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Partial Characterization of a Protease Inhibitor Which Inhibits the Major Endopeptidase Present in the Cotyledons of Mung Beans[1]

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ABSTRACT

Germination of mung beans (*Phaseolus aureus*, Roxb.) is accompanied by an increase in the activity of the endopeptidase involved in storage protein metabolism. Enzyme activity in the cotyledons increases 25-fold during the first 5 days of germination. The cotyledons also contain inhibitory activity against the endopeptidase, and this inhibitory activity declines during germination, suggesting that inhibitors may play a role in regulating the activity of the endopeptidase.

The inhibitory activity against the mung bean endopeptidase is due to the presence of two inhibitors which can be separated by chromatography on Sephadex G-100. The two inhibitors have approximate molecular weights of 12,000 and smaller than 2,000 daltons. The large inhibitor coelutes with trypsin inhibitor on Sephadex G-100, but these two inhibitory activities can be separated by means of a trypsin affinity column.

The inhibitory activity disappears slowly from crude extracts incubated at 6 C and more rapidly when the extracts are incubated at 25 C or 37 C. The disappearance of inhibitory activity is accompanied by a rise of the endopeptidase activity, but an examination of the kinetics of these two phenomena suggests that they are not causally related. Fractionation of the cellular organelles on sucrose gradients shows that the inhibitory activity is not associated with the protein bodies, but rather with the cytosol. Our results suggest that the endopeptidase inhibitor(s) does not regulate the increase in endopeptidase activity which accompanies germination or the metabolism of storage protein. We, therefore, postulate that the inhibitor(s) may function in protecting the cytoplasm from accidental rupturing of the protease-containing protein bodies.

The germination of leguminous seeds is accompanied by the metabolism of the reserve proteins stored in the cotyledons. These reserve proteins are located in special organelles called protein bodies or aleurone grains (5, 20), and these organelles appear to be undergoing autolysis during germination (2, 15). Protein bodies isolated from dry legume seeds contain a variety of hydrolytic enzymes including proteases (6, 12, 13, 21), and it has been suggested that they are capable of autolysis (13) although this process has never been demonstrated *in vitro* (6). Germination is accompanied by a dramatic increase in proteolytic activity in the cotyledons, and we have shown that this is due to the appearance of an endopeptidase (3). The metabolism of the storage proteins is dependent on the appearance of this enzymatic activity in the cotyledons. We have demonstrated both a temporal and a spatial relationship between the appearance of the enzyme activity and the metabolism of the reserve proteins. We have recently purified the endopeptidase to homogeneity and found that more than 95% of the endopeptidase activity in the cotyledons of 5-day-old plants can be accounted for by a single enzyme with an apparent mol wt of 23,000 (Baumgartner and Chrispeels, in preparation).

Cotyledons of leguminous seeds are a rich source of protease inhibitors (10). The inhibitors which inhibit proteases of animal or microbial origin (*e.g.* trypsin inhibitors and chymotrypsin inhibitors) have been studied rather extensively, while relatively little is known about the inhibitors which inhibit endogenous plant proteases (18). Germination is accompanied by a gradual decline in the level of trypsin inhibitors in the cotyledons (7, 16). There is no evidence, however, that the increase in proteolytic activity which also accompanies germination is causally related to the decrease in trypsin-inhibitor activity, and the physiological function of these inhibitors remains unknown (18). Royer *et al.* (17) have shown recently that the removal of trypsin inhibitors from extracts of cowpea cotyledons caused an increase in the caseolytic activity of these extracts. This led them to suggest that protease inhibitors play a role in the activation of pre-existing proteases and in the control of reserve protein metabolism. A similar mechanism has been proposed for the control of reserve protein metabolism in germinating lettuce seeds (18, 19).

As part of our study on the control mechanism involved in storage protein metabolism in germinating legume seeds (3, 4, 6), we have analyzed extracts of cotyledons for the presence of inhibitors of the endopeptidase activity. The evidence presented here indicates that the cotyledons contain such inhibitors but suggests that these inhibitors are not involved in the regulation of the metabolism of storage proteins.

