Plant Physiol. (1986) 81, 901-906
0032-0889/86/8103-0901/06/$01.00/0

Hydrolysis of Storage Proteins in Barley Endosperms[1]

ANALYSIS OF SOLUBLE PRODUCTS

Received for publication December 11, 1985 and in revised form April 2, 1986

VIPIN RASTOGI AND ANN OAKS*
Department of Biology, McMaster University, 1280 Main St. West, Hamilton, Ontario, Canada L8S 4K1

ABSTRACT

Soluble products, released by the hydrolysis of hordeins into the media of barley (*Hordeum vulgare* cv. Perth) half-seeds were analyzed. Large polypeptide fragments (methanol-insoluble) were identified using the Western immunoblot technique with the antibodies prepared against B and C polypeptides of hordein. A number of hordein IgG-reacting bands were noted in the samples from dry kernels. In samples incubated in the absence of gibberellic acid, polypeptide fragments in the size range of 25 to 30 kilodaltons appeared within 24 hours, and those in the size range of 40 kilodaltons became more prominent. In samples incubated in the presence of gibberellic acid, polypeptide fragments in the size range of 45 to 67 kilodaltons were less apparent and those in the size range of less than 15 kilodaltons were more pronounced. The hordein-related polypeptide fragments were present in low amounts after 72 hours in the presence of gibberellic acid. Methanol-soluble peptides were fractionated, on the basis of size, into two broad peaks. In the absence of gibberellic acid, there was no significant change in their profile over a 72 hour incubation period. In the presence of this growth substance, however, there was a decrease in the proportion of large size peptides (50–70 amino acid residues in length), and an increase in the levels of small peptides (15–35 amino acid residues in length) and amino acids. Our interpretation of the results is that the release of the initial large polypeptide fragments from hordein proteins is mediated by a protease(s) whose appearance is not dependent on the exogenously added gibberellic acid. Further hydrolysis is, however, mediated by proteases induced in the presence of this growth substance.

Hordeins, the major storage proteins in barley endosperms, are characterized by their: (a) unusual amino acid composition, *e.g.* up to 75% of the total amino acid residues may be glutamine plus glutamate, proline, and asparagine plus aspartate; and (b) hydrophobicity and solubility in organic solvents or in aqueous buffers in the presence of SDS and urea (20). With the onset of germination, these protein reserves are presumably solubilized initially and then hydrolyzed to small peptides and amino acids. The nature of the proteolytic enzymes involved in the overall hydrolysis of these proteins has not been characterized. On the other hand, a number of proteolytic activities, *i.e.* endoproteases and carboxypeptidases, have been assayed in the extracts of germinated barley endosperms and half-seeds incubated in the presence of GA_3 (3, 8, 12, 16–18, 21, 23, 27). The major proteases appear to be a mixture of at least 5 carboxypeptidases (18, 21, 27). These protease activities have been measured using substrates such as animal proteins, *e.g.* hemoglobin, casein, gelatin, or synthetic dipeptides blocked at either C- or N-terminal[2] residues. With the use of such substrates, one is never sure whether an *in vivo* phenomenon is being examined.

The induction of α-amylase and other hydrolases by GA_3 in barley (cv Himalaya) half-seeds is well documented (4, 8, 12, 19, 21). Perth, the cultivar we used, also requires GA_3 for the induction of α-amylase. Even though an overall hydrolysis of endosperm protein reserves appears to be dependent on the peptide hydrolases induced in the presence of GA_3 (16, 21, 23), its requirement for protease(s) mediating the initial steps of solubilization has not been established. In this paper, we have carried out a detailed analysis of the hydrolytic products in order to determine whether the initiation of the hydrolysis is dependent on the presence of this growth substance. Our results indicate that there are at least two classes of proteases. One, which is involved in the initial hydrolysis, does not require GA_3 for its appearance, and a second, which is active on various water-soluble polypeptide fragments derived from the hordeins, does require the presence of GA_3.

MATERIALS AND METHODS

Plant Material. Barley (*Hordeum vulgare* cv Perth) kernels were obtained from Dr. E. Reinburgs, Department of Crop Sciences, University of Guelph, Guelph, Ontario. Embryo-less kernels were surface-sterilized with 1% NaOCl solution for 30 min, washed with 0.01 N HCl for 30 min, and then rinsed several times with sterile distilled H_2O (4). Routinely 30 half-seeds were incubated at room temperature (22 ± 2°C), in 10 ml of 2 mM acetate buffer (pH 4.8) containing 10 mM of $CaCl_2$ (4). GA_3 at a concentration of 5 μM was added as required. The pH of the media dropped to 3.7 during the incubation period in both control and GA_3-treated samples.

