Plant Physiol. (1983) 71, 82–87
0032-0889/83/71/0082/06/$00.50/0

Characterization of the Isozymes of α-Mannosidase Located in the Cell Wall, Protein Bodies, and Endoplasmic Reticulum of *Phaseolus vulgaris* Cotyledons[1]

Received for publication June 14, 1982 and in revised form September 27, 1982

WILLEM VAN DER WILDEN[2] AND MAARTEN J. CHRISPEELS[3]
Department of Biology, University of California, San Diego, La Jolla, California 92093

ABSTRACT

Cotyledons of maturing *Phaseolus vulgaris* seeds contain three isozymes of α-mannosidase which can be separated by isoelectrofocusing. They have isoelectric points of 5.3, 5.8, and 6.5 to 7.5 and were named I, II, and III in order of ascending pI. All three had an acid pH optimum (4.5) and required Zn^{2+} for maximal activity. Isozymes I and II were present in the protein bodies. Together they accounted for 85% of the total activity. Isozyme III was essentially absent from isolated protoplasts but could be extracted from isolated cell walls. All three isozymes were also found to be associated with the endoplasmic reticulum, and the proportion of the total activity in this fraction decreased from 20% in immature cotyledons to 6% in mature cotyledons. The results are interpreted as evidence that newly synthesized α-mannosidase is sequestered in the lumen of the ER prior to its transport to the protein bodies or the cell wall.

As part of our study of the biosynthesis and transport of proteins which are secreted or accumulate in specific cellular compartments such as protein bodies, we have characterized the different forms, and determined the subcellular location of α-mannosidase in developing cotyledons of *Phaseolus vulgaris*. Development of legume cotyledons is accompanied by the deposition of storage proteins in the protein bodies (see 20), and an increase in the total α-mannosidase activity (22). Recent evidence indicates that protein bodies should be thought of as small protein-filled vacuoles (12) which have the same enzymic complement as the vacuoles of nonstorage parenchyma cells (3, 4, , 26). Cotyledons of legume seeds are particularly rich sources of α-mannosidase, and Paus (23, 24) has shown that there are at least two isozymes of α-mannosidase in *P. vulgaris* cotyledons. α-Mannosidase has been used as a marker enzyme for protein bodies in mung bean (14) and pea (19) cotyledons, while in other tissues the enzyme is found in the vacuole (4, 15). It is generally accepted, however, that plant cells contain two major compartments with acid hydrolases: the cell wall and the vacuole (18). The same enzyme (*e.g.* acid phosphatase) may be present in both compartments. We have examined whether this is also the case for α-mannosidase. Our results show that *P. vulgaris* cotyledons contain at least three isozymes of α-mannosidase. During cotyledon development, two of these isozymes accumulate in the protein bodies and one in the cell wall. All three isozymes are associated with the ER, raising the possibility that the enzymes are sequestered in the lumen of the ER prior to their transport to the protein bodies or the cell wall.

MATERIALS AND METHODS

Plant Material. Seeds of *Phaseolus vulgaris* L. cv Greensleeves were purchased from Burpee Co. (Riverside, CA) and grown as described (5). Seeds were harvested at different stages of maturation. For a few experiments, mature seeds were imbibed overnight in H_2O and germinated in moist vermiculite.

Preparation of Cotyledon Extracts. Cotyledons were homogenized with a conical ground-glass tissue grinder in a medium consisting of 100 mM Tris-HCl (pH 7.4) with 500 mM KCl and 0.1% Triton X-100. The homogenate was centrifuged at 500*g* for 10 min, and then at 150,000*g* for 30 min. The supernatant was dialyzed against 5 mM Tris-HCl (pH 7.4) containing 0.1% Triton X-100 and 500 mM KCl, and used for further analysis.

Homogenization of Cotyledons and Fractionation of Organelles. Procedures to isolate organelles and fractionate them on linear sucrose gradients have been described in detail (11, 25). Basically, the technique involves homogenization in a medium which disrupts the protein bodies (12% sucrose containing 100 mM Tris-HCl, pH 7.4) and either detaches the ribosomes from the RER (1 mM EDTA) or leaves the ribosomes on the ER (3 mM $MgCl_2$). The homogenates are centrifuged at 100*g* for 10 min, and then passed over a Sepharose 4B column to separate the organelle fraction from the soluble proteins. The organelles are then fractionated on linear 16 to 48% (w/w) sucrose gradients centrifuged for 2 h at 150,000*g*. The medium used for homogenization is used for all subsequent manipulations.

For some experiments, ER was isolated on a discontinuous sucrose gradient consisting of 11 ml of 16% (w/w) sucrose and 3 ml of 35% (w/w) sucrose in the EDTA-containing medium. These gradients were centrifuged for 2 h at 82,000*g*.