MATERIALS AND METHODS

Plant Material, Germination, and Extraction of Cotyledons. Seeds of mung beans (*Phaseolus aureus*, Roxb.) were obtained from a local dealer and grown as described in our earlier work (4). The cotyledons were homogenized with a mortar and pestle in 25 mM citrate-phosphate (pH 5.7) containing 10 mM β-mercaptoethanol and 0.02% sodium azide. The homogenate was centrifuged at 20,000*g* for 20 min and the supernatant used as a source of endopeptidase. The enzyme could be stored at 3 to 5 C for up to 2 weeks, but was inactivated by freezing. For studies of endopeptidase inhibitor, the homogenate was incubated for 15 min at 70 C and then centrifuged at 20,000*g* for 20 min. The supernatant was adjusted to pH 4.8 with 1 M citric acid, again clarified by centrifugation, and this supernatant used as a source of inhibitors.

Enzyme Assays. Endopeptidase activity was determined by viscometry as described before (3). Ten ml of 5% gelatin in 25 mM citrate-phosphate buffer (pH 5.7) and 0.02% NaN_3 were mixed with 2 ml of 25 mM citrate-phosphate (pH 5.7) with 10 mM 2-mercaptoethanol containing an appropriate amount enzyme. The reaction was allowed to proceed at 40 C, and the viscosity of the gelatin solution was measured every 2 min for up

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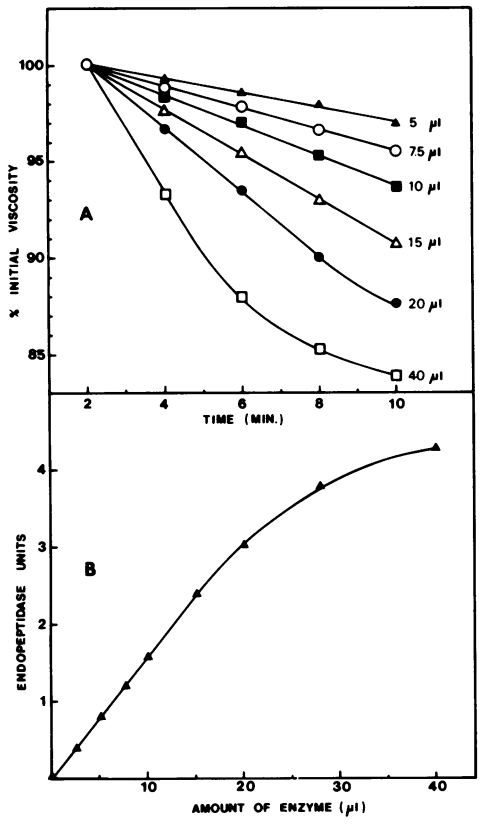

FIG. 1. Determination of endopeptidase activity by viscometry. Ten ml of a 5% solution of gelatin were mixed in the viscometer with 2 ml of buffer containing the enzyme and incubated at 40 C. Viscosity of the mixture was determined 2 min after mixing and was termed "initial viscosity"; viscosity of the mixture was measured at 2-min intervals thereafter. A: Decrease in viscosity with time using different aliquots of enzyme. Results are expressed as per cent of initial viscosity to normalize the curves. B: Relationship between the aliquot of enzyme solution and the calculated enzyme activity using the total 10-min reaction period. Use of shorter reaction times extends the linear portion of the curve.

to 10 min. The decrease in viscosity was linear with time only between 100% and 91% of the initial viscosity (Fig. 1A). Endopeptidase activity is expressed as the change in relative viscosity/hr. A decrease in relative viscosity of 1.0 represents 1 unit of enzyme activity. There was a linear relationship between the amount of enzyme added to the gelatin and the measured enzyme activity, and the method allowed us to measure enzyme activities conveniently and accurately between 0.25 units and 2.5 units/2-ml aliquot (Fig. 1B). Lower activities were measured by extending the total reaction time.

α-Mannosidase activity was determined colorimetrically by the hydrolysis of *p*-nitrophenyl-α-D-mannoside (3). Trypsin-like activity (BAPNA[2]-ase) was measured colorimetrically by the hydrolysis of benzoyl-arginine-*p*-nitroanalide (BAPNA). An aliquot of enzyme was mixed with 1 ml of 2 mM BAPNA in 10 mM phosphate buffer (pH 7.5) and the volume made to 2 ml with water. The reaction mixture was incubated for 60 min at 37 C, and the reaction was stopped by adding 1 ml of 1 N acetic acid. When necessary, the solution was clarified by centrifugation. The absorbance was determined at 410 nm, and the activity expressed as units of absorbance/ml enzyme.

