Plant Physiol. (1985) 79, 867–871
0032-0889/85/79/0867/05/$01.00/0

Messenger RNAs from the Scutellum and Aleurone of Germinating Barley Encode (1→3,1→4)-β-D-Glucanase, α-Amylase and Carboxypeptidase[1]

Received for publication April 22, 1985 and in revised form July 1, 1985

JOHN MUNDY, ANDERS BRANDT, AND GEOFFREY B. FINCHER*[2]
Department of Biotechnology, Carlsberg Research Laboratory (J.M.) *and Department of Physiology* (A.B., G.B.F.), *Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500, Copenhagen Valby, Denmark*

ABSTRACT

Polyclonal antibodies raised against barley (1→3,1→4)-β-D-glucanase, α-amylase and carboxypeptidase were used to detect precursor polypeptides of these hydrolytic enzymes among the *in vitro* translation products of mRNA isolated from the scutellum and aleurone of germinating barley. In the scutellum, mRNA encoding carboxypeptidase appeared to be relatively more abundant than that encoding α-amylase or (1→3,1→4)-β-D-glucanase, while in the aleurone α-amylase and (1→3,1→4)-β-D-glucanase mRNAs predominated. The apparent molecular weights of the precursors for (1→3,1→4)-β-D-glucanase, α-amylase, and carboxypeptidase were 33,000, 44,000, and 35,000, respectively. In each case these are slightly higher (1,500–5,000) than molecular weights of the mature enzymes. Molecular weights of precursors immunoprecipitated from aleurone and scutellum mRNA translation products were identical for each enzyme.

During the germination of barley grain, cell walls, starch, and reserve protein of the endosperm are depolymerized by the action of hydrolytic enzymes. Degradation products are translocated to the embryo where they support seedling growth. Many of the hydrolases which participate in endosperm mobilization are secreted from the aleurone layer in response to the phytohormone gibberellic acid (7, 21). Despite suggestions that the scutellum plays no part in endosperm modification (22), there is increasing evidence that cells of the scutellar epithelial layer are also capable of secreting hydrolytic enzymes, in particular α-amylase and cell wall-degrading enzymes, and that these contribute to the degradation of the endosperm in germinating barley (3, 11, 16, 24). Furthermore, the initial site of protease activity in germinating barley is also adjacent to the epithelial cells of the scutellum (19). Histochemical studies have identified an important role for the scutellum in α-amylase secretion in other cereal grains, including oats, rice, rye, maize, wheat, and sorghum (1, 12, 19).

Two (1→3,1→4)-β-glucan endohydrolase (EC 3.2.1.73) isoenzymes have been purified from extracts of germinating barley (28). The isoenzymes depolymerize (1→3,1→4)-β-glucan, a polysaccharide which constitutes approximately 70% of the endosperm cell wall (9), and exhibit almost identical substrate specificities and kinetic properties (28, 29). However, enzyme I, which has M_r 28,000, a pI of 8.5, and contains approximately 0.7% associated carbohydrate, can be readily distinguished from enzyme II, which has M_r 30,000, a pI of greater than 10, and contains approximately 4% carbohydrate (28). Based on the high degree of amino acid sequence homology (approximately 90%), it may be concluded that the enzymes are derived from different genes which originated by duplication of a common ancestral gene (30). Antibodies raised against the isoenzymes are mutually cross-reactive, presumably because of this sequence homology (28, 30). Similarly, there are a number of α-amylase (EC 3.2.1.1) isoenzymes involved in starch degradation (14, 16), and several carboxypeptidases participate in the hydrolysis of storage proteins in germinating barley (17, 27).

In the present study, mRNA was isolated from barley scutellum and aleurone at various times after the initiation of germination. *In vitro* translation products of the mRNA preparations were immunoprecipitated with antibodies raised against barley (1→3,1→4)-β-glucanase, the α-amylase 2 group of isoenzymes, the major group of α-amylases in germinating barley (10), and against a serine carboxypeptidase (EC 3.4.16.1) which accounts for at least 30% of malt carboxypeptidases (2). Thus, specific antibodies were used to detect aleurone and scutellar mRNAs encoding hydrolytic enzymes which play central roles in cell wall, starch, and protein degradation in germinating barley.