Isolation of Protoplasts and Protein Bodies. Protoplasts and protein bodies were isolated as described (26) for mung bean cotyledons, except that the medium used for the digestion of cell walls contained 0.25% pectinase in addition to the 2% Cellulysin and 0.5% Macerase. All these enzymes were obtained from Calbiochem-Hoechst (La Jolla, CA).

Isoelectrofocusing. Isoelectrofocusing was performed with 7.5% acrylamide gels essentially as described by Aron (2); 10% sucrose was included in all solution to stabilize the pH gradient; 0.1% (w/v) NaOH was used as cathode solution; the starting field current was 1 mamp/gel tube; the samples (100 μl of extract) were polymerized with the gel. After focusing was complete (4 h), the

[1] Supported by grants from the National Science Foundation (PCM 81-14943) (Metabolic Biology) and from the Herman Frasch Foundation to M. J. C.

[2] Present address: Lehrstuhl für Pflanzenphysiologie, Ruhr Universität, Bochum 630, West Germany.

[3] To whom reprint requests should be addressed.

gels were cut into 3-mm slices, and each slice allowed to equilibrate overnight at 5°C with 0.4 ml of distilled H_2O. The pH and the mannosidase activity of the solution were then determined.

Enzyme Activity. α-Mannosidase activity was determined with the chromogenic substrate *p*-nitrophenyl-α-D-mannoside (Sigma) as substrate. Enzyme (0–100 μl), substrate (100 μl of 1 mg/ml) and buffer (100 mM sodium acetate, pH 4.5, with 4 mM $ZnSO_4$ and 0.1% Triton X-100) up to a total volume of 500 μl were incubated at 37°C. The reaction was stopped with 2.5 ml of 200 mM Na_2CO_3 and the A_{410} determined. One unit of activity is defined as a change in absorbance of 1 unit·h^{-1}. The assays of the other enzymes have all been described: NADH-Cyt c reductase (11), leucine aminopeptidase (10), and inosinediphosphatase (7).

Determination of Phaseolin. An approximate measure of the amount of phaseolin in the supernatant and protein body fractions of the Ficoll gradients was obtained by dialyzing these fractions exhaustively (48 h) against several changes of cold deionized H_2O. The dialyzed fractions were centrifuged at 10,000*g* for 20 min and the amount of protein in the pellet determined by the method of Lowry *et al.* (17).

RESULTS

Three Isozymes of α-Mannosidase. Initial experiments showed that although α-mannosidase activity was readily extractable from dry powder of cotyledons, and present in homogenates of developing cotyledons, maximal yield of enzyme was only obtained when both detergent (0.1% Triton X-100) and salt (500 mM KCl were present in the homogenizing medium. The enzyme present in cotyledon extracts had a pH optimum of 4.5, with 50% of maximal activity around pH 5.5 and 3.3. The enzyme required 4 mM $ZnSO_4$ for maximal activity and was normally assayed under those conditions.

Fractionation of extracts by chromatography with DEAE-Sephadex and by gel filtration with Sephacryl-200 showed the presence of only one peak of α-mannosidase activity (data not shown). Isoelectrofocusing of a crude extract indicated the presence of multiple forms of α-mannosidase (Fig. 1). Three forms of α-mannosidase were found in extracts of developing cotyledons: two sharp peaks with pI of 5.3 and 5.8, while the third one made a broad band between pH 6.5 and 7.5. All three isozymes were isolated on a preparative isoelectrofocusing column and shown to have the same pH optimum of 4.5 and to require Zn^{2+} for maximal activity. The three isozymes were therefore assayed under the same conditions.

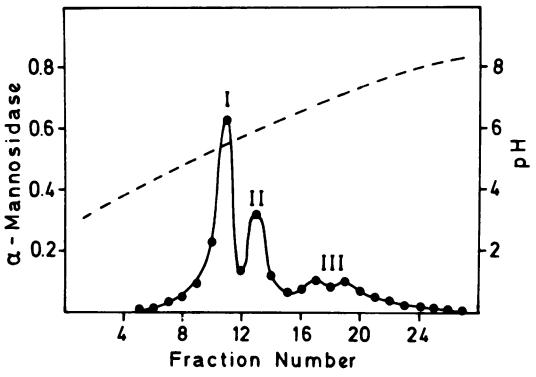

FIG. 1. Isoelectrofocusing of the α-mannosidase present in a total extract of developing *P. vulgaris* cotyledons. α-Mannosidase was extracted from the cotyledons (200–225 mg each) using a medium consisting of 0.1% Triton X-100, 500 mM KCl, and 100 mM Tris-HCl (pH 7.4). The extract was centrifuged for 30 min at 150,000*g*; the supernatant dialyzed against a solution of 0.1% Triton X-100, 500 mM KCl, and 5 mM Tris-HCl (pH 7.4); and the dialysate subjected to isoelectrofocusing in the pH 3 to 10 range. pH gradient (– – –); enzyme activity (——).