Fractionation of Extracts. Endosperm pieces were extracted together with the medium in the presence of 0.5 N NaCl and the crude homogenate was centrifuged at 20,000*g* for 30 min in a Sorvall rotor SS34. The supernatant solutions and pellets were analyzed separately. In the initial experiments involving measurement of total nitrogen, 10% TCA was used precipitate large polypeptides in the supernatant solution. However, in subsequent experiments, 50% methanol was used for this purpose. There were only minor qualitative differences, when polypeptides from the two sets (TCA- and methanol-precipitation) were compared on a polyacrylamide gel. The TCA-soluble and TCA-precipitable materials were analyzed for polypeptide fragments.

Prolamins were extracted at 60°C by shaking the residual pellet in 55% 2-propanol containing 2% β-mercaptoethanol (22). The suspension was centrifuged for 30 min at 10,000*g*. The super-

[1] Supported by an operating grant from the Natural Sciences and Engineering Research Council (Canada (A-2818)). V. R. was a recipient of C. W. Sherman Graduate Scholarship.

[2] C-terminal, carboxyl-terminal; GAR-HRP, goat anti-rabbit IgG horseradish peroxidase conjugate; IgG, α-immunoglobulin; N-terminal, amino-terminal; RuBPCase, ribulose bisphosphate carboxylase.

Table I. *Distribution of Nitrogen in Barley Half-Seeds Incubated in the Presence and Absence of GA_3*

Crude extracts were prepared from endosperm tissue of dry kernels and embryo-less endosperm pieces incubated for various times in the presence and absence of GA_3. Large polypeptides were precipitated with 10% TCA. Prolamins were extracted according to the method of Shewry *et al.* (22). The total nitrogen content was measured according to the method of Kaplan (13). The whole experiment was done two times. Values given represent the means. The variation between two experiments was less than 10%.

Incubation Time	GA_3	Total Nitrogen			
		Water-soluble nitrogen		Water-insoluble nitrogen	
		TCA-precipitable	TCA-soluble	Prolamins	Glutelins
h		*mg/30 endosperms*			
0		0.8	0.9	5.8	4.7
12	−	1.0	0.8	6.0	3.0
12	+	0.9	0.8	6.0	4.0
18	−	0.9	0.8	6.2	4.0
18	+	1.8	1.7	6.1	3.5
24	−	0.9	0.8	6.0	5.1
24	+	1.8	2.0	4.8	2.4
60	−	0.9	0.7	6.0	4.9
60	+	3.8	7.0	2.4	1.9

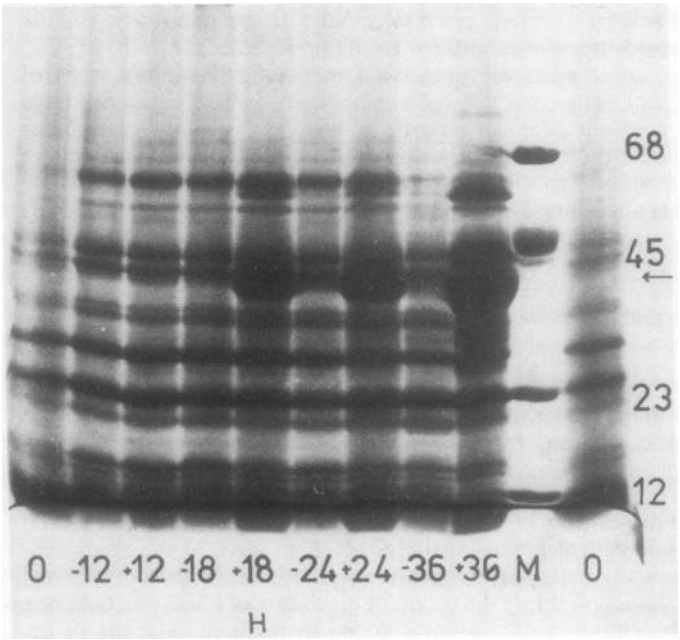

FIG. 1. SDS-PAGE analysis of large polypeptides in the water-soluble fractions prepared from unincubated kernels (0) and endosperms incubated for 12, 18, 24, and 36 h. Incubations were performed both in the presence (+) and absence (−) of GA_3 as described in the "Materials and Methods." Approximately 50 to 75 μl of samples containing 40 to 60 μg of proteins were loaded into different wells. Polypeptides were electrophoresed through polyacrylamide gel in the presence of SDS. Arrow shows position of the α-amylase polypeptide. Protein mole mass (mol wt) standards were BSA (68 kD), ovalbumin (45 kD), trypsinogen (23 kD), and Cyt *c* (12 kD).

natant solution containing prolamins was dialyzed against several changes of distilled H_2O to precipitate the hordein polypeptides. Prolamins, glutelins in the remaining residue, methanol-soluble, and methanol-precipitable materials were analyzed for total nitrogen.