Protease-inhibitor Assays. Endopeptidase-inhibitory activity was assayed by mixing an aliquot of the inhibitor solution with 1 unit of endopeptidase activity and determining the endopeptidase activity of the mixture. One unit of inhibitory activity is defined as the amount of inhibitor which inhibits 1 unit of endopeptidase by 50%. Trypsin-inhibitory activity was measured by mixing an aliquot of the extract with 2 ml of 2 mM BAPNA in 25 mM K-phosphate buffer (pH 7.5) and 4 µg of trypsin dissolved in 0.1 ml of the same buffer. The mixture was incubated for 15 min at 37 C, and the reaction was stopped by the addition of 1 ml of 1 N acetic acid. The absorbance was read at 410 nm. One unit of inhibitory activity was defined as the amount of inhibitor which inhibited the activity of the trypsin by 50%.

Protein Determination. Protein was measured quantitatively according to the method of Lowry *et al.* (11) using BSA as a standard.

Chromatography. Determination of mol wt by gel filtration was done by chromatography on Sephadex G-100 according to the method of Andrews (1) using riboflavin, actinomycin D, Cyt, and pancreatic ribonuclease as standards.

Affinity chromatography was performed with a gel consisting of trypsin covalently linked to Sepharose 4B (Worthington). The gel was equilibrated with 50 mM citrate-phosphate (pH 7.8) containing 100 mM NaCl and packed in a column (1 × 10 cm). Samples were loaded and the column was eluted with the starting buffer until the eluate was free of protein. The buffer was then changed to 0.2 M KCl-HCl (pH 1.6).

Isolation of Protein Bodies. Protein bodies were isolated as described before (4) except that the cotyledons were chopped in 25 mM citrate-phosphate (pH 4.5). The use of a lower pH was first suggested to us by J. M. Mascherpa of the Department of Plant Biology at the University of Geneva. The low pH prevents the globulins from being solubilized and results in a much greater recovery of globulins and other protein body proteins such as α-mannosidase in the protein body band.

RESULTS

Endopeptidase and Endopeptidase Inhibitor in Cotyledons. The increase in endopeptidase activity in the cotyledons of germinating mung beans is shown in Figure 2. The enzyme activity increases gradually during the 1st 3 days of germination, and accumulates more rapidly during the 4th and 5th days of germination. The appearance of enzyme activity could be due to *de novo* synthesis, to the activation of a pre-existing protein (proenzyme), or to the disappearance of enzyme inhibitors. Since cotyledons are known to be rich sources of enzyme inhibitors (10), mixing experiments were carried out to determine whether they contained inhibitory activity against the endopeptidase which accumulates during germination. The results of an experiment in which extracts from cotyledons obtained from seeds germinated for 1 and 5 days were mixed, showed that the former contained inhibitory activity against the endopeptidase present in the latter (Table I). The endopeptidase activity present in 5-day-old cotyledons was progressively inhibited by adding larger and larger aliquots of extract from 1-day-old cotyledons. The enzyme activity present in one 5-day-old cotyledon was completely inhibited by the inhibitory activity present in 10 1-day-old cotyledons. Since some protease inhibitors have been shown to be heat-stable (8, 9), we tested the effect of a 15-min preincubation at different temperatures on the activity of the endopeptidase and its inhibitor(s). The results (Fig. 3) showed that preincubation at 60 C completely inactivated the enzyme activity but reduced the inhibitory activity by only 8%. Preincubation at temperatures above 70 C caused the inhibitory activity to be significantly but not completely inactivated. In subsequent experiments, crude extracts were routinely heated to 60 or 70 C to inactivate the endopeptidase while at the same time preserving the inhibitory activity.

The endopeptidase activity of freshly prepared extracts of cotyledons gradually increased upon standing in the refrigerator (6 to 7 C). The activity generally increased from 50% to 100%

[2] Abbreviation: BAPNA: benzoyl-arginine-*p*-nitroanalide.

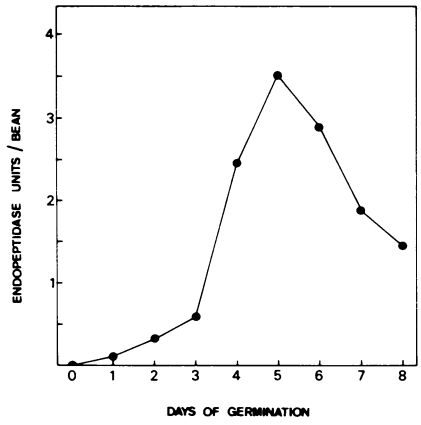

FIG. 2. Time course of endopeptidase activity during germination. Cotyledons were collected at 1-day intervals, and 40 cotyledons were homogenized in 10 ml of buffer and centrifuged at 20,000*g* for 20 min. Endopeptidase activity was determined on the clear supernatant.