To find out if the three isozymes accumulate throughout cotyledon development, cotyledons of different ages were homogenized, and the extracts were assayed for total α-mannosidase activity and subjected to isoelectrofocusing. The results (Table I) showed that α-mannosidase activity increased about 2-fold when expressed on a fresh weight basis, and about 13-fold on a per cotyledon basis. It is interesting to note that there was no further increase in enzyme activity during germination and seedling growth.

The extracts from the cotyledons in different stages of development were subjected to isoelectrofocusing, and the amount of the three isozymes of α-mannosidase was calculated and expressed as units per gram of cotyledon (Fig. 2). The results show that the three forms of the enzyme accumulated throughout cotyledon development. The increase was greater than the increase in fresh weight, especially for forms I and II. α-Mannosidase III increased at the same rate as fresh weight. We also examined the α-mannosidases in the cotyledons of young seedlings and found that they contained forms I and II of the enzyme, but very little form III (data not shown).

α-Mannosidase in the Protein Bodies. To find out which isozymes of α-mannosidase are associated with the protein bodies, protein bodies were isolated in a two-step procedure. Cotyledons slices were digested with cell wall hydrolyzing enzymes, and protoplasts isolated. The protoplasts were dissolved in the homogenization buffer containing 500 mM KCl and 0.1% Triton X-100, and the cleared extract subjected to isoelectrofocusing. The results

Table I. *α-Mannosidase Activity of P. vulgaris Cotyledons at Different Stages of Development*

Stage of Cotyledon Development	α-Mannosidase Activity	
	units/g fresh wt	*units/cotyledon*
78 mg/cotyledon	280	22
148 mg/cotyledon	406	60
272 mg/cotyledon	437	119
487 mg/cotyledon	554	279
24 h germination	544	240
4-d seedling	701	175
8-d seedling	640	130

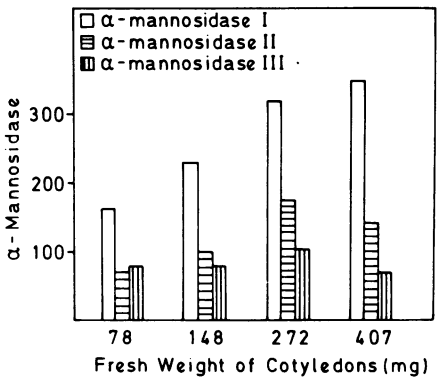

FIG. 2. Levels of α-mannosidase I, II, and III activity during the development of *P. vulgaris* cotyledons. Cotyledons of differing weights were homogenized in a medium consisting of 0.1% Triton X-100, 500 mM KCl, and 100 mM Tris-HCl (pH 7.4). The extracts were centrifuged for 30 min at 150,000*g* and the supernatant dialyzed against 0.1% Triton X-100 and 500 mM KCl in 5 mM Tris-HCl (pH 7.4). The dialysates were subjected to isoelectrofocusing in the pH 3 to 10 range. Gel slices were assayed for α-mannosidase activity and the amount of α-mannosidase I, II, and III in the extracts was calculated based on the peak size. The enzyme activity is expressed as units per g fresh weight.

(Fig. 3) indicate that the protoplasts contained isozymes I and II, but were essentially devoid of III. Another preparation of protoplasts was passed through a narrow syringe rupturing the protoplasts. The broken protoplasts were fractionated on a discontinuous Ficoll gradient consisting of 5% Ficoll over 20% Ficoll in 0.6 M mannitol and 10 mM Mes (pH 5.5). After centrifugation for 20 min at 50g, the protein bodies formed a layer on top of the 20% Ficoll. The gradient was fractionated and the fractions assayed for α-mannosidase, leucine aminopeptidase (a cytosol marker enzyme), and NADH-Cyt c reductase (and ER-marker enzyme). The results (Fig. 4) show that all the leucine aminopeptidase and NADH-Cyt c reductase activity remained at the top of the gradient, while a significant proportion (25%) of the α-mannosidase sedimented with the protein bodies. We also determined the proportion of protein bodies which remained intact during this procedure, by measuring the distribution of the reserve protein phaseolin. Phaseolin is localized in the protein bodies and its presence in the supernatant would be the result of protein body breakage. In the preparation shown in Figure 4, 30% of the phaseolin was found in the protein body fraction, indicating that a significant proportion of the protein bodies remained intact, but that 70% of the protein bodies ruptured during the isolation procedure. The presence of α-mannosidase at the top of the gradient (Fig. 4) is interpreted as resulting from the breakage of protein bodies. Finding 75% of the α-mannosidase activity in the supernatant and 70% of the reserve protein phaseolin, we conclude that about 90% of the α-mannosidase in the protoplasts was in the protein bodies.