Gel Filtration of the Methanol-Soluble Material. The methanol-soluble material was lyophilized and then taken up in a minimum volume of water. The samples were initially passed over a Sephadex G-10 column (1.3 × 90 cm) and the void volume was collected. The void volume was concentrated and passed over a Sephadex G-25 column (1.3 × 90 cm). Fractions of 2.8 ml were collected using a Gilson model 201 fraction collector. Peptide bonds and aromatic amino acids were detected at 210 and 280 nm, respectively.

SDS-PAGE. A discontinuous slab gel system (14) run at pH 8.8 with 12.5% running gel and 4% stacking gel in the presence of SDS was used for analyzing polypeptides. A current of 1.5 m amp/slot (11 slots; slot width, 7.5 mm) was used until the samples migrated through the stacking gel. It was then increased to 3 m amp/slot. The protein bands in the gel were stained with 0.125% Coomassie brilliant blue R (in 50% methanol and 10% acetic acid). A solution containing 7.5% acetic acid and 5% methanol was used for destaining the gel.

Preparations of Antigen. Soluble proteins were first extracted from dry endosperm powder using saline solution. Prolamins were then extracted from the remaining residue using 55% 2-propanol containing 2% β-mercaptoethanol (22). This solution was then dialyzed against several changes of distilled H_2O to precipitate hordeins. The hordein samples were lyophilized, and electrophoresed through polyacrylamide gel under denaturing conditions (14). The major B and C polypeptide bands (ranging in size from 43–67 kD), were cut out. The gel was frozen with liquid N_2, and then pulverized into a fine powder. The hordein polypeptides were finally eluted with acetate buffer (2 mM, pH 4.8) containing 3 to 4 M urea by vigorously shaking the suspension overnight at 37°C.

Immunization. The antigen (eluted hordeins), at a concentration of 5.0 mg/ml, was emulsified with an equal volume of Freund's incomplete adjuvant. The emulsion was injected subcutaneously into 2 New Zealand White rabbits at 4 to 5 sites. Booster injections were given with 1.25 mg of antigen proteins, 21 and 31 d after the first injection.

Enrichment of Hordein-Reacting IgG. Precipitin lines were noted when the crude serum was challenged with both hordein and non-hordein (soluble) proteins extracted from dry endosperms, indicating the presence of hordein-unrelated IgG in the crude serum. The serum was then enriched for hordein IgG by pretreating it with soluble proteins. When this enriched serum was challenged with both types of proteins, a precipitin line was noted only against the hordein proteins. The enriched serum was used as a source of primary antibody in the following immunoblotting experiments.

