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Barley Aleurone Layers Secrete a Nuclease in Response to Gibberellic Acid[1]

PURIFICATION AND PARTIAL CHARACTERIZATION OF THE ASSOCIATED RIBONUCLEASE, DEOXYRIBONUCLEASE, AND 3′-NUCLEOTIDASE ACTIVITIES

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ABSTRACT

Incubation of barley (*Hordeum vulgare* L. cv Himalaya) half-seeds with gibberellic acid enhances the secretion of ribonuclease and deoxyribonuclease from aleurone tissue (MJ Chrispeels, JE Varner 1967 Plant Physiol 42: 398–406; L Taiz, JE Starks 1977 Plant Physiol 60: 182–189). These activities were over 50-fold greater in medium of half-seeds incubated with gibberellic acid than in control medium. Ribonuclease and deoxyribonuclease activities initially appeared in the medium 24 to 48 hours after hormone induction and increased for up to 96 hours. Both activities had a pH optimum of 6.0 and a temperature optimum of 55°C. When the medium from gibberellic acid-treated half-seeds was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, the major ribonuclease and deoxyribonuclease activity bands comigrated. The two enzyme activities remained associated throughout a 2,700-fold purification employing ammonium sulfate fractionation, Heparin-Agarose affinity chromatography, and Reactive Blue 2-Agarose affinity chromatography. Also accompanying the ribonuclease and deoxyribonuclease activities throughout purification was the ability to hydrolyze the 3′-phosphoester linkage of 3′-AMP. The purified protein was composed of a single polypeptide with an apparent molecular weight of 36 kilodaltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. It is concluded that in response to gibberellic acid, barley aleurone tissue secretes a nuclease having ribonuclease, deoxyribonuclease, and 3′-nucleotidase activities.

Heterotrophic growth of cereal seedlings depends upon utilization of proteins, carbohydrates, and other nutrients stored in the endosperm. In barley and wheat, the mobilization of these food reserves appears to be regulated by the hormone gibberellin synthesized in and secreted from the germinating embryo (23). This hormone induces aleurone tissue to synthesize and secrete several hydrolases specific for degrading cell walls and depolymerizing macromolecules stored in the endosperm. These enzymes include α-amylase (5), protease (9) 1,3-β-glucanase (21), (1,3:1,4)-β-glucanase (27), acid phosphatase (1), RNase (5), and DNase (22). Here we report the purification of a nuclease (EC 3.1.30.2) having RNase, DNase, and 3′-nucleotidase activities. This enzyme is apparently synthesized in barley aleurone tissue, and is secreted in response to GA_3. It likely accounts for much of the RNase and DNase activities secreted by this tissue.

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MATERIALS AND METHODS

Materials. Barley (*Hordeum vulgare* L. cv Himalaya) seeds from the 1985 harvest were purchased from the Department of Agronomy and Soils, Washington State University, Pullman, WA. The antibiotic-antimycotic (containing amphotericin B, penicillin, and streptomycin), gibberellic acid, toluidine blue, Torula yeast RNA, salmon testes DNA, Heparin-Agarose, and Reactive Blue 2-Agarose were from Sigma. Ultrapure acrylamide and ultrapure $(NH_4)_2SO_4$ were purchased from Schwarz Mann Biotech, Cambridge, MA. Low mol wt standards were purchased from Bio-Rad, Richmond, CA.

Tissue Incubation. Embryoless half-seeds of barley were surface sterilized as previously described (5), and incubated in a medium containing 0.02 M Na succinate, pH 5.0, 0.02 M $CaCl_2$, 0.38 μg/ml amphotericin B, 150 units/ml penicillin, and 0.15 mg/ml streptomycin. Half-seeds were incubated aseptically at 22 to 25°C on a rotary shaker at 200 rpm. Isolated aleurone layers were prepared as previously described (6) and incubated as above. One hundred half-seeds or aleurone layers were incubated in 10 ml of medium in the presence or absence of GA_3 (1 μM). At the indicated times, the medium was collected and centrifuged at 10,000*g* for 10 min. All centrifugation steps were at 4°C. The supernatant was collected and the volume brought up to 15 ml with cold buffer (0.02 M Na succinate [pH 5.0] and 0.02 M $CaCl_2$) and 15 μl of 2-mercaptoethanol were then added. The medium was stored at −20°C.

