeptidase-like protein that was very similar to type III carboxypeptidases of barley and wheat but had slight differences in both the N- and the C-terminal sequences. The mRNAs of each of these carboxypeptidases were observed in immature grains, and they decreased during maturation. The abundance of mRNA for each class of carboxypeptidase increased again following germination with the same time course and in a tissue-specific manner. The mRNAs for type I and type III-like carboxypeptidases were abundant in germinated embryos composed of leaf, root, and scutellum, whereas the mRNA for type III carboxypeptidase was conspicuous in endosperm that contained the aleurone layer. Altered amounts of mRNA in deembryonated half-grains in response to phytohormones, such as gibberellic acid and abscisic acid, were only detectable in the case of type III carboxypeptidase. Southern blot analysis using rice genomic DNA revealed the simple organization of each gene for these three classes of carboxypeptidases.

CPD is an exopeptidase capable of releasing free amino acids from the C-terminal ends of proteins. Ser CPDs have an active Ser residue necessary for hydrolysis of the peptide bond. Following germination of cereal grains, the catalytic activities of CPDs are assumed to be responsible for the effective mobilization of stored proteins (see Fincher, 1989, for review). Five classes of Ser CPDs (types I–V) have been identified in the germinated grains of wheat (*Triticum aestivum*) (Mikola, 1986), barley (*Hordeum vulgare*) (Mikola, 1983), and rice (*Oryza sativa*) (Doi et al., 1980). Of these, the primary structures of barley type I (Sorensen et al., 1986), type II (Sorensen et al., 1987), and type III (Sorensen et al., 1989) were determined by automated amino acid sequencing of the purified enzymes, and it was proven that these three classes of CPDs originate from different genes. Both type I and type II CPDs in barley are composed of two identical subunits that contain an A- and a B-chain, whereas type III CPD in barley is composed of 411 amino acids and is active in a monomeric form.

The stage and site of synthesis of each class of CPD in germinated grains have been deduced from the various enzymic activities in excised tissues (Mikola and Kolehmainen, 1972; Schroeder and Burger, 1978) and by specific antisera raised against purified enzymes (Mundy et al., 1985). Activities of barley type II CPD are already present in resting grains and decrease after imbibition, whereas those of type I and type III CPDs appear in later stages after germination. Use of specific antisera against purified type I CPD in barley revealed that the bulk of type I CPD was synthesized and secreted from the scutellum of germinated grains. Although these observations provide details of the occurrence of these CPDs at different times and at different sites in cereal grains, it is difficult to determine the real distribution of each class of CPD in germinated grains given their similar substrate specificities. Information about the abundance of mRNAs for several classes of CPDs following germination should provide some clues. The accumulation of mRNA for type I CPD in the scutellum was confirmed by RNA blot analysis with a cDNA probe for barley type I CPD (Ranki et al., 1990). Similar analysis indicated that the amounts of mRNA for type III CPD in the aleurone layer of germinated wheat grains were promoted by GA_3 (Baulcombe et al., 1987).

In this study, we tried to isolate cDNA clones for rice Ser CPDs from independent cDNA libraries prepared from germinated embryos, endosperms plus aleurone layer, and immature grains. Obtained cDNAs were identified into two known CPDs (type I and III CPDs) and one unknown CPD-like protein (type III-like CPD). Each type of encoded CPD had characteristic features of an active Ser CPD, and their mRNAs accumulated in germinated grains in a tissue-specific manner. Temporal and spatial expression observed in several Ser CPDs suggests a distinct physiological role for each class of CPD following germination in cereal grains.

MATERIALS AND METHODS

Plant Material

Rice grains (*Oryza sativa* L. cv Yukihikari; kindly supplied by the Hokkaido Central Agricultural Experiment Station,

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Abbreviations: CPD, carboxypeptidase; WAF, weeks after flowering.

Iwamizawa, Japan) and deembryonated half-grains were surface sterilized in 1% sodium hypochlorite for 20 min, rinsed thoroughly, immersed in water that contained 20 mM $CaCl_2$, 10 μg/ml chloramphenicol, and 25 units/ml nystatin, and germinated in the dark at 27°C. Germinated grains were dissected into leaves, roots, scutella, and endosperms plus the aleurone layer at appropriate times. Immature grains were harvested from rice plants grown in a paddy field at weekly intervals after flowering. Samples were immediately frozen in liquid nitrogen.