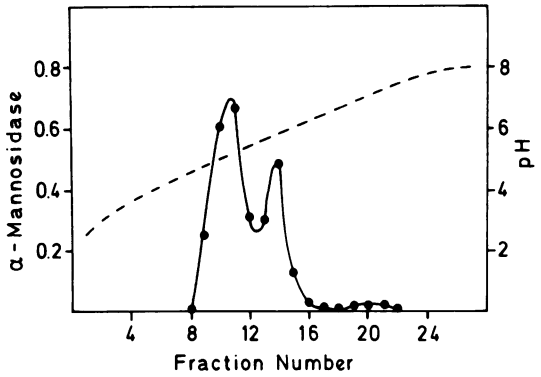

FIG. 3. Isoelectrofocusing of α-mannosidase present in protoplasts obtained from *P. vulgaris* cotyledons. Protoplasts obtained from cotyledons weighing 200 to 250 mg each were incubated for 60 min at 0°C with a solution consisting of 0.1% Triton X-100, 500 mM KCl, and 100 mM Tris-HCl (pH 7.4) to extract all the α-mannosidase present. The suspension was then centrifuged for 30 min at 150,000g; the supernatant dialyzed against 0.1% Triton X-100, 500 mM KCl, and 5 mM Tris-HCl (pH 7.4); and the dialysate submitted to isoelectrofocusing in the pH 3 to 10 range. pH gradient (- - -); enzyme activity (——).

Protein bodies were recovered from a Ficoll gradient (Fig. 4) and extracted for 60 min at 0°C with 0.1% Triton X-100 in H_2O. The mixture was centrifuged at 100,000g for 30 min and the supernatant and pellet collected. The supernatant contained 40% of the α-mannosidase activity, and the pellet the remainder. Increasing the detergent concentration did not change this distribution. The pellet was extracted with 500 mM KCl, and both the detergent extract and the KCl extract subjected to isoelectrofocusing. The results (Fig. 5) show that the detergent extract contained exclusively isozyme I, while the salt extract contained mostly isozyme II. It appears that isozyme I is readily solubilized from the protein bodies when the protein body membrane is removed by detergent treatment, while isozyme II is only solubilized when the storage globulin (phaseolin) is dissolved. These results raise the possibility that α-mannosidase I is associated with the protein body membrane. Protein bodies were isolated as described and treated with 500 mM KCl without Triton X-100. The resulting mixture was layered on a 16 to 48% (w/w) sucrose gradient and centrifuged to equilibrium. Under these conditions, the protein body membranes form a band at a density of 35% (as shown by the turbidity). Assays of α-mannosidase activity showed that all the activity was at the top of the gradient and that none was associated with the protein body membranes (data not shown).

α-Mannosidase in the Cell Wall. The absence of isozyme III from the protoplasts and from the protein bodies may have been due to the inactivation of isozyme III during the preparation of the protoplasts (incubation overnight at 22°C) or to its removal by the cell wall degrading enzymes. To choose between these possibilities, we examined the α-mannosidase associated with isolated cell walls. Cotyledons were homogenized with a conical ground-glass tissue homogenizer in 100 mM Tris-HCl (pH 7.4) and the cell walls sedimented by centrifugation for 5 min at 500g. The cell walls were washed five times by resuspension in cold H_2O and resedimentation at the same speed. The cell wall pellet was then extracted with 500 mM KCl for 60 min at 0°C and the extract assayed for α-mannosidase activity. Cell wall extracts contained

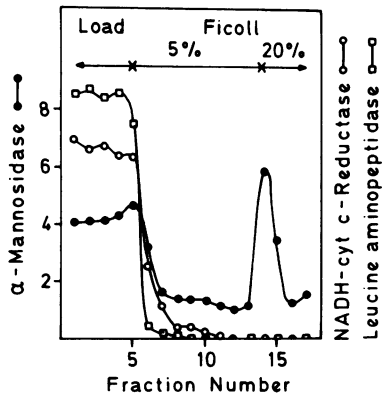

FIG. 4. Distribution of α-mannosidase and other enzymes on a Ficoll gradient. Protoplasts were obtained from cotyledons weighing 225 to 250 mg each. The protoplasts were broken by pressing them through a narrow syringe, and the broken protoplasts were fractionated on a discontinuous Ficoll gradient (0.6 M mannitol, 10 mM sodium Mes, pH 5.5) by centrifugation for 20 min at 50g. The protein bodies formed a layer at the interphase of the 5% and the 20% Ficoll. Fractions were assayed for α-mannosidase, NADH Cyt c-reductase, and leucine aminopeptidase.