RNase and DNase Assays. RNase and DNase were assayed by a modification of the method of Wilson (24). The buffer used consisted of 0.1 M Na succinate (pH 6.0) and 0.01 M KCl. Up to 200 μl of sample were added to 2 ml of ice-cold buffer containing 4 mg/ml RNA (RNase assay) or 2 mg/ml denatured DNA (DNase assay). The DNA was denatured by boiling for 5 min and quenching in ice water prior to addition of sample. Assay tubes were incubated in a 55°C water bath for 30 or 60 min. The reaction was stopped by placing assay tubes in ice water and adding 0.4 ml of ice-cold 25% (v/v) $HClO_4$ containing 0.75% (w/v) uranyl acetate. Samples were centrifuged at 12,000*g* for 5 min, the supernatants collected and diluted 20- to 100-fold with water, and the A_{260} determined. Assays were linear with respect to time and amount of sample. One unit of activity is that amount of enzyme which catalyzes the release of acid-soluble nucelotides at a rate of 1.00 A_{260} unit/min.

3′-Nucleotidase Assay. This assay measures the release of Pi from 3′-AMP. The reaction mixture contained 0.1 M Na succinate (pH 6.0), 0.01 M KCl, and 1 mM 3′-AMP. To 2-ml of ice-cold reaction mixture was added 5 to 25 μl of sample and the mixture was incubated in a 37°C water bath for 30 min. The reaction was stopped by transferring the mixture to ice water,

adding 0.5 ml of cold 40% TCA and vortexing. A 0.1 to 0.5 ml portion of the reaction mixture was assayed for Pi by the method of Chen *et al.* (4). One unit of activitiy is that amount of enzyme which catalyzes the release of 1 μmol of Pi/min.

The pH and temperature optima for RNase and DNase activities were assayed in 60 min incubations as above using the $(NH_4)_2SO_4$ fractionated medium. The pH range tested was 4.5 to 8.5, in 0.5 unit increments. The temperature range tested was 20 to 70°C, in 5° increments. Protein was determined according to Bradford (3) using BSA as standard.

RNase and DNase Activity Gels. SDS-PAGE for RNase and DNase activities were according to the methods of Blank *et al.* (2), except that 0.3 mg/ml RNA or DNA was also added to the stacking gel and upper running buffer. Sample loading buffer consisted of 0.125 M Tris-HCl (pH 6.8), 0.1% SDS, 50% glycerol, and 0.02% bromophenol blue. Samples were not heated after addition of sample loading buffer. After running, gels were rinsed in two changes of 25% isopropanol in 0.01 M Tris-HCl (pH 7.4), followed by two changes of 0.01 M Tris-HCl (pH 7.4), and one rinse in a reaction buffer containing 0.1 M Na succinate (pH 6.0), 0.01 M KCl, 1 mM cysteine, 40 μM $MgCl_2$, and 40 μM Zn acetate. All rinses were for 15 min at room temperature in 250 ml of the respective buffer. This was followed by a 3 h incubation at 55°C in the reaction buffer. Incubated gels were rinsed, stained and destained as previously described (2).

Purification of Enzyme. One thousand half-seeds were incubated as above in each of 30 flasks (1-L) with 100 ml of medium containing 1 μM GA_3. After 24 h, the initial medium was discarded and another 100 ml of medium containing 1 μM GA_3 was added to each flask. Half-seeds were then incubated for another 3 d. The medium was collected and centrifuged for 15 min at 10,000*g*. The supernatant was collected, 0.1% (v/v) 2-mercaptoethanol was added, and the medium was stored at −20°C.

Ammonium Sulfate Fractionation. The medium collected from the above step was precipitated with $(NH_4)_2SO_4$. Protein which precipitated between 50 to 75% saturation was dissolved in water and extensively dialyzed against buffer A (0.05 M Na acetate [pH 6.0], 1 mM cysteine, 2 mM $MgCl_2$, and 0.1 mM Zn acetate). All dialysis procedures were at 4°C.