Western Immunoblotting. The samples were electrophoresed

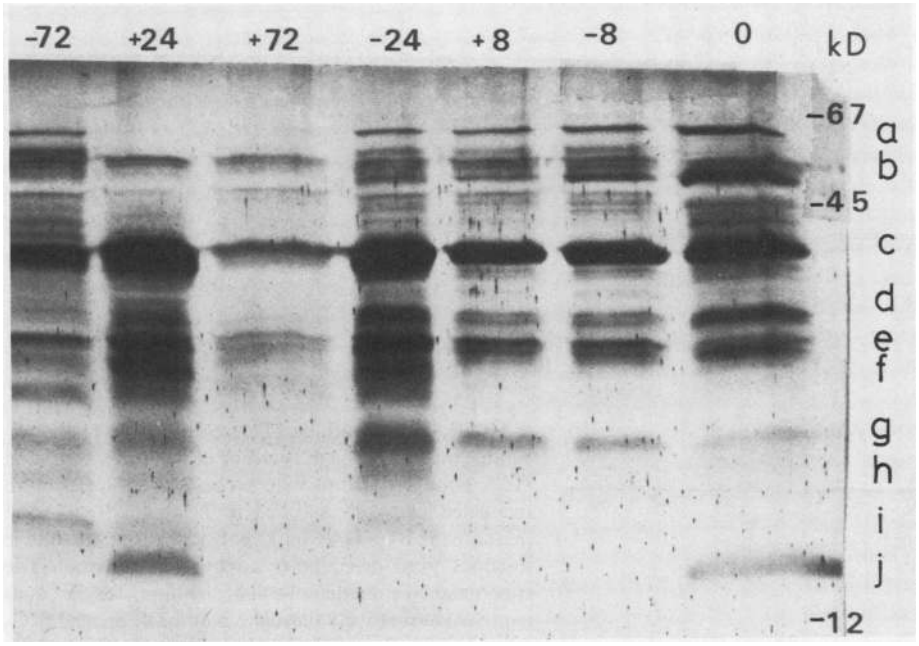

FIG. 2. Western immunoblot analysis of hordein-related polypeptides in the soluble fractions prepared from unincubated kernels (0) and endosperms incubated for 8, 24, and 72 h. Incubations were performed both in the presence (+) and absence (−) of GA_3 as described in the "Material and Methods." Approximately 100 μl samples containing 25 to 50 μg of proteins were loaded into different wells. Polypeptides were first electrophoresed through polyacrylamide gel in the presence of SDS, and then electroblotted on a nitrocellulose filter. The filter was sequentially incubated in the following solutions: 3% gelatin, primary antibodies, and secondary antibodies (details as in the "Material and Methods"). The immunoreactive polypeptides were visible after assaying for peroxidase activity. Alphabetical symbols a to j represent discrete areas on the blot that are referred to in the text. Protein mole mass (mol wt) standards were BSA (67 kD), ovalbumin (45 kD), and Cyt *c* (12 kD).

through a 15% polyacrylamide gel under denaturing conditions. The polypeptides were then electroblotted on to a nitrocellulose filter using a Biorad Trans-Blot Cell. The blotting was performed at 4°C using 60 V and 0.25 amp (25). All subsequent steps were performed at room temperature with slow shaking. The nitrocellulose filter was incubated for 3 h in a solution containing 3% gelatin. It was then incubated for 1 h in a solution containing hordein-related IgG (1/1000 final dilution). After giving two washes for 10 min each with Tris-saline buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.5), the filter was incubated for 1 h in a solution containing GAR-HRP at 1/1000 final dilution. It was again washed twice with Tris-saline buffer. The immunoreactive bands were visible after assaying for the peroxidase activity. The reliability and specificity of bands to primary antibody was tested by performing two control experiments: (a) one in which the filter was incubated with preimmune serum instead of serum containing hordein IgG, and (b) second in which the extracts prepared from barley leaves and roots were used instead of endosperm proteins. The nitrocellulose filter in this case was treated as usual with the serum containing hordein IgG and secondary antibodies. No bands were visible in either of the control experiments.

Total Nitrogen Determination. The samples were digested overnight with concentrated H_2SO_4. The resultant ammonia was trapped into 0.1 N HCl using Conway diffusion dishes. The ammonia was then measured according to the method of Kaplan (13).

RESULTS

GA_3 and Protein Hydrolysis. Total nitrogen stored as protein reserves in the mature barley endosperm is distributed into soluble (saline-buffer extractable) and insoluble (propanol extractable prolamins and the remaining residue) fractions. The distribution of total nitrogen in these two fractions is shown in Table I. In endosperm tissue of dry kernels, approximately 12% of total nitrogen was recovered in the soluble fraction. In this sample, the total nitrogen was distributed equally between the TCA-precipitable and TCA-soluble materials. In the absence of GA_3, there was no significant change in the distribution of total nitrogen during the initial 60 h incubation period. In the GA_3-treated samples, however, 35% of the total nitrogen was recovered in the soluble fraction after a 24 h incubation period. This proportion increased to 70% during the next 36 h. The TCA-soluble material in the samples incubated for 60 h accounted for about two-thirds of the total increase in total nitrogen in the soluble fraction. There was a concomitant decrease in the total nitrogen in the insoluble fraction. This result, therefore, indicates that the overall hydrolysis of endosperm protein reserves is dependent on the presence of GA_3.

Analysis of the Methanol-Insoluble Fraction. *(a) Large Polypeptides.* Large polypeptides precipitated from the soluble fractions were analyzed by SDS-PAGE using 12.5% polyacrylamide gels. A number of discrete polypeptide bands were noted in control samples (Fig. 1). There is the appearance and with time disappearance of a polypeptide at 55 kD in both the presence and absence of GA_3. No other major changes in the profile of polypeptides was seen in the absence of GA_3 during a 36 h incubation period. In the presence of GA_3, the banding pattern remains largely unchanged except for the appearance of a 42 kD band. The intensity of this band increased with longer periods of incubation. Based on the electrophoretic mobility and Western immunoblotting data (V Rastogi, A Oaks, unpublished data), this band is the subunit of α-amylase.

(b) Hordein-Derived Polypeptides. If the polypeptide fragments released at early times from the hydrolysis of hordeins retain the antigenicity of the hordein proteins, then the antibodies raised against hordeins should serve to detect the hydrolysis products. Soluble proteins were prepared from endosperm tissue of dry kernels (0) and half-seeds incubated for 8, 24, and 72 h in the presence and absence of GA_3. A number of hordein IgG-reacting polypeptide bands were seen on the immunoblots of control samples (lane 0; Fig. 2). The presence of hordein-related polypeptides in this sample could be due to (i) sharing of common epitopes by soluble proteins and hordeins; (ii) prematurely aborted hordein polypeptides during seed development, which are extractable with the aqueous buffer; and/or finally (iii) partial hydrolysis of hordeins, which may begin during the later stages of caryopsis development.