Isolation of DNA and RNA

High mol wt DNA was prepared from 7-d-old seedlings by the procedure of Murray and Thompson (1980). Total RNA was extracted from frozen samples by the SDS-phenol method, as described by Harris and Dure (1978). Poly(A)-enriched RNA was obtained by batchwise precipitation with latex resin conjugated with oligo(dT) (Oligotex-dT30; Japan Roche, Tokyo, Japan).

Construction and Screening of cDNA Libraries

Double-stranded cDNA was synthesized from each sample of poly(A)-enriched RNA that had been prepared from 2-d-germinated embryos, endosperms plus aleurones, and immature grains (2 WAF) by the procedure of Gubler and Hoffman (1983). The cDNAs supplemented with *Eco*RI adapters were cloned into the *Eco*RI site of λgt11 and packaged in accordance with the manufacturer's instructions (Amersham, Buckinghamshire, UK). The cDNA libraries were screened by stepwise hybridizations according to the general procedure of Sambrook et al. (1989). At the first stage, cDNA clones encoding type I CPD were selected with a synthetic oligonucleotide probe for barley type I CPD. A 34-mer oligonucleotide (5'GGGCATGGATTGCAGATCTG ACGGCAGCGTTGTC3') corresponding to the sequence of a cDNA clone for type I CPD in barley (Doan and Fincher, 1988) was end labeled with [γ-^{32}P]ATP (4000 mCi/mM; ICN, Costa Mesa, CA) and T4 polynucleotide kinase and subjected to the plaque hybridization. The cDNA fragments excised from the cDNA clones for rice type III CPD (Washio and Ishikawa, 1992) and from the positive clone that had been shown to encode type I CPD of rice were labeled by the random priming method (Feinberg and Vogelstein, 1984) using [α-^{32}P]dCTP (3000 mCi/mM, ICN) and the Klenow fragment of DNA polymerase from *Escherichia coli*, and they were used in subsequent screening for the cDNA clones that encoded other classes of CPDs.

Sequence Analysis

The cDNA fragments derived from positive clones were subcloned into the pBluescript plasmid (Stratagene, La Jolla, CA), and their nucleotide sequences were determined (Sanger et al., 1977). The nucleotide and predicted amino acid sequences were subjected to a search for homologies in the data bases of GenBank, EMBL, NBRF, and SwissProt, using the GENETYX software package (Software Development, Tokyo, Japan).

DNA and RNA Blot Analysis

Fragments of genomic DNA that had been digested to completion with several restriction enzymes were separated by electrophoresis on a 0.8% agarose gel. Samples of RNA, denatured in the presence of formaldehyde, were subjected to electrophoresis on a 1.2% agarose gel that contained 0.66 M formaldehyde. After electrophoresis, nucleic acids were blotted onto nylon membranes (Hybond-N, Amersham) and hybridized with a ^{32}P-labeled probe prepared from each class of rice CPD as follows. The *Hin*dIII-*Sph*I fragment (415 bp, nucleotides 469–883), the *Xho*I-*Eco*RI fragment (492 bp, nucleotides 1237–1728), and the *Eco*RI-*Hin*dIII fragment (444 bp, nucleotides 1160–1603) were prepared from cDNA clones that encoded type I, type III, and type III-like CPD, respectively, and each was labeled by the random priming procedure. These DNA fragments were designed to minimize cross-hybridization between sequences. Hybridizations were performed using the general procedure described by Sambrook et al. (1989).