Heparin-Agarose Affinity Chromatography. The dialyzed medium from the preceding step was divided in half, and each portion of approximately 80 ml (10.4 mg protein/ml) was applied to a column (2.5 × 10 cm) packed with 25 ml of Heparin-Agarose equilibrated with buffer A at room temperature. As the sample was loading onto the column the eluant was collected. After the sample was loaded, 10 ml fractions were collected eluting with 200 ml of buffer, followed by 100 ml of buffer A containing 0.3 M KCl, followed by 100 ml of buffer A containing 0.6 M KCl. The column was run at a flow rate of 2 ml/min. Fractions were assayed for RNase and DNase activities and their A_{260} determined. Fractions in which RNase and DNase activities coeluted were pooled.

Reactive Blue 2-Agarose Affinity Chromatography. The pooled fractions from the preceding step (69 ml, 0.208 mg protein/ml) were applied to a column (1.5 × 10 cm) packed with 5 ml of Reactive Blue 2-Agarose equilibrated with buffer A containing 0.6 M KCl at room temperature. The column eluant was collected as the sample was loaded. After the sample was loaded, 5 ml fractions were collected eluting with 25 ml of buffer A containing 0.6 M KCl, followed by 25 ml of buffer A containing 0.75 M KCl, followed by 50 ml of buffer A containing 3 M KCl. The column was run at a flow rate of 2 ml/min. Fractions were assayed for RNase and DNase activities and those in which the activities coeluted were pooled and extensively dialyzed against buffer A.

The dialyzed sample (22 ml, 0.04 mg protein/ml) from the above step was divided into three portions. Each portion was applied to a column (1 × 10 cm) packed with 2 ml of Reactive Blue 2-Agarose equilibrated with buffer A at room temperature. The column eluant was collected as the sample was loaded. Then 2 ml fractions were collected eluting with 10 ml of buffer A, followed by 10 ml buffer A containing 0.75 M KCl, followed by 10 ml buffer A containing 0.75 M KCl and 5 mg/ml RNA, followed by 10 ml of buffer A containing 3 M KCl. The column was run at a flow rate of 0.5 ml/min. Fractions were assayed for RNase and DNase activities, and those in which both activities coeluted were pooled and extensively dialyzed against buffer A.

Determination of Molecular Weight and Enzyme Purity. A small portion of the dialyzed sample from each step of the purification procedure was added to an equal volume of electrophoresis sample loading buffer (0.25 M Tris-HCl [pH 6.8], 1% 2-mercaptoethanol, 2% SDS, 50% glycerol, and 0.02% bromophenol blue) and heated at 100°C for 5 min. The sample was analyzed by SDS-PAGE using a 12.5% acrylamide slab resolving gel according to the procedure of Laemmli (14), and proteins were visualized using the silver stain method of Morrissey (17).

RESULTS

When barley half-seeds were incubated in control medium, RNase and DNase activities in the medium were low (Table I). Incubation with GA_3 increased levels of RNase and DNase activities in the medium. During the first 24 h of incubation in the presence of hormone, half-seeds secreted only small amounts of RNase and DNase. However, relatively large amounts of both activities were secreted during the following 72 h of incubation. Incubation of half-seeds with GA_3 for 4 d caused more than a 50-fold increase in the secretion of RNase and DNase. Similar results were obtained using isolated aleurone layers, except that the timing of GA_3-enhanced secretion of RNase and DNase was earlier than for half-seeds (Table I).

The first evidence that GA_3 enhanced the secretion of an enzyme which hydrolyzes both RNA and DNA was, that in crude medium, the pH and temperature optima for both RNase and DNase activities were the same. The pH optimum was 6.0 (Fig. 1) and the temperature optimum was 55°C (Fig. 2). The medium from half-seeds incubated with GA_3 was analyzed by SDS-PAGE. Staining these gels for RNase and DNase activities showed that the major RNase activity band and the DNase activity band comigrated (Fig 3), each having an apparent mol

Table I. *Effect of GA_3 on the Secretion of RNase and DNase from Barley Half-Seeds and Isolated Aleurone Layers*

Barley half-seeds and isolated aleurone layers were incubated in medium in the absence or presence of GA_3 (1 μM). At indicated times, the medium was collected and assayed for RNase and DNase activities according to "Materials and Methods."