No significant change compared to the controls (0) was noted in samples incubated for 8 h in the presence and absence of GA_3. In samples incubated for 24 h in the absence of GA_3, however, a major band at around 40 kD (band c; Fig. 2) was more prominent. In addition, polypeptide bands in the size range of 25–30 kD (bands between f and g) appeared. In samples incubated for 24 h in the presence of GA_3, large polypeptide bands

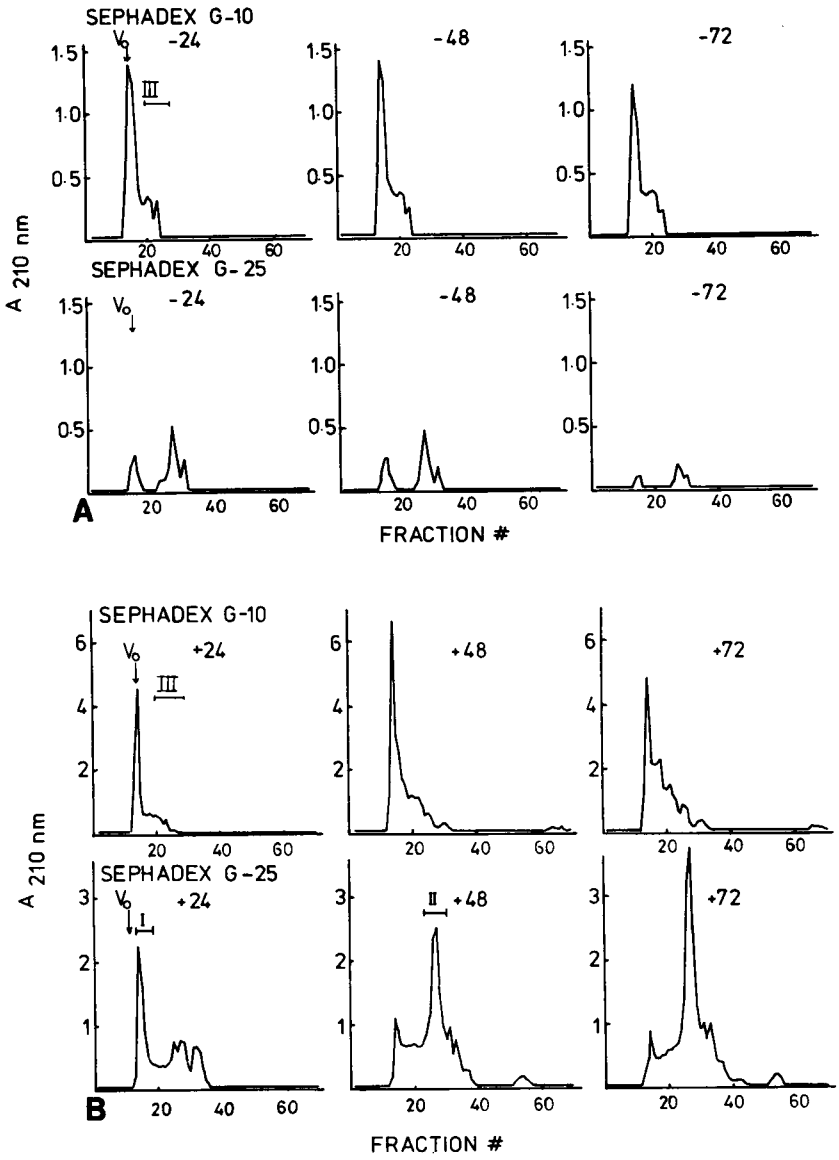

FIG. 3. Elution profile of peptides in the methanol-soluble fraction through columns of Sephadex G-10 and G-25. Soluble fractions were prepared from half-kernels incubated for 24, 48, and 72 h in the absence (−; A) and presence (+; B) of GA_3. Large polypeptides in the soluble fractions were precipitated using 50% methanol. The supernatant, *i.e.* methanol-soluble fraction, was chromatographed successively through columns of Sephadex G-10 and G-25. Peptide bonds were read at 210 nm. Bars with I, II and III represent the fractions pooled in 'peak I', 'peak II', and 'peak III', respectively. V_0 is the void volume.

in the size range of 45 to 67 kD (a, b, and c bands) were much less pronounced. In addition, polypeptide bands of low mol wt that appeared as a smear rather than discrete bands (less than 15 kD; i–j), were observed. There was no significant difference in the banding pattern of immunoreactive polypeptide bands in samples incubated for 24 or 72 h in the absence of GA_3. In the presence of GA_3, however, most of the hordein IgG-reacting polypeptide bands were either absent or present in low amounts when samples were incubated for 72 h. This result showed that an initial release of large polypeptide fragments from hordein proteins was independent of GA_3. Further hydrolysis of the water-soluble polypeptide fragments derived from hordein proteins is, however, dependent on the proteases induced in the presence of this plant growth substance.