RESULTS

Identification of Rice Ser CPDs

Several candidate cDNAs that appeared to encode a Ser CPD and/or a related protein were arranged in three groups. Two of them showed clear identity in terms of nucleotide and amino acid sequences to cereal type I and type III CPDs, respectively. The existence of the gene for type III CPD in rice was presented in our previous study (Washio and Ishikawa, 1992). The message transcribed from the gene for type I CPD is estimated to be about 2.0 kb in length and to encode 510 amino acids (M_r 55,709; Fig. 1). It was known that the precursor sequence of barley type I CPD was divided into three parts: the A-chain, the linker peptide, and the B-chain (Doan and Fincher, 1988). The same distribution of protein-coding regions was also found in the mRNA for rice type I CPD, and the extent of homology of the amino acid sequence between these two CPD I was 81.6%. The similarities are also exemplified by their linker peptides, but a five-amino acid insertion (R-G-S-R-P, amino acid positions 357–361) is observed on the C-terminal side of the linker peptide in rice type I CPD. In addition to the amino acid sequences similar to that of barley type I CPD, an N-terminal extension can be found in the type I CPD of rice. Charged residues (Arg) are followed by a cluster of hydrophobic residues, such as Ala, Val, Leu, etc., showing a characteristic of a signal peptide (Watson, 1984).

Another group of cDNA clones included sequences that exhibited a close correlation in terms of nucleotide and amino acid sequences with peptidase domains in cereal type III CPDs. The mRNA for type III-like CPD in rice encodes 429 amino acids (M_r 47,745; Fig. 1), which correspond to the amino acid sequences of type III CPDs in cereals. However, similarities are not apparent at either the N- or C-terminal ends. Identities at the C-terminal end between type III-like and type III CPDs in rice (Washio and Ishikawa, 1992) are scarcely detectable, and two extra potential sites for glycosylation (N-X-S/T) are found near the C terminus of the type III-like CPD. The N-terminal prosequences in type III CPD

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Type I carboxypeptidase

R-CPD1 MARRGRRSLASPAVAIALFVFLAYGGGGGGGGVCEAAPASAVVKSVPGFDGALPSKHYAG  60
B-CPD1                                    ..QG.E.TGL.............  24
W-CPD2                                  .EPSGH.ADRIARL..-QP.VDFDM.S.  27

R-CPD1 YVTVEEQHGRNLFYYLVESERDPAKDPLVLWLNGGPGCSSFDGFVYEHGPFNFESGGSAK 120
B-CPD1 ....D.G.........V.......G...V.............................V.  84
W-CPD2 .I..D.GA..S...L.Q.APE.AQPA.............V-AYGASEELGA.RVKPRGA  86

R-CPD1 SLPKLHLNPYSWSKVSSVIYLDSPAGVGLSYSKNTSD-YNTGDLKTAADSHTFLLKWFQL 179
B-CPD1 ..........A.....TM.................V.....E.......T.......... 143
W-CPD2 G.---V..E.R.N..AN.LF........F..TNTS..I.TS..NR..H..YA..A...ER 143

R-CPD1 YPEFLSNPFYIAGESYAGVYVPTLSHEVVKGLHDGVKPTINFKGYMVGNGVCDTVFDGNA 239
B-CPD1 ............................IQG.A.....................I..... 203
W-CPD2 F.HYKYRD..........H...E..Q----LV.RSKN.V..L..F.....LI.DYH.YVG 199

R-CPD1 LVPFAHGMALISDDIYQEAQTACHGNYWNTTTDKCENALYKVDTSINDLNIYDILEPCYH 299
B-CPD1 ........G....E...Q.S.S.......A.DG..DT.IS.IESL.SG............ 263
W-CPD2 TFE.WWNHGIV...T.RRLKE..LHDSFIHPSPA.DA.TDVATAEQGNIDM.SLYT.VCN 259

R-CPD1 SKTIKKVTPANTKLPKSFQHLGTTTKPLAVRTRMHGRAWPLRAPVRAGRVPSWQEFARGS 359
B-CPD1 .RS..E.NLQ.S...Q..KD....N..FP.....L..........K.........V.--- 320
W-CPD2 lTSS------------------------------------------------------- 263

R-CPD1 RPSGVPCMSDEVATAWLNNDDVRAAIHAQPVSSIG-SWLICTNVLDFI-HDAG-SMISYH 416
B-CPD1 --.............D.AA..S.....S..A...P..L..DK.Y.V.........A.. 375
W-CPD2 TG.YD..-TERYS..YY.RR..QM.L..NVTGAMNYT.AT.SDTINTHW...PR..LPIY 322