Incubation Time	Half-Seeds		Aleurone Layers	
	Control	GA_3	Control	GA_3
h	*RNase activity (ΔA_{260}/min)*[a]			
24	1.5	5	0.5	18
48	1.5	37	1.5	36
72	1.5	64	5.5	112
96	1.5	74	ND[c]	ND
	DNase activity (ΔA_{260}/min)			
24	<0.25[b]	0.25	<0.25	3.0
48	<0.25	9	<0.25	7.5
72	<0.25	35	<0.25	43
96	<0.25	42	ND	ND

[a] RNase and DNase activities were for 50 half-seeds or 50 aleurone layers. [b] Values were below the sensitivity of the assay which was for 50 half-seeds or aleurone layers (ΔA_{260}/min) = 0.25. [c] Values at these time points were not determined.

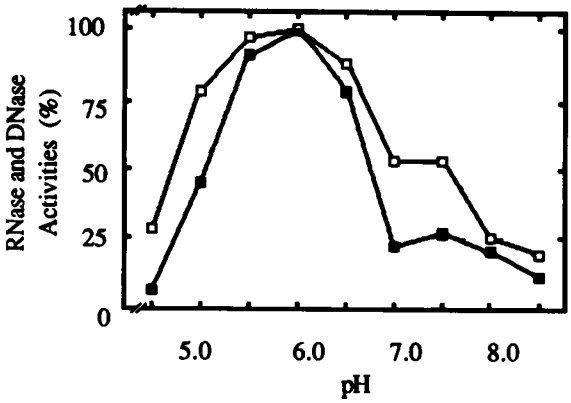

FIG. 1. Effect of pH on RNase and DNase activities. Medium from GA_3-treated barley half-seeds was assayed for RNase and DNase activities at different pH values as described in "Materials and Methods." Buffers contained 0.01 M KCl and were 0.1 M: Na succinate for pH 4.5 to 6.5; imidazole-HCl for pH 6.5 to 7.5; Tris-HCl for pH 7.5 to 8.5. Activities are in percent of the maximum obtained for RNase (□), 0.53 unit, and for DNase (■), 0.58 unit.

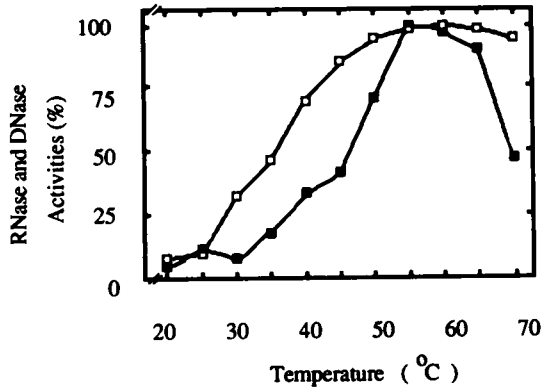

FIG. 2. Effect of temperature on RNase and DNase activities. Medium from GA_3-treated barley half-seeds was assayed for RNase and DNase activities at different temperatures as described in "Materials and Methods." Activities are in percent of the maximum obtained for RNase (□), 1.4 units, and for DNase (■), 1.6 units.

wt of 36 kD. Two other RNase activity bands were observed with apparent mol wt of 25 kD and 20 kD. The relative proportions of the three RNase activity bands varied with experiments, however the 36 kD RNase activity band was always prominent. RNase and DNase activities observed following SDS-PAGE was substantially less than those observed when the medium was analyzed by non-denaturing PAGE (data not shown).

The steps used in the purification of the enzyme are summarized in Table II. Each step of the purification was assessed by SDS-PAGE (Fig. 4). The crude medium from half-seeds incubated between 24 to 96 h after addition of GA_3 was substantially enriched for RNase and DNase (*i.e.* during the initial 24 h incubation, many of the soluble endosperm and cell wall proteins were removed). The crude medium was centrifuged to remove particulates (Fig. 4, lane 1), and the supernatant was fractionated with $(NH_4)_2SO_4$. The protein which precipitated between 50 to 75% $(NH_4)_2SO_4$ saturation was dissolved in water and dialyzed against buffer A (Fig. 4, lane 2). The dialyzed material from the $(NH_4)_2SO_4$ step was applied to a Heparin-Agarose column and eluted with stepwise increases in the KCl concentration of buffer A (Fig. 5). Two RNase activity peaks were resolved. The first peak eluted at 0.3 M KCl and had very little DNase activity associated with it. Increasing the KCl concentration to 0.6 M resulted in a second peak of RNase activity, with which the