MATERIALS AND METHODS

Preparation of Aleurone and Scutellum. Proanthocyanidin-free barley seeds (*Hordeum vulgare* L. cv Bonus, mutant ant13) harvested in Denmark in 1981 were obtained from Dr. John Ingversen, Carlsberg Plant Breeding, Denmark. The proanthocyanidin-free mutant was used to obviate difficulties caused by polyphenolic compounds, which inactivated mRNA extracted from aleurone layers of wild type seeds.

Seeds were surface sterilized with 0.2% (w/v) silver nitrate, rinsed thoroughly, and steeped for 24 h in sterile water containing 100 μg chloramphenicol/ml, 100 units penicillin/ml, and 100 units nystatin/ml (28). After steeping, the seeds were germinated in the dark at 15°C on filter paper moistened with sterile distilled H_2O containing antibiotics. Two, 5, and 8 d after the initiation of germination, roots and shoots were removed from the seeds (approximately 1000 at each time interval) and the scutella excised with a scalpel. Adhering pericarp-testa was removed and isolated scutella were immediately frozen in liquid N_2. Examination by light microscopy showed them to be essentially free of starchy endosperm, pericarp-testa, and other embryonic tissue.

[1] Supported in part by grants from the Australian Research Grants Scheme (to G. B. F.) and the Biomolecular Engineering Program of the Commission of the European Communities (research contract GB 1-4-024-DK) to Professor D. von Wettstein.

[2] Permanent address: Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia.

Following excision of the scutellum, the starchy endosperm was squeezed out of the remainder of the seed. The aleurone layer, together with the pericarp-testa, was frozen in liquid N_2. Some starch remained associated with the aleurone layer.

Isolation of mRNA. Frozen tissue was ground to a powder under liquid N_2, thawed into 10 volumes 10 mM Tris-HCl (pH 8.5), containing 5 M guanidinium chloride, 5 mM EGTA, 100 mM β-mercaptoethanol, 0.1% lauryl sarcosine, and 0.01% octanol (6, 20) and the suspension further homogenized in a Sorvall Omnimixer at maximum speed for 2.5 min. Debris was removed by centrifugation and polymeric material precipitated with 0.75 volume ethanol at −20°C for 16 h.

The pellet was resuspended in 25 mM trisodium citrate (pH 7.0) containing 5 M guanidinium chloride, 5 mM DTT, and 10 mM EDTA (6), reprecipitated with 0.5 volume ethanol, dried, and redissolved in 100 mM Tris-HCl buffer (pH 9.0) containing 100 mM NaCl and 2 mM EDTA. Redistilled phenol (0.5 volume, saturated with the same buffer) and 0.5 volume chloroform (containing 2% iso-amyl alcohol) were added, thoroughly mixed, and the phases separated by centrifugation at 10,000*g* for 10 min. The aqueous phase was removed and re-extracted with phenol/chloroform until denatured protein was no longer detected at the interface. Nucleic aids were recovered by precipitation with 0.1 volume 3 M sodium acetate-acetic acid (pH 4.5) and 2.5 volumes ethanol at −20°C for 16 h.

Polyadenylated mRNA was isolated on poly(U)-Sepharose 4B (Pharmacia Fine Chemical Co.) equilibrated in 10 mM Tris-HCl buffer (pH 7.5), containing 0.7 M NaCl and 1 mM EDTA. The total RNA fraction was heated at 68°C for 10 min, cooled, and applied to the column. The column was washed with buffer until all material absorbing at 260 nm had been eluted. Bound material was eluted with 98% formamide. Polyadenylated mRNA was precipitated with 0.1 volume 3 M sodium acetate-acetic acid (pH 4.5) and 2.5 volumes ethanol for 16 h at −20°C and redissolved in water.