R-CPD1 KNLTGQGYRAFIYSGDHDMCVPYTGTEAWTRSLGYGVIDSWRPWHLNGQVSGYTQGYEHG 476
B-CPD1 ....S.....I.F.........F..S....K......V......IT........E..... 435
W-CPD2 RE.IAA.L.IWVF...T.AV..L.A.RYSIGA..LPTTT..Y..YDDQE.G.WS.V.-K. 381

R-CPD1 LTFATIKGAGHTVPEYKPQESLAFYSRWLAGSKL*                          510
B-CPD1 .....................AF.............*                          469
W-CPD2 ..LVSVR....E..LHR.RQA.VLFQYF.Q.KPMPGQTKNAT*                   423

Type III carboxypeptidase

R-CPD3  MATARVSLILLVVVLAASACAEGLRLPRDAKFPAAQAERLIRSLNLLPKEAGPTGAGDVP  60

R-CPD3  SVAPGELLERRVTLPGLPQGVGDLGHHAGYYRLPNTHDARMFYFLFESRGKK-ED-PVVI 118
R-CPD3L          MA·GKSGGSSAE.................L...F.....S.G..D....  50
B-CPD3               .....E..A.....................F.....................  45

R-CPD3  WLTGGPGCSSELAVFYENGPFTISNNMSLAWNKFGWDTISNIIFVDQPTGTGFSYSSDDR 178
R-CPD3L .............L.......H.AD....V..D....QE..L.Y.............NP. 110
B-CPD3  ....................A.....V.......K.....................  105

R-CPD3  DTRHDETGVSNDLYSFLQVFFKKHPEFAKNDFFITGESYAGHYIPAFASRVHQGNKANEG 238
R-CPD3L .......A.......A...A..TE..N.....Y.....................YK...NS.. 170
B-CPD3  ............D.....................I.....................K...  165

R-CPD3  IHINLKGFAIGNGLTDPAIQYKAYTDYALDMNLIKKSDYDRINKFIPPCEFAIKLCGTNG 298
R-CPD3L .............................S...G..T..QFN....IV.T..L.......S. 230
B-CPD3  T.............................E....Q.A..E.....................  225

R-CPD3  KASCMAAYMVCNSIFSSIMKLVGTKNYYDVRKECEGKLCYDFSNLEKFFGDKAVKEAIGV 358
R-CPD3L TI..LG..V...L.C...ETII.K.....I..P.V.S....L..M...LQL.S.R.SL.. 290
B-CPD3  ............T..N.....................................RQ.....  285

R-CPD3  GDLEFVSCSTTVYQAMLTDWMRNLEVGIPALLEDGINVLIYAGEYDLICNWLGNSRWVHS 418
R-CPD3L ..IQ.....P.......L............E...ND.K.....................N. 350
B-CPD3  ..I.......S.....................................................  345

R-CPD3  MEWSGQKDFVSSHESPFVVDGAEAGVLKSHGPLSFLKVHNAGHMVPMDQPKASLEMLRRF 478
R-CPD3L .....KEA....S.E..T...K...I...Y.........D.............VA....M.W 410
B-CPD3  .........AKTA..S.L..D.Q.........A.....................A.......  405

R-CPD3  TQGKLKEEWLAELPEQPMYAAM*                                      500
R-CPD3L .S.N.SNASSSFQRLDFTM*                                         429
B-CPD3  ......                                                       411
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Figure 1. Comparison of amino acid sequences of cereal Ser CPDs. Each amino acid sequence predicted from the cDNA sequence for the type I and the type III CPDs in rice is aligned with those of the respective barley type I (B-CPD1; Doan and Fincher, 1988), wheat type II (W-CPD2; Liao et al., 1992) and those of rice type III-like (R-CPD3L), and barley type III CPDs (B-CPD3; Sorensen et al., 1989). Identical and similar amino acids are indicated by dots and shaded letters. Amino acid motifs of putative active sites (hexapeptide, His and Asp residues) are in bold letters. The linker peptide of the type I CPDs and potential sites for glycosylation (N-X-S/T) are underlined and wavy-lined, respectively. The nucleotide sequence data will appear in the DDBJ, EMBL, and GenBank nucleotide sequence data banks with the accession numbers D17586, D17587, and D10985 for rice type I, type III-like, and type III CPDs, respectively.