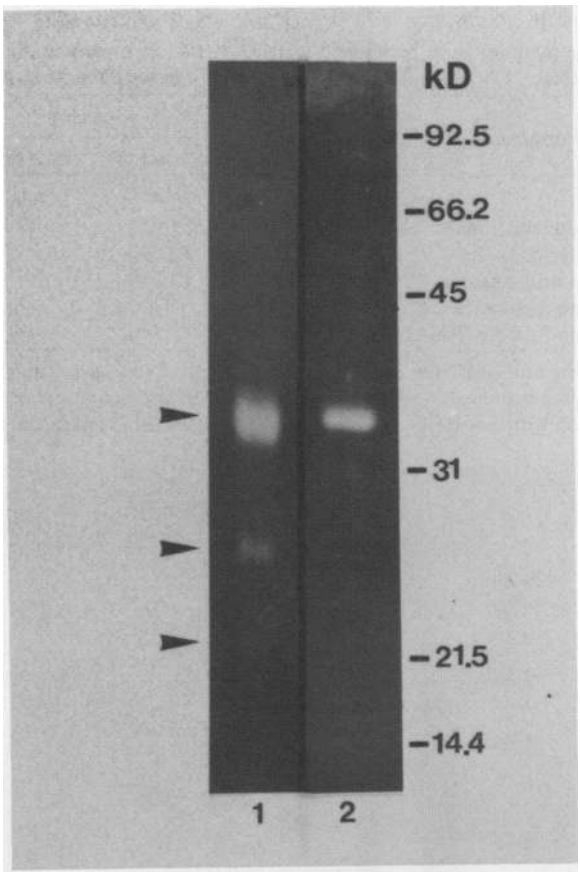

FIG. 3. Composite of RNase and DNase activity gels. Medium from GA_3-treated barley half-seeds was fractionated using $(NH_4)_2SO_4$ and run on SDS-polyacrylamide gels (12.5%) containing either RNA or DNA as described in "Materials and Methods." Lane 1, RNase activity gel; lane 2, DNase activity gel; samples for activity gel analyses were from the same medium. The arrows indicate the migration of the three RNase activities observed in lane 1. The migration of mol wt markers is indicated on the right.

DNase activity coeluted. These data are in agreement with that obtained from activity gels (Fig. 3); *i.e.* the presence of more than one RNase isozyme, and the association of DNase activity with the major RNase activity.

Fractions from Heparin-Agarose chromatography in which RNase and DNase activities coeluted were pooled (Fig. 4, lane 3) and directly applied to a Reactive Blue 2-Agarose column equilibrated with buffer A containing 0.6 M KCl. The column was eluted with stepwise increases in the KCl concentration of buffer A (Fig. 6). RNase and DNase activities coeluted at 3 M KCl, and the peak fractions were pooled as indicated and dialyzed against buffer A. This peak still contained one major and two minor polypeptides (Fig. 4, lane 4). Final protein purification was accomplished by chromatography over Reactive Blue 2-Agarose. Once again, neither activity was eluted at 0.75 M KCl. However when eluted with 0.75 M KCl and 5 mg/ml RNA, both RNase and DNase activities coeluted (Fig. 7). Both activities were completely removed from the column by this procedure, as no RNase or DNase activity was further eluted with 3 M KCl. All of the protein in this activity peak migrated as a single band with an apparent mol wt of 36 kD (Fig. 4, lane 5). (The two bands at approximately 60 and 67 kD are known artifacts of silver stained gels (15) and also appear in lanes to which only sample loading buffer was applied; Fig. 4, lane 6). The purified protein was also capable of hydrolyzing the 3′-phosphoester linkage of 3′-AMP. This activity had remained associated with

Table II. *Purification of RNase, DNase, and 3'-Nucleotidase from Medium in Which Barley Half-Seeds Were Incubated in the Presence of GA_3*

The medium from barley half-seeds (3×10^4) incubated in the presence of 1 μM GA_3 according to the "Materials and Methods" was collected, and RNase, DNase, and 3'-nucleotidase activities were followed during enzyme purification.