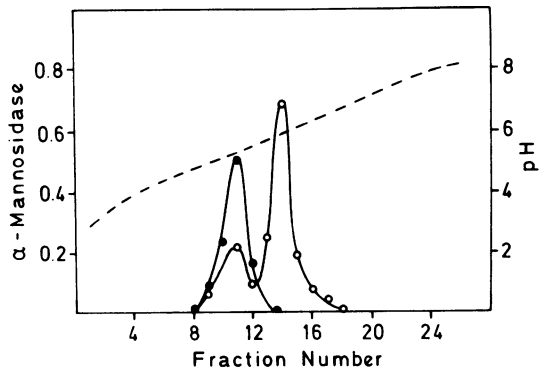

FIG. 5. Isoelectrofocusing of α-mannosidase present in protein bodies obtained from *P. vulgaris* protoplasts. Protein bodies isolated as described in Figure 4 were incubated for 60 min at 0°C with 0.1% Triton X-100, centrifuged for 30 min at 150,000g, and the supernatant subjected to isoelectrofocusing. The pellet was resuspended in 500 mM KCl, incubated for 60 min at 0°C, and the suspension centrifuged for 30 min at 150,000g. The supernatant was subjected to isoelectrofocusing. pH gradient (- - -); Triton X-100 extract (●); KCl extract (○).

7 to 9% of the total activity in the cotyledons. Isoelectrofocusing of the cell wall extracts (Fig. 6) showed that the α-mannosidase formed a broad peak from pH 6.5 to 7.5 which corresponds to the α-mannosidase III of Figure 1.

α-Mannosidase in the Endoplasmic Reticulum. Homogenization of cotyledons in 12% sucrose containing 100 mM Tris-HCl (pH 7.4) and 1 mM EDTA results in nearly total breakage of the protein bodies. When such an extract was first centrifuged at 100*g* for 10 min, and then passed over a Sepharose 4B column, a significant proportion of α-mannosidase activity remained with the organelles (Fig. 7). The proportion of α-mannosidase in the organelle fraction dropped from 20.4% in cotyledons weighing 65 mg to 6.4% in mature cotyledons weighing 350 mg.

The organelles obtained from the Sepharose 4B column were fractionated on isopycnic sucrose gradients to determine with which organelles α-mannosidase activity was associated. Cotyledons were homogenized in 12% sucrose containing 100 mM Tris-HCl (pH 7.4), and containing either 1 mM EDTA or 3 mM $MgCl_2$. The homogenates were then fractionated on Sepharose 4B columns equilibrated with the same media, and the organelle fractions recovered. The organelles were fractionated on isopycnic sucrose gradients (16–48% w/w in the same media) and the fractions assayed for NADH-Cyt *c* reductase (an ER marker enzyme) and inosine diphosphatase (a Golgi marker enzyme). The results (Fig. 8) show that in the presence of EDTA, all three

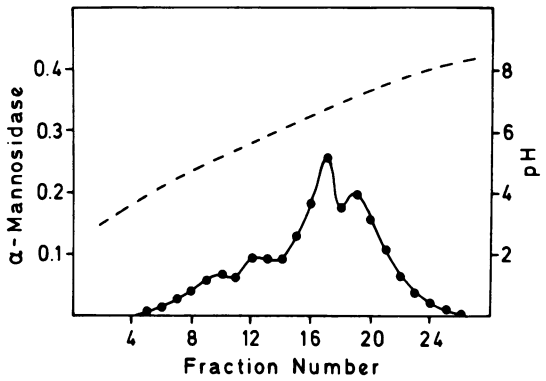

FIG. 6. Isoelectrofocusing of α-mannosidase associated with cell walls obtained from *P. vulgaris* cotyledons. A cell wall preparation obtained from cotyledons weighing 250 to 275 mg each was incubated for 60 min at 0°C with 500 mM KCl, centrifuged for 10 min at 1,000*g*, and the supernatant subjected to isoelectrofocusing in the pH 3 to 10 range. pH gradient (– – –); enzyme activity (——).

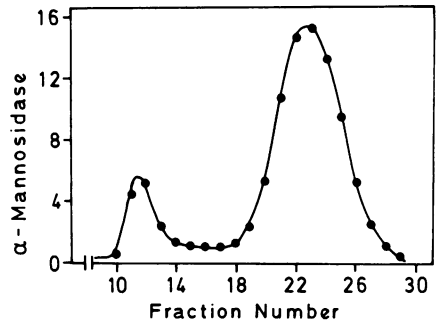

FIG. 7. Distribution of α-mannosidase after fractionation of a cotyledon extract on a Sepharose 4B column. Cotyledons (four, 650 mg total) were homogenized in 12% sucrose containing 100 mM Tris-HCl and 1 mM EDTA. The homogenate was centrifuged at 100*g* for 10 min and loaded on a Sepharose 4B column (16 × 200 mm). The column was eluted with the homogenization medium, and 1-ml fractions were collected. The fractions were assayed for α-mannosidase activity which is expressed as units/fraction.