Preparation of Antibodies. Polyclonal antibodies against (1→3,1→4)-β-glucanases were raised in rabbits using isoenzyme I as the antigen; the antibodies cross-react with both (1→3,1→4)-β-glucanase isoenzyme I and II (28). A 20 to 40% saturated $(NH_4)_2SO_4$ precipitate of serum was used without further purification. Barley α-amylase was purified by affinity chromatography on cycloheptaamylose-Sepharose followed by CM-cellulose ion exchange chromatography (25). Antibodies against α-amylase 2 were purified from rabbit serum by Protein A-Sepharose 4B chromatography. Antibodies against purified malt carboxypeptidase (2) were custom made by Dakopatts A/S, Denmark and crude serum was used without further purification.

***In Vitro* Translations and Immunoprecipitation.** *In vitro* translations of mRNA were performed using the rabbit reticulocyte lysate system and L-[^{35}S]methionine (23). Lysate (N90) and L-[^{35}S]methionine (1300 Ci mmol^{-1}) were from Amersham. The standard assay consisted of 2 μl mRNA preparation (approximately 1 μg RNA), 7.5 μl lysate, and 1.5 μl [^{35}S]methionine incubated at 30°C for 1 h. Control assays, from which mRNA was omitted, were routinely used to identify background incorporation. Samples were removed for the determination of net incorporation of radioactivity into 10% TCA-insoluble material, for analysis of the translation products by gel electrophoresis, and for immunoprecipitations.

Immunoprecipitation of specific proteins was performed according to Jonassen *et al.* (15). *In vitro* translation products were diluted to 600 μl with 500 mM Tris-HCl buffer (pH 7.5), containing 0.15 M NaCl, 2% Triton X-100, and 10 mM methionine. Protein A-Sepharose (50 μl hydrated gel; Pharmacia Fine Chemicals) suspended in the same buffer (approximately 50% suspension) was gently mixed with the translation products at room temperature for 30 min to remove proteins binding nonspecifically to Protein A-Sepharose. After removal of the gel, immunoprecipitation was performed by the sequential addition of preimmune serum (20 μl, 5–10 mg/ml protein concentration) for 60 min and Protein A-Sepharose (50 μl hydrated gel) for 30 min. Protein A-Sepharose was recovered by centrifugation and washed thoroughly with buffer. Residual *in vitro* translation products in the supernatant were immediately treated with 20 μl antibody solution (5–10 mg/ml protein concentration) and Protein A-Sepharose as described. Immunoprecipitations with different antibodies could be performed sequentially on the same *in vitro* translation products.

Gel Electrophoresis. Samples (approximately 50 μl) were heated at 100°C for 3 min with 25 μl 0.2 M Tris-HCl buffer (pH 8.8) (containing 10 mM EDTA, 1% methionine, 0.5 M sucrose, 0.05% bromophenol blue), 2 μl 0.5 M DTT, and 10 μl 10% SDS. Electrophoresis was performed in 12.5% polyacrylamide gels containing 1% SDS (8); gels were treated with sodium salicylate prior to fluorographic detection of radioactivity (4). The ^{14}C-labeled protein markers for mol wt calibration were from Amersham (mixture CFA 626).

RESULTS

***In Vitro* Translation Products.** The polypeptides synthesized *in vitro* using the rabbit reticulocyte lysate system programmed with mRNA from scutellum and aleurone are compared in Figure 1. The major translation products from scutellar mRNA are at M_r 40,000 and 24,500. The M_r 40,000 product appears much less abundant among *in vitro* translation products from aleurone mRNA, which include major polypeptides of M_r 57,000, 44,000, 35,000, 33,000, and 24,500 (Fig. 1). The polypeptide at M_r 57,000 is absent from scutellar products, while the polypeptide at M_r 44,000 is much more abundant in the aleurone mRNA products (Fig. 1). Thus, the mRNA preparations from aleurone and scutellum of germinating barley contain different mRNA species and the relative abundance of mRNA species