Purification Step	Total Protein	RNase		DNase		3'-Nucleotidase	
		Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity
	mg	*units*[a]	*units/mg*[b]	*units*	*units/mg*	*units*[c]	*units/mg*
Crude medium	4,945.320	55,912	11.3	17,352	3.5	844	0.17
$(NH_4)_2SO_4$	1,664.000	32,000	19.2	20,267	12.2	1,707	1.0
Heparin-Agarose	14.352	13,340	929	14,260	994	437	30
Blue-Agarose (KCl)[d]	0.882	5,320	6,031	5,040	5,714	102	115
Blue-Agarose (RNA)[e]	0.264	3,740	14,167	2,493	9,444	99	375

[a] One unit of RNase or DNase activity is equal to a ΔA_{260}/min of 1. [b] RNase, DNase, and 3'-nucleotidase specific activities are expressed in units/mg protein. [c] One unit of 3'-nucleotidase activity is equal to the release of 1 μmol Pi/min. [d] Eluted from Reactive Blue 2-Agarose column with 3 M KCl. [e] Eluted from Reactive Blue 2-Agarose column with 0.75 M KCl and 5 mg/ml RNA.

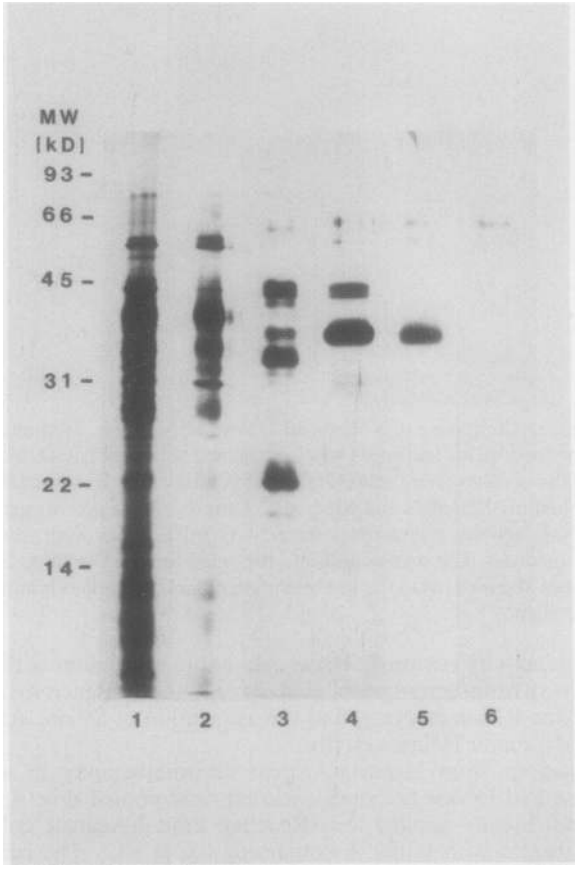

FIG. 4. Composite of SDS-PAGE analysis assessing the steps used for purification of barley nuclease. Portions of the samples following the various purification steps listed in Table II were analyzed by SDS-PAGE on 12.5% gels and polypeptides were visualized with silver stain as described under "Materials and Methods." Lane 1, crude medium; lane 2, material from 50 to 75% $(NH_4)_2SO_4$ precipitation; lane 3, pooled fractions from Heparin-Agarose affinity chromatography (Fig. 5); lane 4, pooled fractions from Reactive Blue 2-Agarose affinity chromatography eluting with 3 M KCl (Fig. 6); lane 5, pooled fractions from Reactive Blue 2-Agarose affinity chromatography eluting with 0.75 M KCl and 5 mg/ml RNA (Fig. 7); lane 6, sample loading buffer, MW, mol wt standards.

the RNase and DNase activities throughout the purification procedures (Table II).

DISCUSSION

Barley aleurone nuclease purified to homogeneity represents over a 2,700-fold increase in DNase specific activity (Table II).