Table I. *Effect of Mixing Extracts of Cotyledons Obtained from Seeds Germinated for 1 and 5 Days on Endopeptidase Activity*

The extracts were obtained by homogenizing 250 cotyledons in 25 ml.

Mixing Ratio 5 Day:1 Day	Expected Endopeptidase Activity	Measured Endopeptidase Activity	Per Cent Inhibition
ml	*units*	*units*	
0.1:0	2.58	2.58	0
0.1:0.2	2.58	2.04	21
0.1:0.4	2.58	1.5	42
0.1:0.6	2.58	1.11	57
0.1:0.8	2.58	0.36	86
0.1:1.0	2.58	0	100

over a period of 24 hr, and then remained stable for up to 2 weeks (data not shown). It seemed likely that this increase was due to a gradual disappearance of the inhibitory activity, and that these two processes were causally related. This possibility was tested by examining the endopeptidase activity and the endopeptidase-inhibitory activity in extracts made from cotyledons obtained from beans germinated for 2, 4, and 6 days. Such extracts have different levels of endopeptidase activity initially. The results (Table II) show that the levels of endopeptidase activity in all three extracts gradually increased upon standing at 6 C, and that the inhibitory activity decreased. The kinetics of this decrease did not correspond to the kinetics of the increase in enzyme activity. Indeed, most of the inhibitory activity disappeared during the first 7 hours, while there was a substantial increase in enzyme activity between 7 and 24 hr after homogenization.

The effect of temperature on the decrease of the inhibitory activity was determined by incubating extracts at 0 C (ice bucket), 25 C, and 37 C. The results showed that the inhibitor content of the extracts decreased during the incubation, and that the rate and the extent of this decrease depended on the temperature and the length of the incubation (Fig. 4). No decrease was observed if the extract was heated to 70 C for 15 min prior to incubation at 37 C. This suggests that the disappearance of the inhibitory activity was due to a heat-labile factor, possibly an enzyme.

Characterization of Inhibitory Activity. Gel filtration on Sephadex G-100 was used to determine the molecular size of the inhibitors. Crude extracts of 1-day imbibed seeds were centrifuged at 20,000*g* for 20 min, heated to 70 C for 15 min, centrifuged again, and fractionated on Sephadex G-100. The results (Fig. 5) showed two peaks of endopeptidase-inhibitory activity: a large inhibitor with an apparent mol wt of 12,000, and a small inhibitor with an apparent mol wt smaller than 2,000

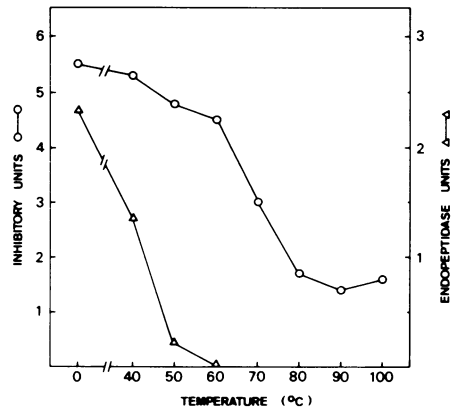

FIG. 3. Effect of heating on the activity of endopeptidase and the endopeptidase inhibitor(s). Endopeptidase was obtained by extracting cotyledons obtained from seeds germinated for 5 days. Endopeptidase inhibitor was obtained by extracting cotyledons obtained from seeds germinated for 1 day. Extracts were heated for 15 min at the temperatures indicated prior to assay.

Table II. *Effect of Incubation of Crude Homogenates at 7 C on Levels of Endopeptidase and Inhibitory Activity*

One hundred cotyledons were homogenized in 20 ml. The cleared extracts were kept in the refrigerator for the times indicated. At these times, enzyme activity was determined and an aliquot of the extract was heated to 65 C for 15 min. Inhibitory activity was determined on an aliquot of the heated extract. These plants, grown in winter, grew slower than those used for the experiment in Figure 1 and had comparable physiological ages of 1.5, 3, and 4.5 days.