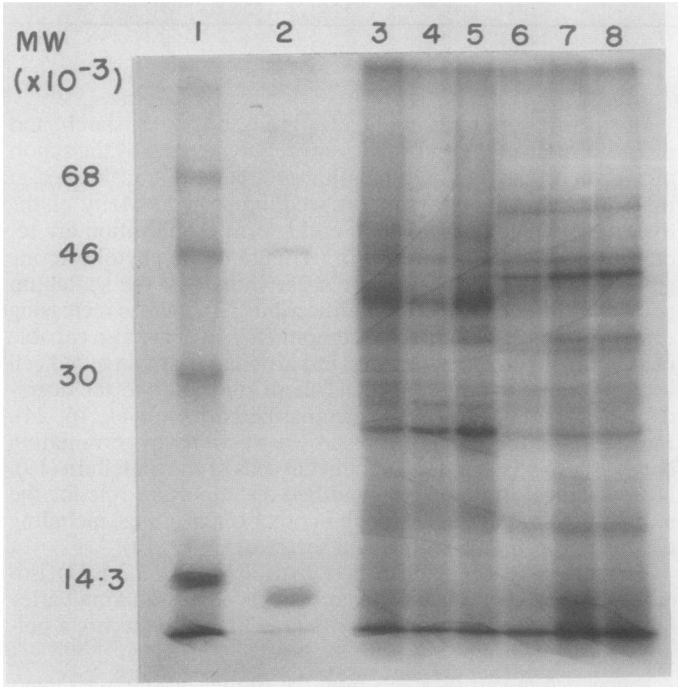

FIG. 1. Fluorogram of SDS-PAGE gel showing *in vitro* translation products of mRNA isolated from the aleurone and scutellum of germinating barley. Lane 1, [^{14}C] mol wt markers; 2, control containing no mRNA; 3 to 5, scutellum mRNA at 2, 5, and 8 d germination; 6 to 8, aleurone mRNA at 2, 5, and 8 d germination.