Analysis of the Methanol-Soluble Fraction. The initial calibration of the Sephadex G-10 column showed that amino acids and peptides of less than 9 amino acid residues eluted together. Therefore the fractions containing peptides of less than 9 amino acid residues in length and amino acids were pooled and analyzed as one group (Fig. 3; peak III). Larger peptides (greater than 9 amino acid residues) eluted with the void volume (V_0). This was then passed over a Sephadex G-25 column. Two broad peaks were observed (Fig. 3, A and B). There was a shift from larger polypeptides (peak I) to smaller polypeptides (peak II) with time when half-seeds were incubated in the presence of GA_3 (Fig. 3B). Peptides in peak I ranged from 50 to 70 amino acid residues in length and those in Peak II, from 15 to 35 amino acid residues in length. In the absence of GA_3, there was no significant change in the profile and/or proportion of peptides in both the peaks during the 72 h incubation period (Fig. 3A). In the presence of GA_3, peptides in 'peak I' accounted for 43% of total nitrogen in the methanol-soluble fraction at 24 h, and about 11% by 72 h of incubation (Table II). During this period, the total nitrogen increased in 'peak II' from 42% to 50% and in 'peak III' from 2% to 26%.

DISCUSSION

The technique of incubating embryo-less endosperm pieces has been used by several workers in studies related to the induction of hydrolytic enzymes by GA_3. The best studied example is the induction of α-amylase (4, 12, 19). The appearance of gliadin- and hemoglobin-degrading protease activity (8, 12, 23), and carboxypeptidase(s) activity (21, 27) in barley endosperm tissue has also been examined. The hydrolytic products of barley storage proteins, released in the media have, however, not been described. Following incubation in the presence of GA_3, protein

Table II. *Balance Sheet of Total Nitrogen in Different Fractions Prepared from Barley Half-Kernels*

Crude extracts were prepared from endosperm tissue of dry kernels and embryo-less endosperm pieces incubated in the presence and absence of GA_3. After centrifugation, supernatant solution (SF, soluble fraction) and residue (pellet) were separated. Large polypeptides in the soluble fraction were precipitated with 50% methanol. The supernatant solution (MSF, methanol-soluble fraction) was passed successively over Sephadex G-10 and G-25 columns. Amino acids + peptides of less than 9 amino acid residues ('peak III') were collected after filtration through a Sephadex G-10 column, and peptides fractionated on the Sephadex G-25 column were pooled ('peak I' and 'peak II'). The total nitrogen content was measured in each fraction.

Sample	Pellet	Total Nitrogen SF	Total Nitrogen MSF	'Peak I'	'Peak II'	'Peak III'
			mg/30 endosperms			
Initial	10.5	1.4	0.5	—[a]	—	—
−24 h	11.4	1.6	0.7	0.2	0.4	0.03
+24 h	8.1	3.7	2.1	0.9	0.8	0.04
−72 h	10.5	1.6	0.6	0.1	0.4	0.05
+72 h	2.3	10.9	7.2	0.8	3.5	1.90

[a] Not done.

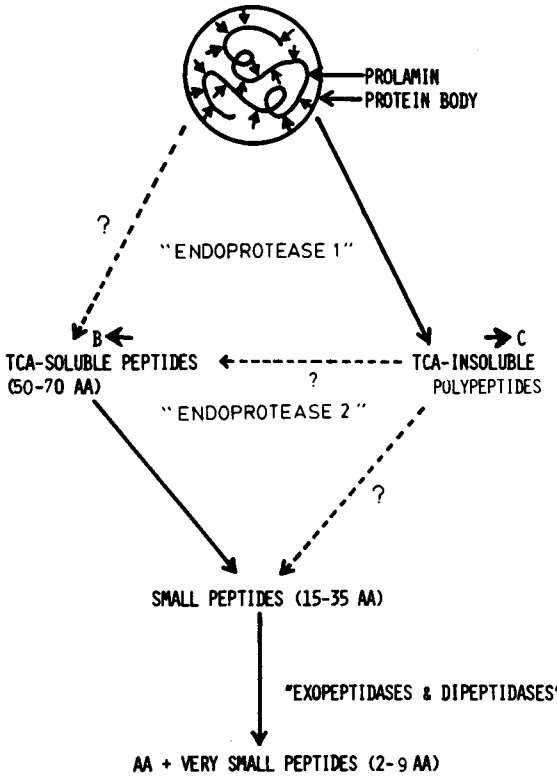

FIG. 4. Proposed model for overall hydrolysis of hordeins in barley endosperms. Broken arrows with (?) sign represent the hypothetical steps. Solid arrows represent the possible steps, which are consistent with the evidence presented in this paper. AA = amino acids. Small arrows on the prolamin polypeptide in protein body represent the possible cleavage sites at the junctions of repeating octapeptide units, which are present in the hordein polypeptides (6). Whether or not these cleavages occur depends on the accessibility of the amino acid residues to the active sites of protease(s) mediating this step. B and C represent the two alternative types of products (see Discussion) released by the initial endoprotease activity.

hydrolysis is initiated both in the aleurone and starchy endosperm tissues. Thus, it is possible that the hydrolysis products released from proteins in the aleurone layers contribute in part to the observed changes in total nitrogen and peptides.