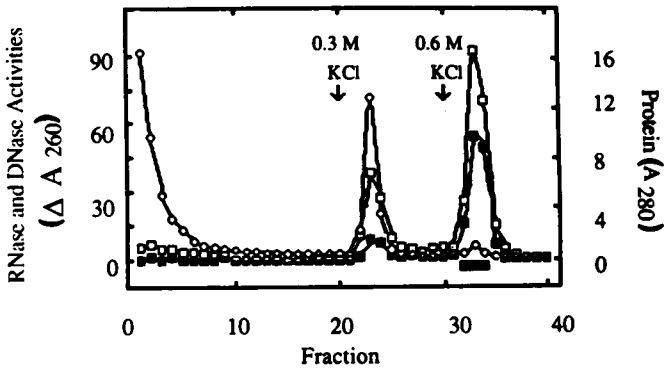

FIG. 5. Heparin-Agarose affinity chromatography. The dialyzed material which precipitated between 50 to 75% $(NH_4)_2SO_4$ saturation was applied to a column (2.5 × 10 cm) packed with 25 ml of Heparin-Agarose equilibrated with buffer A. The column was eluted with stepwise increases in the KCl concentration (arrows). The flow rate was 2 ml/min and 10 ml fractions were collected. Each fraction was assayed for RNase (□) and DNase (■) activities and protein (◇) as described in "Materials and Methods." RNase and DNase activities are expressed as the ΔA_{260}/h for a 2-ml reaction mixture. The bar indicates the fractions that were pooled for further analysis.

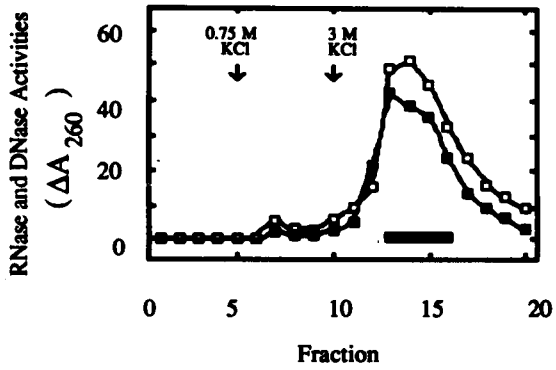

FIG. 6. Reactive Blue 2-Agarose affinity chromatography. The pooled material from Heparin-Agarose affinity chromatography (Fig. 5) was applied to a column (1.5 × 10 cm) packed with 5 ml of Reactive Blue 2-Agarose equilibrated with buffer A containing 0.6 M KCl. The column was eluted with stepwise increases in the KCl concentration (arrows). The flow rate was 2 ml/min and 5 ml fractions were collected. Each fraction was assayed for RNase (□) and DNase (■) activities as described under "Materials and Methods" and the legend to Figure 5. The bar indicates the fractions which were pooled for further analysis.

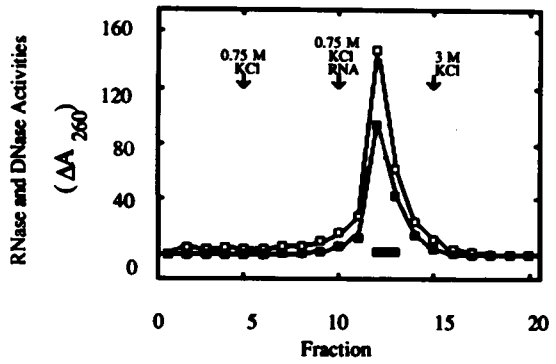

FIG. 7. Reactive Blue 2-Agarose affinity chromatography. The pooled and dialyzed material from Reactive Blue 2-Agarose affinity chromatography (Fig. 6) was rechromatographed through a column (1 × 10 cm) packed with 2 ml of Reactive Blue 2-Agarose equilibrated with buffer A. The column was eluted with buffer A alone, buffer A containing 0.75 M KCL (first arrow), buffer A containing 0.75 M KCl and 5 mg/ml RNA (second arrow), and buffer A containing 3 M KCl (third arrow). The flow rate was 0.5 ml/min and 2 ml fractions were collected. Each fraction was assayed for RNase (□) and DNase (■) activities as described under "Materials and Methods" and the legend to Figure 5. The bar indicates the fractions which were pooled for further analysis.