Days of Germination	Time of Incubation at 7 C	Endopeptidase Units/Bean	Inhibitor Units/Bean
	hr		
2	0	0.07	2.28
	7	0.07	0.6
	24	0.16	0.0
	72	0.15	0.0
4	0	0.80	1.14
	7	1.34	0.06
	24	2.28	0.0
	72	2.24	0.0
6	0	2.64	0.29
	7	3.94	0.04
	24	5.04	0.0
	72	5.04	

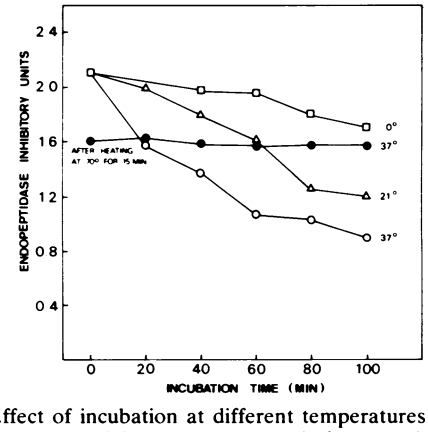

FIG. 4. Effect of incubation at different temperatures on the endopeptidase-inhibitory activity. An extract made from cotyledons of seeds germinated for 1 day was incubated at different temperatures, and the activity was determined immediately after the incubation. An aliquot of the extract was heated to 70 C for 15 min prior to incubation at 37 C.

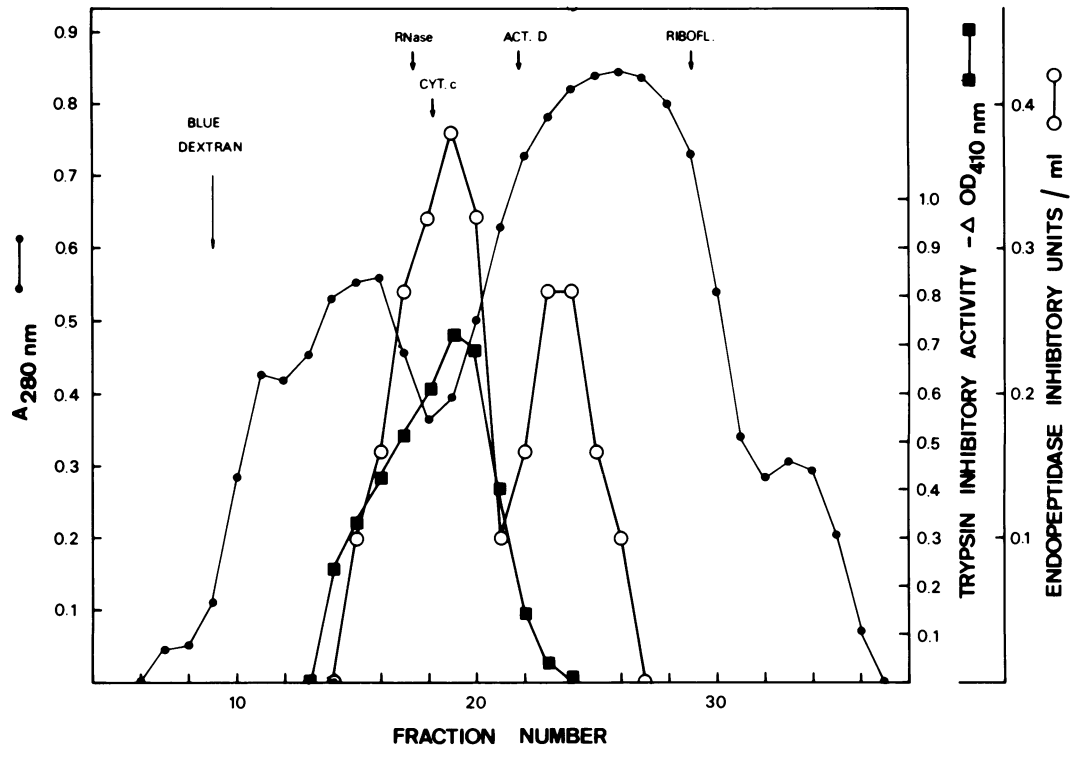

FIG. 5. Fractionation of endopeptidase-inhibitory and trypsin-inhibitory activity on Sephadex G-100. The extract was obtained from cotyledons of seeds which had been germinated for 24 hr. The extract was heated for 15 min at 70 C to stabilize the endopeptidase inhibitory activity.

daltons. Trypsin-inhibitor activity co-eluted with the large endopeptidase inhibitor suggesting that both inhibitory activities might be associated with the same molecule. The fractions containing both the large endopeptidase inhibitor and the trypsin inhibitor were combined and fractionated on an affinity column consisting of trypsin covalently linked to Sepharose 4B. The elution pattern is shown in Figure 6. The large endopeptidase inhibitor was not retained or retarded by the trypsin-Sepharose column, while all of the trypsin-inhibitory activity was bound and later released when the column was washed with a 0.2 M KCl-HCl buffer at pH 1.6. This result suggests that the two protease-inhibitory activities are associated with two distinct molecules.