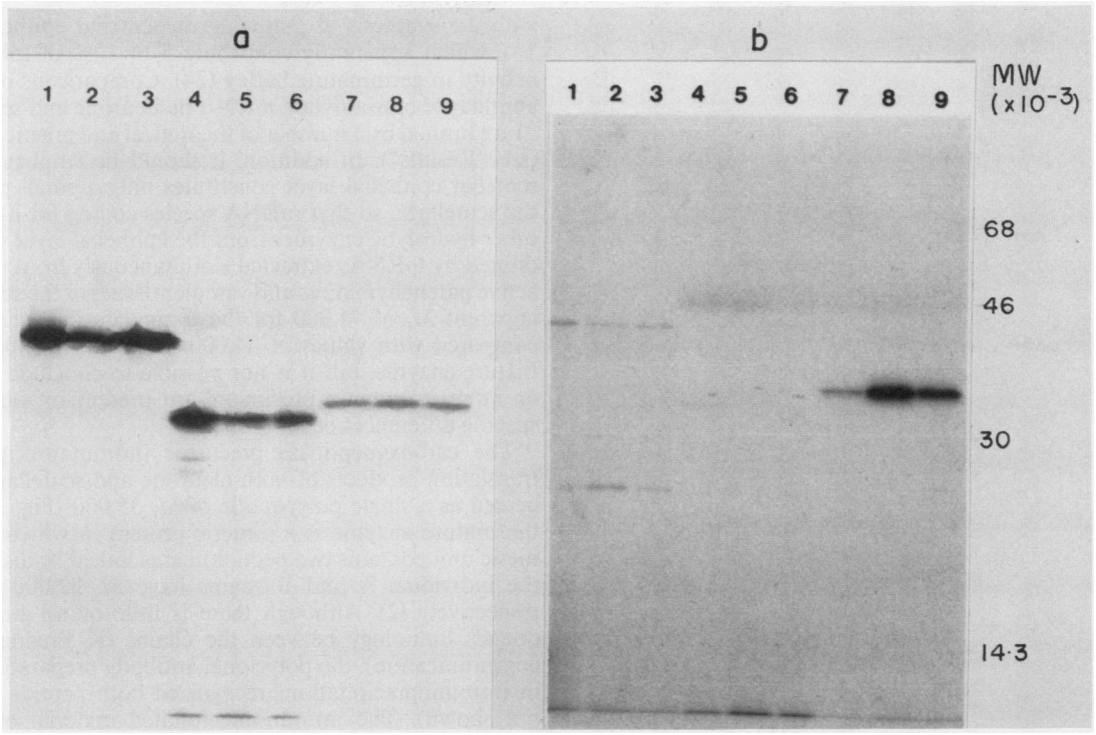

FIG. 2. Fluorogram of SDS-PAGE gel showing polypeptides immunoprecipitated from *in vitro* translation products of (a) aleurone mRNA and (b) scutellar mRNA. Lanes 1 to 3, α-amylase (immunoprecipitates at 2, 5, and 8 d germination; 4 to 6, (1→3,1→4)-β-D-glucanase immunoprecipitates at 2, 5, and 8 d germination; 7 to 9, carboxypeptidase immunoprecipitates at 2, 5, and 8 d germination.

common to both preparations varies between tissues.

Immunoprecipitations. The polypeptides immunoprecipitated from *in vitro* translation products of aleurone mRNA with polyclonal antibodies raised against (1→3,1→4)-β-glucanase, α-amylase, and carboxypeptidase are shown in Figure 2a. The major polypeptide immunoprecipitated with antibodies to (1→3,1→4)-β-glucanase has M_r 33,000; other lower mol wt polypeptides are also evident (Fig. 2a). The lower mol wt polypeptides may be due to premature chain termination during translation, to proteolytic degradation of the major polypeptide during the immunoprecipitation procedure, or to the presence of contaminating, nonspecific antibodies in the preparation used here. However, in immunoprecipitations performed with other antibody preparations, a single polypeptide of M_r 33,000 was observed (data not shown). The immunoprecipitated α-amylase precursor has M_r approximately 44,000, while antibodies to malt carboxypeptidase bound to a protein of M_r approximately 35,000 in the *in vitro* translation products from aleurone mRNA (Fig. 2a).

Polypeptides immunoprecipitated from translation products of scutellar mRNA are shown in Figure 2b. To permit a semiquantitative comparison between scutellar and aleurone immunoprecipitates, the same amount of translation products were subjected to immunoprecipitation (185,000 net cpm) and the exposure times of fluorographs were the same (Fig. 2). The (1→3,1→4)-β-glucanase immunoprecipitate from scutellum shows an extremely faint band at M_r 33,000 (Fig. 2b), which was clearly visible after longer exposure or when more material was loaded on the gel. Thus, in Figure 3, where polypeptides immunoprecipitated from 600,000 cpm of scutellar *in vitro* translation products are compared with those immunoprecipitated from 30,000 cpm of aleurone products, the mobilities and intensities of the (1→3,1→4)-β-glucanase immunoprecipitates are similar.

The polypeptide of M_r 44,000 immunoprecipitated with antibodies to α-amylase can be seen in Figure 2b but appears to be relatively less abundant than in aleurone products (Fig. 2a). Similar differences are observed in the relative intensities of the protein of M_r 44,000 in the total products of scutellar and aleurone mRNA (Fig. 1). When α-amylase immunoprecipitated from aleurone translation products was compared with that immunoprecipitated from approximately 20 times as much scutellar mRNA product, the mobilities of the immunoprecipitated polypeptides, as well as their intensities, were similar (Fig. 3). The origin of the associated band of M_r approximately 25,000, which is visible in the α-amylase immunoprecipitate in Figure 2b but is not present in other α-amylase immunoprecipitations (Figs. 2a and 3), is unknown.

The polypeptides immunoprecipitated with antibodies to carboxypeptidase from the scutellar mRNA translation products had M_r approximately 35,000 (Fig. 2b). The abundance of this mRNA species appears to be relatively higher in scutellar than in aleurone preparations and, in contrast to the aleurone translation products, the carboxypeptidase band immunoprecipitated from scutellar mRNA products is more intense than the (1→3,1→4)-β-glucanase and α-amylase bands (Fig. 2).