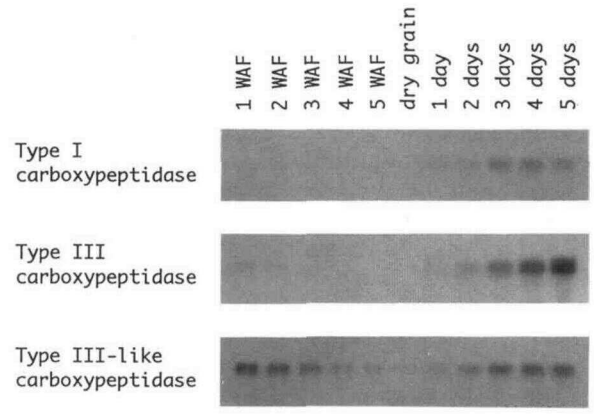

Figure 2. Time courses of the accumulation of mRNAs for rice grain carboxypeptidases. Poly(A)-enriched RNA (0.5 μg/lane) prepared from immature grains (1–5 WAF) or total RNA (5 μg/lane) extracted from germinated grains (dry grains, 1–5 d) was subjected to electrophoresis, blotted, and hybridized to the probe indicated to the left of each panel.

do not exist in the type III-like CPD of rice. The first initiation codon (ATG) of type III-like CPD was determined from the deduced open reading frame in the full-length cDNA sequence, which was obtained by the technique of rapid amplification of cDNA ends (Frohman et al., 1988; data not shown), and it corresponds to the conserved nucleotide sequence found in many organisms (Kozak, 1981). The decided first ATG codon is suitable for the N-terminal end of mature type III CPD in barley and no N-terminal extension is found. In spite of the divergence at both terminal sequences, type III-like CPD has a hexapeptide that includes a Ser residue (G-E-S-Y-A-G, amino acid positions 146–151). This Ser residue, in association with His-393 and Asp-336, are applicable to the three key residues of wheat CPD II that form the "catalytic triad" required for full activity of Ser CPDs (Galjart et al., 1990; Liao et al., 1992).

Accumulation of the mRNAs

The accumulation of mRNAs for each class of CPD occurs with a similar time course during development and following germination of rice grains (Fig. 2). The amount of mRNAs for the three classes of CPDs in early immature grains (1 WAF) are low, although there is a higher proportion of type III-like CPD, allowing detection by RNA blot analysis with total RNA fractions. This mRNA abundance for the three

CPDs decreases as the rice grains mature. At the postgermination stage, detectable signals for transcripts of all three CPDs appear on the 1st d after imbibition, and increasing amounts of the three mRNAs can be detected for the next 4 d. A slight decline in the mRNA for type I CPD is observed on d 5.

It seems likely that each class of cereal CPDs is expressed in a tissue-specific manner in germinated grains according to reports of the mRNAs for barley type I (Ranki et al., 1990) and some type III (Baulcombe et al., 1987; Washio and Ishikawa, 1992) CPDs. To examine this hypothesis, we performed RNA blot analysis using several samples excised from 2-d-germinated grains (Fig. 3). The similarity in terms of the amino acid sequence between type III and type III-like CPDs suggests a similar function, but an unexpected distribution of their mRNAs in the germinated grains was demonstrated. The bulk of mRNA for type III CPD accumulated in endosperms plus the aleurone layer, and fluctuating amounts of the mRNA in deembryonated half-grains upon treatments with phytohormones (GA_3, ABA) were only noted for the type III CPD. In contrast, the accumulation of mRNA for the type III-like CPD was observed in germinated embryos with slightly larger amounts in scutella. The transcripts of the type I CPD were concentrated in leaves and scutella, in agreement with a previous report for barley type I CPD (Ranki et al., 1990).

Organization of the Genes

Although the spatial and temporal divergence of the accumulation of the mRNAs suggested the existence of a gene family for each class of CPD, the results of Southern analysis were in clear contrast to such expectations. A single band in each lane indicated that the gene for each class of CPD was unique in the rice genome (Fig. 4). The existence of a unique rice gene for type III CPD (Washio and Ishikawa, 1992) and also for type I CPD (Washio and Ishikawa, 1994) has been proposed. Therefore, the transcript for each class of CPD at various stages and in various tissues in rice grains should be attributable to the differential expression of individual corresponding gene.