The two inhibitors separated by Sephadex G-100 column chromatography were collected, lyophilized, and tested separately to determine the extent to which they inhibited the endopeptidase activity. The results (Fig. 7) indicated a distinct difference between the two inhibitors. Adding increasing amounts of the large inhibitor to the enzyme resulted in the complete inhibition of endopeptidase activity, while the small inhibitor did not inhibit enzyme activity by more than 30%, even at high levels of inhibitor.

Metabolism and Subcellular Localization of Inhibitor. To determine whether these inhibitors might play a role in regulation of endopeptidase activity, we determined the levels of inhibitor activity in the cotyledons in the course of germination. The inhibitor-containing extracts were assayed before and after dialysis. Dialysis removed the small inhibitor completely (data not shown). The activity of the large nondialyzable inhibitor decreased gradually from the 1st day to the 6th day of germination, by which time it reached about 20% of its original value (Fig. 8). There was a similar gradual decline in the total inhibitory activity (data not shown). Trypsin-inhibitory activity remained constant for 3 to 4 days and then declined gradually. The disappearance of the endopeptidase inhibitors superficially supports the notion that they might be involved in regulating endopeptidase activity. It should be noted, however, that the inhibitory activity disappeared gradually from the 1st day onward while endopeptidase

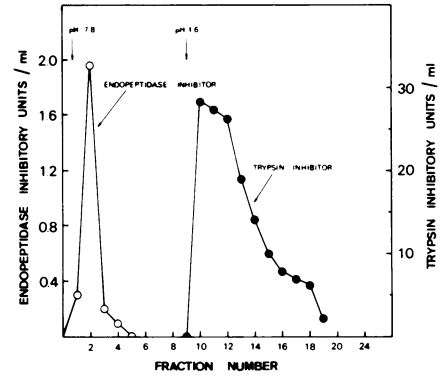

FIG. 6. Fractionation of endopeptidase and trypsin-inhibitory activity by affinity chromatography on a Sepharose 4B-trypsin column. Fractions containing the large endopeptidase inhibitor and the trypsin inhibitor (see Fig. 4) were pooled, frozen, lyophilized, and loaded on a Sepharose-trypsin column. The eluate was assayed for both endopeptidase and trypsin-inhibitory activity. The inhibitors were loaded on the column in 50 mM citrate phosphate (pH 7.8) containing 100 mM NaCl. The column was washed with the same buffer and then eluted with 0.2 M KCl-HCl (pH 1.6) to remove the bound trypsin inhibitor.

levels remained low until the 3rd day and then increased sharply on the 4th and 5th days of germination.

The hydrolysis of the storage protein probably occurs within the protein bodies, since the protein body membrane remains intact throughout this process (2, 15). Protein bodies have been shown to contain low levels of proteases, and this has led to the suggestion that they are autolytic organelles (13). If the increase in endopeptidase activity is due to the disappearance of the inhibitors, both should be located in the same cellular compartment. The subcellular localization of the inhibitory activity was studied by fractionating a homogenate of cotyledons obtained from 1-day seedlings on a 20% to 90% linear sucrose gradient. The distribution of protein (primarily globulin), trypsin-inhibitor