This enzyme also exhibited RNase and 3′-nucleotidase activities. During purification of the nuclease however, RNase and 3′-nucleotidase activities did not have as great an increase in their respective specific activities compared with DNase. Similar results were observed during the purification of wheat nuclease (7). In the case of barley, this is likely due to the presence of other RNase (Fig. 3A) and phosphatase (1) activities in the crude medium.

This enzyme appears to belong to a group of plant nucleases referred to as nuclease I (26). Enzymes in this group share several characteristics: (a) they can catalyze the hydrolysis of DNA, RNA, and the 3′-phosphoester linkage of 3′-nucleotides; (b) they fall within a mol wt range of 31 to 39 kD; (c) they have a pH optimum between 5.0 and 6.5; (d) they are endonucleases; and (e) the products of nucleic acid hydrolysis are 5′-nucleotides. We have demonstrated that barley nuclease has characteristics (a) to (c) of nuclease I enzymes. In addition, this enzyme is an endonuclease against single-stranded and double-stranded DNA (data not shown). Although the products of nucleic acid hydrolysis have yet to be characterized, this enzyme has tentatively been designated nuclease I (EC 3.1.30.2).

The barley enzyme purified in this report is probably identical to the nuclease enzymes partially purified from barley seeds (19) and malt (20). These other enzymes were at most, purified 260-fold, and the purity of the proteins was never assessed. Nuclease I enzymes have also been purified from plants other than barley. The best characterized nuclease I enzymes are from mung bean sprouts (10, 11, 13, 16) and wheat seedlings (7, 12), whereas similar enzymes have been isolated from corn seedling roots (25), cultured tobacco cell medium (18), and oat leaves (28).

The secretion of barley nuclease I from isolated aleurone layers was a GA_3-dependent process. Whether the synthesis of this enzyme was also GA_3-dependent was not investigated. A nuclease with similar properties has been reported in extracts of dry barley seeds (19). The specific tissue(s) to which the nuclease was localized was not determined. In addition, the total activity of nuclease reported in dry seeds was about 2% of that secreted by a similar number of half-seeds incubated with GA_3 for 4 d. This indicates that nuclease I is synthesized in aleurone layers during seed germination.

Incubation of barley aleurone layers in medium without GA_3 increased total RNase (5) and DNase (22) activities (*i.e.* that in the aleurone layer combined with that secreted into the incubation medium) approximately 5-fold over a 2 d period. Most of these activities remained within the aleurone layers. Incubation in the presence of GA_3 caused an additional 2-fold increase in total RNase and DNase activities and induced the secretion of these enzymes. It was therefore suggested that RNase and DNase were synthesized in the absence of added GA_3, but that their secretion was dependent on the hormone. Caution must be used when interpreting data obtained with isolated aleurone layers. The physical process of detaching aleurone layers from the starchy endosperm caused 4- and 10-fold increases in α-amylase and RNase activities, respectively (5). Since many plant tissues produce RNase in response to wounding (28), the production of RNase by isolated aleurone layers incubated in medium without GA_3 might reflect the wounded status of this tissue.

The dependence of RNase and DNase secretion on GA_3, and the similarities in the kinetics of appearance of the two activities in the medium (5, 22) led Taiz and Starks (22) to suggest that these activities were due to nuclease I. We have demonstrated that nuclease I is secreted from barley aleurone layers, and that its secretion is dependent on GA_3. In addition, the kinetics of appearance of the GA_3-induced nuclease I activities in the medium is similar to those reported for the GA_3-induced RNase and DNase. If the activities reported for RNase and DNase are those of nuclease I, then GA_3 may have little effect on its synthesis.

In conclusion, barley aleurone tissue secretes nuclease I in response to GA_3. This enzyme was purified to homogeneity. It is a single polypeptide with an apparent mol wt of 36 kD, and has RNase, DNase, and 3′-nucleotidase activities. We propose that, during germination, nuclease I is secreted from barley aleurone tissue and hydrolyzes remnant nucleic acids in the endosperm, the products of which may contribute to heterotrophic embryonic growth (8).

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