In making such comparisons between the intensities of immunoprecipitate bands on the fluorographs (Figs. 2 and 3), the relative methionine contents of the polypeptides synthesized in the rabbit reticulocyte lysate-[^{35}S]methionine system must be taken into account. The mature forms of (1→3,1→4)-β-D-glucanase have 10 to 11 methionine residues per enzyme molecule (28), the α-amylase primary translation product has seven methionine residues (5), while the two chains of mature malt carboxypeptidase have only three methionines each (2). On a molar basis, carboxypeptidase chains will therefore be less intense than (1→3,1→4)-β-glucanase or α-amylase.

DISCUSSION

In each case the mol wt of immunoprecipitated enzyme precursor was higher than the mature enzyme, presumably due to

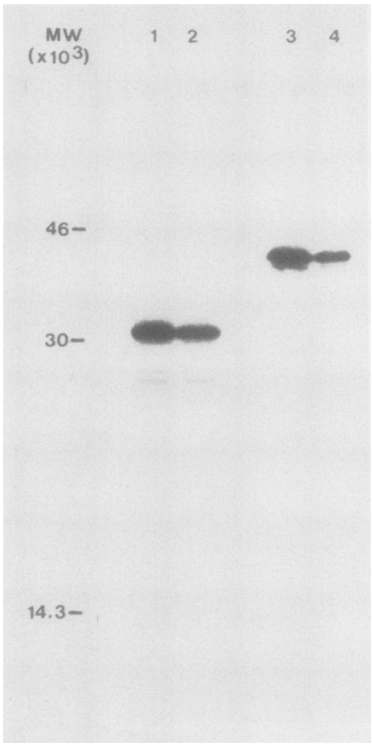

FIG. 3. Comparison of (1→3,1→4)-β-D-glucanase and α-amylase immunoprecipitates from *in vitro* translation products of scutellum and aleurone mRNA. Lane 1, (1→3,1→4)-β-D-glucanase immunoprecipitate from 6×10^5 cpm scutellum products; 2, (1→3,1→4)-β-D-glucanase immunoprecipitate from 3×10^4 cpm aleurone products; 3, α-amylase immunoprecipitate from 6×10^5 cpm scutellum products; 4, α-amylase immunoprecipitate from 3×10^4 cpm aleurone products.

the retention of NH_2-terminal signal peptides on the unprocessed *in vitro* translation products. No differences in molecular sizes of precursors immunoprecipitated from aleurone or scutellar mRNA translation products were detected. Precursors of (1→3,1→4)-β-glucanases immunoprecipitated from both aleurone and scutellum mRNA translation products were seen as a major band of approximately 33,000 (Figs. 2 and 3). Mature isoenzyme I (M_r 28,000) and isoenzyme II (M_r 30,000) are significantly smaller than this precursor polypeptide (28). However, it is not possible to conclude whether this band represents precursor for isoenzyme I, isoenzyme II, or both, since the amino acid sequence homology at both the NH_2^- (30) and COOH-termini (unpublished data) of the two isoenzymes indicates that their precursors are likely to have identical chain lengths and that resolution of mature isoenzymes on SDS-polyacrylamide gels may be attributable simply to different degrees of glycosylation of the primary translation products (28). The detection of mRNA encoding (1→3,1→4)-β-glucanase in aleurone and scutellar mRNA confirms a role for both tissues in the synthesis of hydrolytic enzymes which participate in degradation of starchy endosperm cell walls. The role of the scutellum in (1→3,1→4)-β-glucanase secretion has been demonstrated independently in experiments with isolated aleurone layers and scutella, which clearly show that one (1→3,1→4)-β-glucanase isoenzyme is synthesized predominantly in the aleurone, while the other is secreted from the scutellum (26).