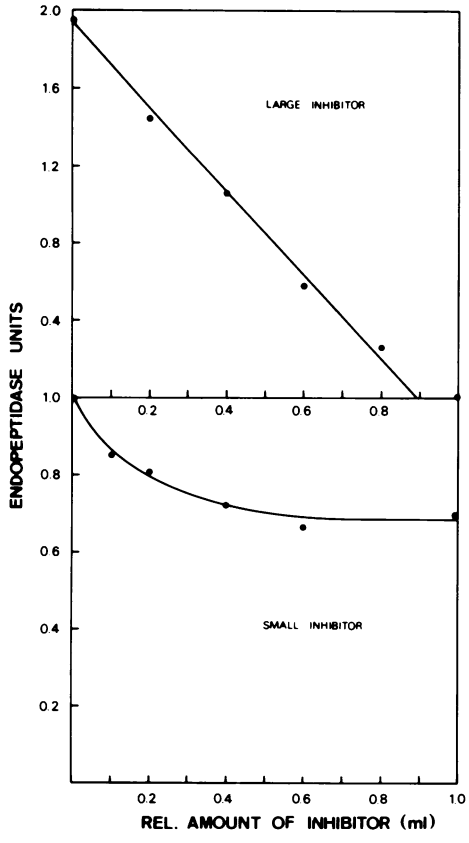

FIG. 7. Inhibition of endopeptidase by the large and the small inhibitors. The inhibitors were separated by gel filtration and the fractions pooled and lyophilized. Aliquots of the concentrated inhibitor solutions were mixed with a standard amount of endopeptidase and the remaining enzymic activity determined.

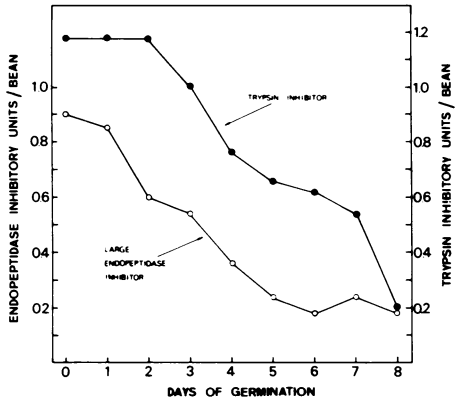

FIG. 8. Changes in the levels of trypsin inhibitor and large endopeptidase inhibitor during germination. The extracts were heated to 70 C for 15 min and cleared by centrifugation. This step inactivated about half the inhibitory activity (cf. Fig. 3 and units/bean in Table II). The levels of the large inhibitor were determined after dialysis of the extracts. Dialysis completely removed the small inhibitor.

activity, α-mannosidase, BAPNA-ase, and endopeptidase inhibitor is shown in Figure 9. The enzyme α-mannosidase is a marker for protein bodies, while BAPNA-ase is a marker for the cytosol (6). Mung bean protein bodies banded at a density of 1.26 to 1.28 (4) as indicated by the presence of a large protein band and the activity of α-mannosidase. BAPNA-ase was found exclusively at the top of the gradient. Endopeptidase-inhibitory activity was found exclusively at the top of the gradient and was not associated with the protein bodies. Trypsin inhibitor, like α-mannosidase, was associated with both the protein bodies and the cytosol.

DISCUSSION

Storage protein mobilization in germinating mung beans cotyledons depends on the increase in endopeptidase activity in the tissue (3). Whether this increase in enzyme activity is due to *de novo* synthesis of the protein, the activation of an inactive proenzyme, or the disappearance of an inhibitor remains to be demonstrated. Since cotyledons of leguminous seeds are rich sources of protease inhibitors, we decided to investigate the last possibility. The inhibitors of animal and microbial proteases present in plants have been extensively studied (10), but relatively little is known about inhibitors of plant proteases, or about their role in the regulation of protein metabolism in plants (18). In this paper, we report the existence in mung bean cotyledons or a protease inhibitor which inhibits the major endopeptidase in the same tissue. This inhibitor has an approximate mol wt of around 12,000 daltons, is relatively heat-stable, and completely inhibits the activity of the endopeptidase if added in sufficient excess. The inhibitory activity in the cotyledons declines during germination, and this is one of the few reports (14) in which the appearance of a proteolytic activity during plant development can be correlated with the disappearance of an inhibitor of that enzymic activity.

Increases in proteolytic activity during germination have been observed in the cotyledons of a variety of legumes. This increase in proteolytic activity is paralleled by a decrease in trypsin-inhibitory activity (7, 16), but there is no evidence that these two events are causally related. Indeed, trypsin-like activity (BAPNA-ase) in cotyledons also declines during germination (7). The increase in proteolytic activity which accompanies germination in mung bean is due to an increase in endopeptidase activity, and the evidence presented here suggests that the trypsin inhibitor does not inhibit the activity of the tissue's own major protease. Indeed, fractionation by affinity chromatography clearly showed that the fractions which contained endopeptidase inhibitor were devoid of trypsin inhibitor, and trypsin-inhibitor-rich fractions did not inhibit the endopeptidase. Com-