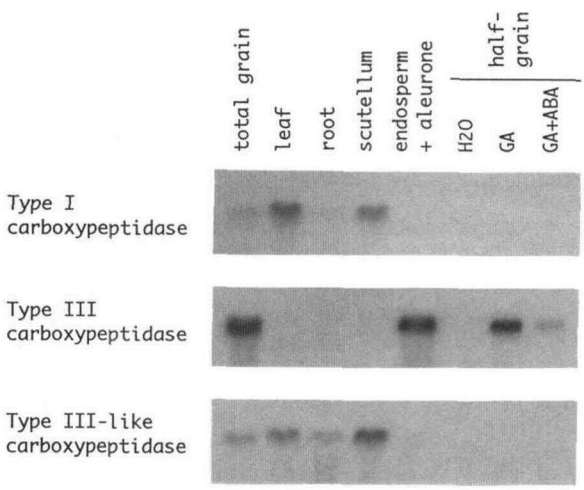

Figure 3. Tissue-specific or hormone-dependent amounts of the mRNAs for rice grain CPDs. Total RNA (5 μg/lane) extracted from germinated grains (2.5 d) from excised tissues of 2.5-d-germinated grains (leaf, root, scutellum, endosperm), or from deembryonated half-grains, which had been incubated in the absence (H_2O) or in the presence of 10^{-5} M GA_3 (GA) or 10^{-5} M GA_3 plus 10^{-4} M ABA (GA+ABA) for 2.5 d, was subjected to electrophoresis, blotted, and subjected to hybridization with the probe indicated to the left of each panel.

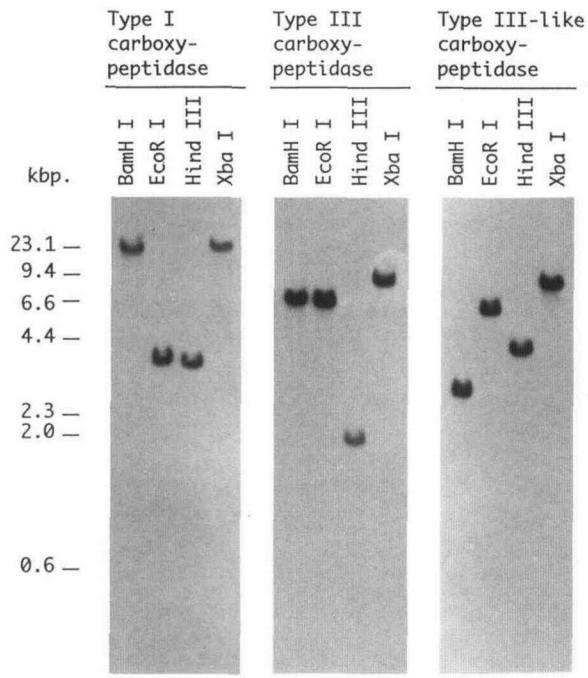

Figure 4. Southern blot analysis showing the organization of genes for rice CPDs. Rice genomic DNA (10 μg/lane) was digested to completion with the restriction endonuclease indicated above each lane, fractionated by electrophoresis on a 0.8% agarose gel, and transferred to a nylon membrane filter. The filter was hybridized with the probe indicated above each panel. Size markers (λ/*Hin*dIII fragments, in kbp) are shown to the left.

DISCUSSION

Following the germination of cereal grains, effective declines in storage proteins are thought to result from the concerted actions of Cys proteinases and CPDs (Preston and Kruger, 1979; Dunaevsky and Belozersky, 1989; Segundo et al., 1990). Insoluble storage proteins are first hydrolyzed by Cys proteinases and then degraded into soluble smaller peptides or amino acids by the subsequent action of CPDs. The appearance of mRNAs for several Cys proteinases has been reported in germinated grains of barley (Rogers et al., 1985; Koehler and Ho, 1990), wheat (Cejudo et al., 1992), and rice (Watanabe et al., 1991). In the present study, rice mRNAs for three types of Ser CPDs were identified during the later period after germination. The existence of type I CPD, which is a predominant type of CPD in germinated cereal grains, was found in barley (Mikola, 1983) and wheat (Mikola, 1986). In rice grains, there is no information about type I CPD, with

the exception that a CPD appears in germinated grains and leaves with enzymic properties similar to those of barley type I CPD (Doi et al., 1980). The similarities in terms of the primary structure and the mRNA appearance between rice and barley type I CPDs (Doan and Fincher, 1988; Ranki et al., 1990) suggest that the common type I CPD is functional following germination of cereal grains.