An immunological approach was adopted to detect large polypeptide fragments released from the hordein proteins. The same technique has been used previously by other groups to study the turnover of RuBPCase and hydrolysis of proteins in a dicotyledonous storage tissue (7, 24, 27). Using antibodies against RuBPCase, Tang and Huffaker (24) identified the degradation products of the enzyme on western immunoblots. Antibodies against the crystalloid storage proteins were employed by Gifford *et al.* (7) to identify the hydrolytic fragments released from the castor bean cotyledons. Wilson *et al.* (27) used a similar technique to identify hydrolysis products of glycinin and conglycinin during germination of soybean seedlings.

As seen in Figure 2, a number of hordein IgG-reacting polypeptide bands are present in the soluble protein fraction from endosperm tissue of dry kernels. However, the interesting observation is the fact that when embryo-less endosperms were incubated in the absence of GA_3, there were shifts in some of the polypeptide bands. For example, polypeptide bands in the size range of 25 to 30 kD appear. In addition, a polypeptide band around 40 kD increased in its intensity. Since these polypeptide fragments react with the hordein antibodies, it is likely that they are derived from the hordein proteins and therefore represent the initial hydrolytic products. Our interpretation is that an endoprotease activity is involved in the release of these initial hydrolytic fragments. In a related cereal, corn, an endopeptidase activity has been reported to be involved in the hydrolysis of denatured zeins (10). However, in that system, carboxypeptidase activities are less apparent (28). In noncereals, such as pea (1), pumpkin (9), mung bean (2), and castor bean (7), good evidence exists in support for the involvement of an endoprotease activity for hydrolysis of the native storgae proteins.

It appears that in the presence of GA_3, polypeptide fragments in the size range of 15 kD or less are produced (Fig. 2). It is possible that these low mol wt polypeptide fragments are derived from those released in the absence of GA_3. With longer periods of incubation in the presence of GA_3, the levels of peptides in 'peak I' decreased and those in 'peak II' and 'peak III' increased (Fig. 3B). It is tempting to speculate that large polypeptide fragments released in the absence and/or presence of GA_3 serve as substrates for the proteases involved in the production of peptides recovered in 'peaks I and II.' Hammerton and Ho (8) have identified three endoprotease-like activities in the media of incubated aleurone layers, and it is possible that these enzymes are active in some of the steps described above.

The different sets of reactions are summarized in a model presented in Figure 4. According to this model, an endoprotease, whose appearance is not dependent on the presence of GA_3, and, which is induced when water is added to the system, is involved in the initial stages of hydrolysis (endoprotease I). The products of this step are either larger polypeptide fragments (in the size range of 40 kD and 25–30 kD) or peptides recovered in 'peak I.' Subsequently, proteases induced in the presence of GA_3, endoprotease 2, are probably involved in the release of peptides recovered in 'peak II' and perhaps 'peak III.' This class of endoprotease appears to require GA_3 for its induction. Exopeptidases and dipeptidases may also be active in the release of amino acids and very small peptides. Even though the steps involved in the hydrolysis of hordeins as shown in the model are not definitive, with our approach and that of Hammerton and Ho (8), we are now in a position to test specific details of this model.

Finally, it must be noted that a substantial proportion of total nitrogen was accounted for by peptides in 'peak II,' both at 24

and 72 h. Because of the high levels of carboxypeptidases, at 72 h after incubation, one would have expected amino acids to be the major component in the soluble fraction. However, since total nitrogen in 'peak III' containing amino acids + peptides of less than 9 residues, is only 26% of that in the methanol-soluble fraction (Table II), the role of carboxypeptidases (16–18) in the hydrolysis of storage proteins remains doubtful. Presence of high levels of peptides in soluble extracts of germinating endosperms and an active peptide uptake system in the scutellum tissue has been demonstrated earlier (11, 26). Our results, therefore, support the observations of Higgins and Payne (11) and are consistent with the idea that peptides may be the principal hydrolytic products transported across the scutellum to the growing embryo by uptake mechanisms similar to those described by Walker-Smith and Payne (26).