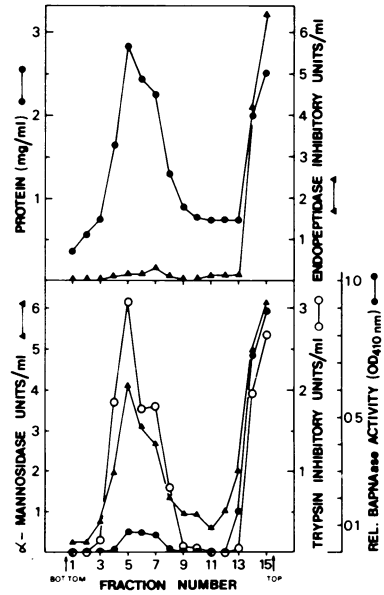

FIG. 9. Fractionation of cytoplasmic organelles on isopycnic sucrose gradients. Cotyledons (5 g) obtained from beans which had been germinated for 24 hr were chopped and the homogenate, after a 3-min centrifugation at 1000g, was layered on a linear 50% to 90% sucrose gradient.

mercial soybean trypsin inhibitor, furthermore, failed to inhibit the endopeptidase activity even if added at concentrations far above those normally present in the tissue. In an elegant study, Royer *et al.* (17) showed that the removal of trypsin inhibitor from extracts of cowpea cotyledons increased the caseolytic activity of these extracts, without affecting the BAPNA-ase activity. The enzyme(s) responsible for caseolytic activity were not identified, and it is, therefore, not clear which enzyme(s) increased in activity when the trypsin inhibitors were removed.

The endopeptidase inhibitor is heat-labile and probably a protein. Inhibitory activity decreased rapidly when crude extracts were incubated at physiological temperatures or even at 0 C. This decrease itself was due to a heat-labile factor, as heating the crude extracts to 70 C for 15 min stabilized the inhibitory activity against any further decay. It seems unlikely that the inactivation of the inhibitors is due to the endopeptidase itself. Indeed, extracts containing almost no endopeptidase activity (2-day extracts, Table II) lost their inhibitory activity quite rapidly.

Does the decay of the endopeptidase inhibitor, either *in vivo* or *in vitro*, play a role in unmasking the activity of the endopeptidase? In a similar series of experiments, Shain and Mayer (19) measured protease activity and protease inhibitors in germinating lettuce seeds. They observed a 50-fold increase in trypsin-like activity during the 1st 3 days of germination and a complete disappearance of the trypsin-inhibitory activity during the 1st day of germination. This and other data led them to conclude that the increase in enzyme activity is causally related to the decrease in inhibitory activity. On the basis of somewhat similar data for the mung bean endopeptidase and its inhibitor, we have come to the opposite conclusion.

In vitro incubation of the extracts at 7 C results in the disappearance of the inhibitory activity and a simultaneous increase in endopeptidase activity. However, the kinetics of these two processes are not similar. Inhibitory activity declines much faster than enzyme activity increases (Table II). Furthermore, extracts from cotyledons obtained from seeds germinated for 2 days lose 2.28 units of inhibitory activity per bean, but acquire less than 0.1 unit of enzyme activity, while 6-day extracts lose only 0.3 units of inhibitory activity but acquire 2.4 units of enzyme activity.

Secondly, the *in vivo* kinetics of inhibitor decay and enhancement of enzyme activity are also quite different. The inhibitory activity starts to decrease soon after germination and decreases gradually until the 5th or 6th day of germination. Enzyme activity, however, increases very slowly at first (first 3 days), then increases quite rapidly during the 4th and 5th days of germination.

Finally, the subcellular localization of the inhibitory activity suggests that its function is in the cytosol, and not in the protein bodies where storage protein hydrolysis occurs. Our own preliminary experiments show that the endopeptidase is synthesized in the course of germination and localized in the protein bodies. This would be the expected subcellular location of the inhibitor if it were involved in the regulation of the activity of the endopeptidase.

Proteases and their inhibitors have been studied in a large number of microorganisms (8) and have often been found to be located in separate compartments: the enzymes in vacuoles or lysosomes, and the inhibitors in the cytosol. It has been suggested that the role of these inhibitors is to protect the cytoplasm against the accidental rupture of the protease-containing vesicles. That may also be the function of this endopeptidase inhibitor in mung beans.

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