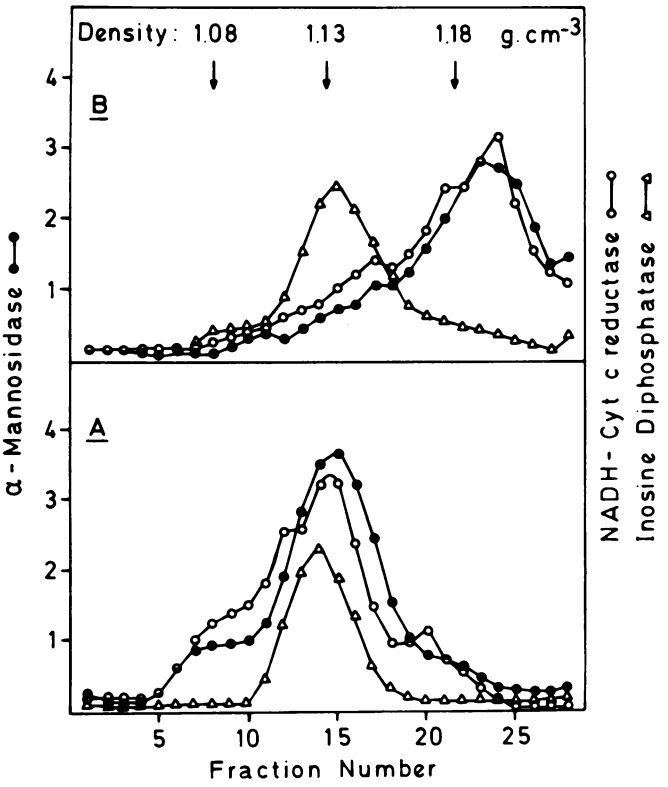

FIG. 8. Fractionation of organelles on isopycnic sucrose gradients either in the presence of 3.0 mM $MgCl_2$ (top) or 1 mM EDTA (bottom). Cotyledons weighing 225 to 250 mg each were homogenized and the homogenate fractionated as in Figure 7 except that two media were used. One medium contained 1 mM EDTA, the other one 3 mM $MgCl_2$. The organelles were fractionated on isopycnic linear sucrose gradient (16–48%) in the same media. The gradients were centrifuged for 2 h at 82,000*g*, the fractions were collected and assayed for α-mannosidase activity in the presence of 0.1% Triton X-100 and for NADH Cyt *c* reductase and inosinediphosphatase (IDPase). Enzyme activities are expressed in relative units.

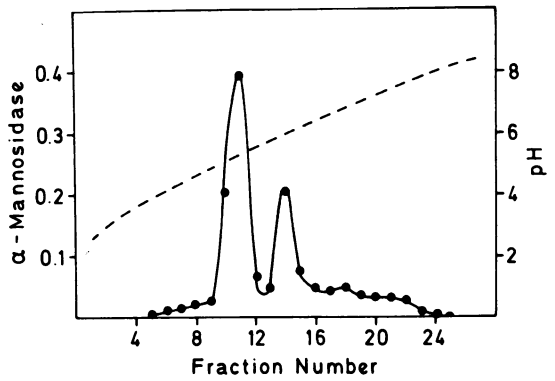

FIG. 9. Isoelectrofocusing of α-mannosidase present in ER obtained from *P. vulgaris* cotyledons. ER was isolated on a discontinuous sucrose gradient, incubated for 60 min at 0°C with a solution consisting of 0.1% Triton X-100, 500 mM KCl, and 100 mM Tris-HCl (pH 7.4) to extract the mannosidase present. The suspension was then centrifuged for 30 min at 150,000*g*, and the supernatant, after being dialyzed against a solution consisting of 0.1% Triton X-100, 500 mM KCl, and 5 mM Tris-HCl (pH 7.4), was submitted to isoelectrofocusing in the pH 3 to 10 range. pH gradient (– – –), enzyme activity (——).

enzymes banded at a density of 1.13 g·cm^{-3}; in the presence of 3 mM $MgCl_2$ α-mannosidase and NADH-Cyt *c* reductase banded at a density of 1.19 g·cm^{-3}. Cyt oxidase, a marker for the mitochondria, bands at 1.18 g·cm^{-3} in both types of gradients (data not shown). These data strongly indicate that most of the α-mannosidase is associated with the RER. The organelle fraction was incubated with 0.1% Triton X-100, 500 mM KCl, and 100 mM Tris-HCl (pH 7.4) and the extracts subjected to isoelectrofocusing. The results (Fig. 9) showed that forms I, II, and III of α-mannosidase were associated with the ER.