It is well established that mRNA encoding α-amylase is abundant in aleurone cells of germinating barley (13, 18), where the development of α-amylase mRNA has been studied in detail (5). The presence of α-amylase precursors among the *in vitro* translation products of scutellar mRNA (Figs. 2b and 3) is consistent with the synthesis of α-amylase in scutellar epithelial cells (11, 16), which apparently contribute 5 to 10% of total α-amylase activity in germinating barley (24). Comparisons of the relative abundance of α-amylase mRNA in aleurone and scutellum (Fig. 3) are limited by a number of theoretical and practical constraints (see "Results"). In addition, it should be emphasized that the scutellar epithelial layer constitutes only a small proportion of the scutellum, so that mRNA species coding for α-amylase and other hydrolytic enzymes from the epithelial layer will be highly diluted by mRNAs extracted simultaneously from metabolically active parenchymatous and vascular tissues of the scutellum. The apparent M_r of 44,000 for the α-amylase 2 precursor may be compared with values of 44,000 (14) and 42,500 (18) for the mature enzyme, but it is not possible to conclude whether one or more isoenzyme precursors are present or whether tissue-specific differences occur (14, 16).

The carboxypeptidase precursor immunoprecipitated from translation products of both aleurone and scutellar mRNA appeared as a single polypeptide of M_r 35,000 (Fig. 2). However, the mature enzyme is a dimeric protein in which each monomeric unit contains two peptide chains linked by disulfide bonds; the individual A and B chains have M_r 32,000 and 19,000, respectively (2). Although there is little or no amino acid sequence homology between the chains (K Breddam, personal communication), the polyclonal antibody preparation used here in immunoprecipitations recognized both peptide chains (data not shown). The immunoprecipitated material of M_r 35,000 probably represents the precursor form of the carboxypeptidase A chain. The origin of the B chain remains obscure, although it is formally possible that it could be derived through processing a separate precursor protein of identical mol wt (M_r 35,000).

On the basis of the quantitative and qualitative differences between polypeptides synthesized from scutellar and aleurone mRNA, we conclude that the two tissue preparations do not substantially contaminate each other. Thus, the results suggest that mRNA species encoding (1→3,1→4)-β-D-glucanase, α-amylase, and carboxypeptidase are present in both the aleurone and scutellum of germinating barley. The mRNAs encoding (1→3,1→4)-β-D-glucanase and α-amylase 2 are found predominantly in the aleurone, while the mRNA encoding carboxypeptidase is more abundant in the scutellum. These observations indicate the major sites of synthesis of the three hydrolases, which are involved in cell wall, starch, and protein degradation in the endosperm of germinating barley.

Acknowledgments—We thank Dr. Klaus Breddam for providing purified carboxypeptidase and Ms. Annette Hansen for her skilled assistance in preparing the tissues for mRNA extractions.

LITERATURE CITED

1. AISIEN AO, GH PALMER 1983 The sorghum embryo in relation to the hydrolysis of the endosperm during germination and seedling growth. J Sci Food Agric 34: 113–121
2. BREDDAM K, SB SORENSEN, M OTTESEN 1983 Isolation of a carboxypeptidase from malted barley by affinity chromatography. Carlsberg Res Commun 48: 217–230
3. BRIGGS DE, J MACDONALD 1983 Patterns of modification in malting barley. J Inst Brew 89: 260–273
4. CHAMBERLAIN JP 1979 Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. Anal Biochem 98: 132–135
5. CHANDLER PM, JA ZWAR, JV JACOBSEN, TJV HIGGINS, AS INGLIS 1984 The effects of gibberellic acid and abscisic acid on α-amylase mRNA levels in barley aleurone layers studies using an α-amylase cDNA clone. Plant Mol Biol 3: 407–418
6. CHIRGWIN JM, AE PRZYBYLA, RJ MACDONALD, WJ RUTTER 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294–5299
7. CHRISPEELS MJ, JE VARNER 1967 Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol 42: 398–406
8. CHUA N-H, P BENNOUN 1975 Thylakoid membrane polypeptides of *Chlamy-*