The primary structure of wheat type III CPD was originally presented by Baulcombe et al. (1987), and it is a product of a GA_3-responsive gene. In the same report, they pointed out the presence of leaf-specific mRNA species similar to but not identical with wheat type III CPD. We selected one novel cDNA class encoding a CPD-like protein that was very similar to cereal type III CPDs. Several key residues (Ser-His-Asp, see "Results") capable of forming the catalytic triad are found in the amino acid sequence of type III-like CPD of rice. Similar catalytic triads were also noted in the crystal structures of other hydrolytic enzymes, termed "α/β hydrolases" (Breddam, 1986), such as acetylcholine esterase from *Torpedo californica* (Sussman et al., 1991) and lipase from *Geotrichum candidum* (Schrag et al., 1991). However, the hexapeptide (G-E-S-Y-A-G) containing the active Ser residue is correctly found in each CPD class, and the high degree of homology (>80%) is observed throughout the amino acid sequences between rice type III-like CPD and barley mature type III CPD (Fig. 1). Given the separate abundance of the mRNA for type III-like CPD and that of type III CPD, type III-like CPD is assumed to be one of Ser CPDs that appeared in nonaleurone tissues of germinated grains.

Abundant mRNAs for the three classes of CPDs were observed in the aleurone layer and the scutellum, as expected from the general concept that several hydrolytic enzymes, such as α-amylase (Ranjhan et al., 1992) and (1–3,1–4)-β-glucanase (Fincher, 1989), that participate in the postgermination program originated from these two tissues. The accumulation of mRNAs for the three CPDs, mainly for the type III-like CPD, is further found in nongerminated tissues (leaves, roots, immature grains). This ubiquitous expression of Ser CPDs suggests additional functions for these enzymes. In fact, the peptidolytic activities of the CPDs have been shown to be involved in both the intracellular turnover of proteins and the protein maturation, such as a limited processing on the C-terminal end of proteins (Breddam, 1986; Galjart et al., 1990; Søgaard et al., 1991).

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LITERATURE CITED

Baulcombe DC, Barker RF, Jarvis MG (1987) A gibberellin responsive wheat gene has homology to yeast carboxypeptidase Y. J Biol Chem **262:** 13726–13735

Breddam K (1986) Serine carboxypeptidases. A review. Carlsberg Res Commun **51:** 83–128

Cejudo FJ, Murphy G, Chinoy C, Baulcombe DC (1992) A gibberellin-regulated gene from wheat with sequence homology to cathepsin B of mammalian cells. Plant J **2:** 937–948

Doan NP, Fincher GB (1988) The A- and B-chains of carboxypeptidase I from germinated barley originate from a single precursor polypeptide. J Biol Chem **263:** 11106–11110

Doi E, Komori N, Matoba T, Morita Y (1980) Some properties of carboxypeptidase in germinating rice seeds and leaves. Agric Biol Chem **44:** 77–83

Dunaevsky YE, Belozersky MA (1989) The role of cysteine proteinase and carboxypeptidase in the breakdown of storage proteins in buckwheat seeds. Planta **179:** 316–322

Feinberg AP, Vogelstein B (1984) Addendum: a technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem **137:** 266–267

Fincher GB (1989) Molecular and cellular biology associated with endosperms mobilization in germinating cereal grains. Annu Rev Plant Physiol Plant Mol Biol **40:** 305–346

Frohman MA, Dush MK, Martin GR (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene specific oligonucleotide primer. Proc Natl Acad Sci USA **85:** 8998–9002

Galjart NJ, Gillemans N, Meijer D, d'Azzo A (1990) Mouse "protective protein." J Biol Chem **265:** 4678–4684