DISCUSSION

The results presented here indicate that cotyledons of *P. vulgaris* contain three isozymes of α-mannosidase; two of these are associated with the protein bodies and the third one with the cell wall. In addition, we found that the ER contains small amounts of all three isozymes of the enzyme. The two isozymes of the enzyme identified and characterized by Paus (24) are probably our forms I and II. She found that yellow wax beans contain two forms of α-mannosidase with pI of 5.1 and 6.1. These two forms have similar molecular radii (Mr 220,000–210,000) and similar amino acid compositions, but differ in their carbohydrate content (8.3% and 16.5%). The absence of our isozyme III from the preparation of Paus may be due to the method of extraction or the subsequent treatment of the extract. We found that 500 mM salt was necessary to extract isozyme III from the cell wall (although lesser amounts of isozyme III were solubilized in other media) and that isozyme III was not recovered when extracts were dialyzed exhaustively against H_2O. The identification of these three isozymes does not exclude the possibility that other forms of α-mannosidase may be present in the cells. The identification of an α-mannosidase which is involved in processing the oligosaccharide side-chains of mannose-containing glycoproteins (see 16) indicates that other α-mannosidases may yet be found in these cells. However, the relatively large amount of α-mannosidase in the ER may make it difficult to identify such specific α-mannosidases.

All three forms of α-mannosidase were found associated with the RER as shown by the experiments in which the density of the ER on sucrose gradients was shifted by inclusion of 3 mM $MgCl_2$ or 1 mM EDTA in the homogenization media. Under our conditions, the ER stripped of its ribosomes has a density of 1.13 g·cm^{-3}, the same as that of the Golgi. Using pea cotyledons, Nagahashi and Beevers (21) found a density of 1.12 g·cm^{-3} for stripped ER and a density of 1.15 to 1.16 g·cm^{-3} for the Golgi. These differences are probably due to differences in the methods used to prepare the organelles.

Proteins which are secreted or are transported to vacuoles or protein bodies are made on polysomes bound to ER membranes and are found in association with the ER (see 8). In particular, there is now considerable evidence that the proteins which accumulate in the protein bodies are sequestered within the lumen of the RER (3, 6, 9, 11, 25). For many of these proteins, in particular legumin and vicilin of *Pisum sativum*, and phaseolin and phytohemagglutinin of *P. vulgaris*, we have evidence that this association is transient, indicating that these proteins move through the ER. The presence of the three isozymes of α-mannosidase in the ER is an indication that they may be synthesized on the RER, and will be transported from the ER to their respective destinations. This raises the important question of how and where the sorting of the proteins is to be carried out. In animal cells, protein sorting appears to be a function of the Golgi apparatus (see 13). We did not find a peak of α-mannosidase activity associated with the Golgi apparatus (inosine diphosphatase, density 1.13 g·cm^{-3}) when 3 mM $MgCl_2$ was included in the medium. This indicates that the α-mannosidase which is on its way to the protein bodies may not pass through the Golgi, or that the Golgi is a very small rapidly turning over compartment in comparison to the ER. When studying the transport of storage protein we also found that there was no peak of newly-synthesized storage protein in the region of the gradient which contained the inosine diphosphatase (11). Such observations neither support nor exclude the possibility that protein transport to the protein bodies is mediated by the Golgi.

The accumulation of α-mannosidase takes place exclusively during the period of seed development. We found no increase during germination and seedling growth. A similar result was obtained with mung bean cotyledons (10). Neely and Beevers (22) found a 2-fold increase in the α-mannosidase activity of pea cotyledons during the first 4 d of seedling growth, while Agrawal and Bahl (1) observed a dramatic (30-fold) increase in the specific activity of α-mannosidase in the cotyledons of *P. vulgaris*. It is not clear from the data of Agrawal and Bahl (1) to what extent this increase in specific activity is due to more enzyme per cotyledon, or to the catabolism of reserve protein. In plants where cotyledons only have a storage function (*e.g.* mung bean and *P. vulgaris*) the accumulation of α-mannosidase appears to be confined to the seed maturation stage.

These experiments open the way to investigate the important problem of protein sorting in plant cells. If different isozymes of α-mannosidase are located in different lytic compartments, it may be possible to identify the structural features of the molecules which direct their transport to these compartments.

Acknowledgment—It is a pleasure to thank Bobbie Bradford for her expert technical assistance with most of these experiments.