domonas reinhardii: wild type and mutant strains deficient in photosystem II reaction centres. Proc Natl Acad Sci USA 72: 2175–2179
9. FINCHER GB 1975 Morphology and chemical composition of barley endosperm cell-walls. J Inst Brew 81: 116–122
10. GIBBONS GC 1979 On the localisation and transport of α-amylase during germination and early seedling growth of *Hordeum vulgare*. Carlsberg Res Commun 44: 353–366
11. GIBBONS GC 1981 On the relative role of the scutellum and aleurone in the production of hydrolases during germination of barley. Carlsberg Res Commun 46: 215–225
12. GLENNIE CW, J HARRIS, NVDW LIEBENBERG 1983 Endosperm modification in germinating sorghum grain. Cereal Chem 60: 27–31
13. HIGGINS TJV, JA ZWAR, JV JACOBSEN 1976 Gibberellic acid enhances the level of translatable mRNA for α-amylase in barley aleurone layers. Nature 260: 166–168
14. JACOBSEN JV, TJV HIGGINS 1982 Characterization of the α-amylases synthesized by aleurone layers of Himalaya barley in response to gibberellic acid. Plant Physiol 70: 1647–1653
15. JONASSEN I, J INGVERSEN, A BRANDT 1981 Synthesis of SPII albumin, β-amylase and chymotrypsin inhibitor CI-1 on the polysomes from the endoplasmic reticulum of barley endosperm. Carlsberg Res Commun 46: 175–181
16. MACGREGOR AW, FH MACDOUGALL, C MAYER, J DAUSSANT 1984 Changes in levels of α-amylase components in barley tissues during germination and early seedling growth. Plant Physiol 75: 203–206
17. MIKOLA J 1983 Proteinases, peptidases and inhibitors of endogenous proteinases in germinating seeds. *In* J Daussant, J Mosse, J Vaughan, eds, Seed Proteins. Academic Press, New York, pp 35–52
18. MOZER TJ 1980 Partial purification and characterization of the mRNA for α-amylase from barley aleurone layers. Plant Physiol 65: 834–837
19. OKAMOTO K, H KITANO,T AKAZAWA 1980 Biosynthesis and excretion of hydrolases in germinating cereal grains. Plant Cell Physiol 21: 201–204
20. OKITA TW, FC GREENE 1982 Wheat storage proteins: isolation and characterization of the gliadin messenger RNAs. Plant Physiol 69: 834–839
21. PALEG LG 1960 Physiological effects of gibberellic acid. II. On starch hydrolyzing enzymes of barley endosperm. Plant Physiol 35: 902–906
22. PALMER GH 1982 A reassessment of the pattern of endosperm hydrolysis (modification) in germinated barley. J Inst Brew 88: 145–153
23. PELHAM HRB, RJ JACKSON 1976 An efficient mRNA-dependent translation system from reticulocyte lysates. Eur J Biochem 67: 247–256
24. RANKI H, T SOPANEN 1984 Secretion of α-amylase by the aleurone layer and the scutellum of germinating barley grain. Plant Physiol 75: 710–715
25. SILVANOVICH MP, RD HILL 1976 Affinity chromatography of cereal α-amylase. Anal Biochem 73: 430–433
26. STUART IM, L LOI, GB FINCHER 1985 Development of (1→3,1→4)-β-D-glucan endohydrolase isoenzymes in isolated scutella and aleurone layers of barley (*Hordeum vulgare*). Plant Physiol. In press
27. WINSPEAR MJ, KR PRESTON, V RASTOGI, A OAKS 1984 Comparisons of peptide hydrolase activities in cereals. Plant Physiol 75: 480–482
28. WOODWARD JR, GB FINCHER 1982 Purification and chemical properties of two 1,3:1,4-β-glucan endohydrolases from germinating barley. Eur J Biochem 121: 663–669
29. WOODWARD JR, GB FINCHER 1982 Substrate specificities and kinetic properties of two (1→3,1→4)-β-D-glucan endo-hydrolases from germinating barley (*Hordeum vulgare*). Carbohydr Res 106: 111–122
30. WOODWARD JR, FJ MORGAN, GB FINCHER 1982 Amino acid sequence homology in two 1,3;1,4-β-glucan endohydrolases from germinating barley (*Hordeum vulgare*). FEBS Lett 138: 198–200