Gubler U, Hoffman BJ (1983) A simple and very efficient method for generating cDNA libraries. Gene **25:** 263–269

Harris TJV, Dure LD III (1978) Developmental regulation in cotton seed germination: polyadenylation of stored messenger RNA. Biochemistry **17:** 3250–3256

Koehler SM, Ho THD (1990) Hormonal regulation, processing, and secretion of cysteine proteinases in barley aleurone layers. Plant Cell **2:** 769–783

Kozak M (1981) Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. Nucleic Acids Res **9:** 5233–5252

Liao DI, Breddam K, Sweet RM, Bullock T, Remington SJ (1992) Refined atomic model of wheat serine carboxypeptidase II at 2.2-Å resolution. Biochemistry **31:** 9796–9812

Mikola J, Kolehmainen L (1972) Localization and activity of various peptidases in germinating barley. Planta **104:** 167–177

Mikola L (1983) Germinating barley grains contain five acid carboxypeptidases with complementary substrate specificities. Biochim Biophys Acta **747:** 241–252

Mikola L (1986) Acid carboxypeptidases in grains and leaves of wheat, *Triticum aestivum* L. Plant Physiol **81:** 823–829

Mundy J, Brandt A, Fincher GB (1985) Messenger RNAs from the scutellum and aleurone of germinating barley encode (1–3,1–4)-β-D-glucanase, α-amylase, and carboxypeptidase. Plant Physiol **79:** 867–871

Murray MG, Thompson WF (1980) Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res **8:** 4321–4325

Preston KR, Kruger JE (1979) Physiological control of exo- and endoproteolytic activities in germinating wheat and their relationship to storage protein hydrolysis. Plant Physiol **64:** 450–454

Ranjhan S, Karrer EE, Rodriguez RL (1992) Localizing α-amylase gene expression in germinated rice grains. Plant Cell Physiol **33:** 73–79

Ranki H, Sopanen T, Voutilainen R (1990) Localization of carboxypeptidase I in germinating barley grain. Plant Physiol **93:** 1449–1452

Rogers JC, Dean D, Heck GR (1985) Aleurain: a barley thiol protease closely related to mammalian cathepsin H. Proc Natl Acad Sci USA **82:** 6512–6516

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA **74:** 5463–5467

Schrag JD, Li Y, Wu S, Cygler M (1991) Ser-His-Glu triad forms the catalytic site of the lipase from *Geotrichum candidum*. Nature **351:** 761–764

Schroeder RL, Burger WC (1978) Development and localization of carboxypeptidase activity in embryo-less barley half- kernels. Plant Physiol **62:** 458–462

Segundo BS, Casacuberta JM, Puigdomènech P (1990) Sequential expression and differential hormonal regulation of proteolytic activities during germination in *Zea mays* L. Planta **181:** 467–474

Søgaard M, Olsen FL, Svensson B (1991) C-terminal processing of

barley α-amylase 1 in malt, aleurone protoplasts, and yeast. Proc Natl Acad Sci USA **88:** 8140–8144

Sorensen SB, Breddam K, Svendsen I (1986) Primary structure of carboxypeptidase I from malted barley. Carlsberg Res Commun **51:** 475–485

Sorensen SB, Svendsen I, Breddam K (1987) Primary structure of carboxypeptidase II from malted barley. Carlsberg Res Commun **52:** 285–295

Sorensen SB, Svendsen I, Breddam K (1989) Primary structure of carboxypeptidase III from malted barley. Carlsberg Res Commun **54:** 193–202

Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, Silman I (1991) Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. Science **253:** 872–879

Washio K, Ishikawa K (1992) Structure and expression during the germination of rice seeds of the gene for a carboxypeptidase. Plant Mol Biol **19:** 631–640

Washio K, Ishikawa K (1994) Cloning and sequencing of the gene for type I carboxypeptidase in rice. Biochim Biophys Acta **1199:** 311–314

Watanabe H, Abe K, Emori Y, Hosoyama H, Arai S (1991) Molecular cloning and gibberellin-induced expression of multiple cysteine proteinases of rice seeds (oryzains). J Biol Chem **266:** 16897–16902

Watson MEE (1984) Compilation of published signal sequences. Nucleic Acids Res **12:** 5145–5164