LITERATURE CITED

1. Basha SMM, L Beevers 1975 The development of proteolytic activity and protein degradation during the germination of *Pisum sativum* L. Planta 124: 77–87
2. Baumgartner B, MJ Chrispeels 1977 Purification and characterization of vicilin peptidohydrolase, the major endopeptidase in the cotyledons of mung bean seedlings. Eur J Biochem 77: 223–233
3. Burger WC, N Prentice, J Kastenschmidt, JD Huddle 1966 Partial purification of proteases from germinated barley. Crop Sci 4: 546–554
4. Chrispeels MJ, JE Varner 1967 Hormonal control of enzyme synthesis: on the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiol 42: 1008–1016
5. Dierks-Ventling C, K Cozens 1982 Immunochemical crossreactivity between zein, hordein, and gliadin. FEBS Lett 142: 147–150
6. Forde BG, M Kries, MS Williamson, RP Fry, J Pywell, RP Shewry, N Bunce, BJ Miflin 1985 Short tandem repeats shared by B- and C- hordein cDNAs suggest a common evolutionary origin for two groups of cereal storage proteins genes. EMBO J 4: 9–15
7. Gifford DJ, HC Imeson, JD Bewley 1985 The castor bean crystalloid protein complex: nature and kinetics of its hydrolysis following seed imbibition. Plant Physiol 77: S-399
8. Hammerton RW, THD Ho 1986 Hormonal regulation of the development of protease and carboxypeptidase activities in barley aleurone layers. Plant Physiol 80: 629–637
9. Hara I, H Matsubara 1980 Pumpkin (*Cucurbita* sp) seed globulin V. Proteolytic activities involved in globulin degradation in ungerminated seeds. Plant Cell Physiol 21: 219–232
10. Harvey BMR, A Oaks 1974 The hydrolysis of endosperm protein in *Zea mays*. Plant Physiol 53: 454–457
11. Higgins CF, JW Payne 1980 The peptide pools of germinating barley grains: Relationship to hydrolysis and transport of storage proteins. Plant Physiol 67: 785–792
12. Jacobsen JV, JE Varner 1967 Gibberellic acid-induced synthesis of protease by isolated aleurone layers of barley. Plant Physiol 42: 1596–1600
13. Kaplan A 1965 Urea nitrogen and urinary ammonia. In S Meits, ed, Standard Methods of Clinical Chemistry, Academic Press, New York, pp 245–246
14. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680–685
15. Laurière M, J Mossé, J Daussant 1983 Physicochemical and immunochemical studies of the hordeins with low electrophoretic mobility at acidic pH. Biochim Biophys Acta 748: 254–262
16. Mikola J 1983 Proteinases, peptidases, and natural inhibitors of endogenous proteinases in germinated seeds. In J Daussant, J Mosse, J Vaughan, eds, Seed Proteins, Academic Press, New York, pp 35–52
17. Mikola J, L Kolehmainen 1972 Localization and activity of various peptidases in germinating barley. Planta 104: 167–177
18. Mikola L 1983 Germinating barley grains contain five carboxypeptidases with complementary substrate specificity. Biochim Biophys Acta 747: 241–252
19. Mozer TJ 1980 Control of protein synthesis in barley aleurone layers by the plant hormones gibberellic acid and abscisic acid. Cell 20: 479–485
20. Pernolet JC, J Mossé 1983 Structure and location of legume and cereal seed storage proteins. In J Daussant, J Mossé, J Vaughan, eds, Seed Proteins, Academic Press, New York, pp 155–192
21. Schroeder RL, WC Burger 1978 Development and localization of carboxypeptidase activity in embryo-less barley half-kernels. Plant Physiol 62: 458–462
22. Shewry PR, JM Field, MA Kirkman, BJ Miflin 1980 The extraction, solubility, and characterization of two groups of barley storage polypeptides. J Exp Bot 31: 393–407
23. Sundblom N-O, J Mikola 1972 On the nature of proteinases secreted by the aleurone layer of barley grain. Physiol Plant 27: 281–284
24. Tang AB, RC Huffaker 1985 Immuno identification of *in vivo* ribulose bisphosphate carboxylase (RuBPCase) degradation products in barley chloroplasts. Plant Physiol 77: S-168
25. Towbin H, T Staehelin, J Gordon 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76: 4850–4854
26. Walker-Smith DJ, JW Payne 1983 Peptide uptake in germinating barley embryos involved in dithiol-dependent transport protein. FEBS Lett 160: 25–30
27. Wilson, KA, J Chen, B Rightmire, A Tan-Wilson 1985 Differential metabolism of glycinin and conglycinin chains during germination. Plant Physiol 77: S-407
28. Winspear MJ, KR Preston, V Rastogi, A Oaks 1984 Comparisons of peptide hydrolases in cereals. Plant Physiol 75: 480–482