LITERATURE CITED

1. AGRAWAL KML, OP BAHL 1968 Glycosidases of *Phaseolus vulgaris*. II. Isolation and general properties. J Biol Chem 243: 103–111
2. ARON CG 1973 A new method for the detection of isoelectrically focused proteins in polyacrylamide gels. ISCO Applications Research Bulletin No. 12.
3. BAUMGARTNER B, KT TOKUYASU, MJ CHRISPEELS 1978 Localization of vicilin peptidohydrolase in the cotyledons of mung bean seedling by immunofluorescence microscopy. J Cell Biol 79: 10–19
4. BOLLER T, H KENDE 1979 Hydrolytic enzymes in the central vacuole of plant cells. Plant Physiol 63: 1123–1132
5. BOLLINI R, MJ CHRISPEELS 1979 The rough endoplasmic reticulum is the site of reserve-protein synthesis in developing *Phaseolus vulgaris* cotyledons. Planta 146: 487–501
6. BOLLINI R, W VAN DER WILDEN, MJ CHRISPEELS 1982 A precursor of the reserve-protein phaseolin is transiently associated with the endoplasmic reticulum of developing *Phaseolus vulgaris* cotyledons. Physiol Plant 55: 82–92
7. BOWLES DJ, H KAUSS 1976 Characterization, enzymic and lectin properties of isolated membranes from *Phaseolus aureus*. Biochim Biophys Acta 43: 360–374
8. CHRISPEELS MJ 1980 The endoplasmic reticulum. *In* NE Tolbert, ed, The Biochemistry of Plants, Vol 1. Academic Press, New York, pp 390–412
9. CHRISPEELS MJ, R BOLLINI 1982 Characteristics of membrane-bound lectin in developing *Phaseolus vulgaris* cotyledons. Plant Physiol 70: 1425–1428
10. CHRISPEELS MJ, D BOULTER 1975 Control of storage protein metabolism in the cotyledons of germinating mung bean: role of endopeptidase. Plant Physiol 55: 1031–1037
11. CHRISPEELS MJ, TJV HIGGINS, S CRAIG, D SPENCER 1982 Role of the endoplasmic reticulum in the synthesis of reserve proteins and the kinetics of their transport to protein bodies in developing pea cotyledons. J Cell Biol 93: 5–14
12. CRAIG S, DJ GOODCHILD, C MILLER 1980 Structural aspects of protein accumulation in developing pea cotyledons. II. Three-dimensional reconstructions of vacuoles and protein bodies from serial sections. Aust J Plant Physiol 7: 329–337
13. FARQUHAR MG, GE PALADE 1981 The Golgi apparatus (complex)-(1954–1981)-from artifact to center stage. J Cell Biol 91: 77–103
14. HARRIS N, MJ CHRISPEELS 1975 Histochemical and biochemical observations on storage protein metabolism and protein body autolysis in cotyledons of germinating mung bean. Plant Physiol 56: 292–299
15. HECK V, E MARTINOIA, P MATILE 1981 Subcellular localization of acid proteinase in barley mesophyll protoplasts. Planta 151: 198–200
16. HUBBARD SC, RJ IVATT 1981 Synthesis and processing of asparagine-linked oligosaccharides. Annu Rev Biochem 50: 555–583
17. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
18. MATILE P 1975 The lytic compartment of plant cells. Springer-Verlag, New York
19. MIFLIN BJ, SR BURGESS, PR SHEWREY 1981 The development of protein bodies in the storage tissues of seeds: subcellular separations of homogenates of barley, maize and wheat endosperm and of pea cotyledons. J Exp Bot 32: 199–219
20. MILLERD A 1975 Biochemistry of legume seed proteins. Annu Rev Plant Physiol

26: 53–72
21. NAGAHASHI L, L BEEVERS 1978 Subcellular localization of glycosyl transferases involved in glycoprotein biosynthesis in the cotyledons of *Pisum sativum* L. Plant Physiol 61: 451–459
22. NEELY RS, L BEEVERS 1980 Glycosidases from cotyledons of *Pisum sativum* L. J Exp Bot 31: 299–312
23. PAUS E 1977 α-Mannosidase from *Phaseolus vulgaris*. Composition and structural properties. Eur J Biochem 73: 155–161
24. PAUS E, TB CHRISTENSEN 1972 α-Mannosidase from *Phaseolus vulgaris*. Purification and characterization. Eur J Biochem 25: 308–314
25. VAN DER WILDEN W, NR GILKES, MJ CHRISPEELS 1980 The endoplasmic reticulum of mung bean cotyledons. Role in the accumulation of hydrolases in protein bodies during seedling growth. Plant Physiol 66: 390–394
26. VAN DER WILDEN W, EM HERMAN, MJ CHRISPEELS 1980 Protein bodies of mung bean cotyledons as autophagic organelles. Proc Natl Acad Sci USA 77: 428–432