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# Biochemical and Ultrastructural Trends in Proteolysis of the β-subunit of 7S Protein in the Cotyledons During Germination of Soybean Seeds

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**ABSTRACT: Antibodies raised against the purified β-subunit of β-conglycinin were used in immunohistochemical studies to monitor the pattern of β-conglycinin mobilization in the cotyledons during soybean [*Glycine max* (L.) Merr.] seed germination. Western blot analysis revealed that the break down of the β-subunit of β-conglycinin commenced as early as 2 days after seed imbibition (DAI). Concurrent with the degradation of the β-subunit of β-conglycinin, accumulation of 48, 28, and 26 kD proteolytic intermediates was observed from 2 to 6 DAI. Western blot analysis also revealed that the acidic subunit of glycinin was mobilized earlier than the basic subunit. The basic glycinin subunit was subjected to proteolysis within 2 DAI resulting in the appearance of an intermediate product approximately 2 kD smaller than the native basic glycinin subunit. In contrast to the major seed storage proteins, lipoxygenase was subjected to limited proteolysis and was detected even after 8 DAI. The first sign of β-conglycinin breakdown was observed near the vascular strands and proceeded from the vascular strands towards the epidermis. Protein A-gold localization studies using thin sections of soybean cotyledons and antibodies raised against the β-subunit of β-conglycinin revealed intense labeling over protein bodies. A pronounced decrease in the protein A-gold labeling intensity over protein bodies was observed at later stages of seed germination. The protein bodies, which were converted into a large central vacuole by 8 DAI, contained very little 7S protein as evidenced by sparse protein A-gold labeling in the vacuoles.**

***Keywords*** **:** β-conglycinin; *Glycine max*; storage protein, seed germination.

Seeds serve as storage organs for reserve materials such as protein, oil, and starch. Storage reserves are rapidly mobilized during germination and provide the necessary energy and nutrients for seedling growth. Salt-soluble globulins are the major seed storage proteins of soybean. The soybean globulins are classified into 7S and 11S, and together make up 70% of the total seed protein (Nielsen, 1996). The 11S globulins are named glycinin, while the 7S globulins are known as β-conglycinin. The glycinin and β-conglycinin, which are synthesized during seed development, are deposited within vacuolar compartments resulting in specialized structures called protein bodies or protein storage vacuoles (PSVs) (Horisberger *et al.*, 1996).

Numerous biochemical investigations have been carried out on the mobilization of legume seed storage proteins (Wilson, 1986; Muntz, 1996). These studies have established that the break down of storage proteins occurs within the protein bodies, and this process is initiated 2 to 3 days after imbibition (DAI). Proteases responsible for initiating the breakdown of storage proteins are synthesized *de novo* on the rough endoplasmic reticulum (rER) and transported to the protein bodies through the secretory pathway (Muntz, 1996).

Catsimpoolas *et al.* (1968) investigated the fate of soybean seed storage proteins during germination by employing disc electrophoresis and disc immunoelectrophoresis. They reported that the 7S and 11S globulins are metabolized at different rates. The 7S globulin was reported to disappear after 9 DAI, while the 11S globulins were present even after 16 DAI. Subsequently, several studies were carried out on the proteolysis of soybean seed storage proteins during germination (Madden *et al.*, 1985; Hartl *et al.*, 1986; Qi *et al,* 1992; Wilson *et al.*, 1986; Wilson *et al.*, 1988; Seo *et al.*, 2001). These studies led to the following observations: (i) glycinin and β-conglycinin undergo limited specific proteolysis during germination leading to intermediate polypeptides, (ii) the α and α' subunits are degraded at a faster rate than the β-subunit of β-conglycinin, (iii) the degradation of the acidic chain of glycinin is initiated faster than that of basic chain of glycinin, and (iv) protease C1 catalyzes the proteolytic cleavage of the α and α subunits of β-conglycinin.

Most investigations on mobilization of soybean seed proteins have been at the biochemical level. Information obtained by immunocytochemical localization of storage proteins in cotyledons at different stages of seedling growth, in conjunction with the biochemical data, should provide a better understanding of the mechanism responsible for the mobilization of the storage proteins. In this study, a com-

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bined biochemical and ultrastructural approach has been taken to systematically examine the mobilization of storage proteins in the mesophyll cells of soybean cotyledons.

## MATERIALS AND METHODS

### Chemicals

Acrylamide, bis-acrylamide, and sodium dodecyl sulfate (SDS), molecular weight markers, affinity purified goat anti-rabbit IgG-horseradish peroxidase conjugate, and 4-chloro-1-naphthol were purchased from Bio-Rad (Richmond, CA). Nitrocellulose membrane (0.45 μm) was from MidWest Scientific (St. Louis, MO). Osmium tetroxide, glutaraldehyde, and Spurrs low viscosity resin were purchased from Polysciences, Inc. (Warrington, PA). Protein A-gold (10nm) and most other reagents were purchased from Sigma Chemicals Co. (St. Louis, MO).

### Plant material

Pioneer Brand 4362 soybean seeds were surface sterilized by immersion for 5 min in 95% ethanol followed by 50% Chlorox and rinsed in an excess of distilled water. Seeds were germinated in Petri plates (10 seeds/plate) containing 1% agar in water at 30°C in dark. Cotyledons were harvested at specified time periods and used immediately for ultrastructural studies or stored at -80°C until used.

### Preparation of protein extracts

Individual cotyledons harvested at different DAI were ground to a fine powder under liquid nitrogen using a mortar and pestle. The fine powder was extracted with 1 ml of SDS-sample buffer (60 mM Tris-HCl, pH 6.8, 2% SDS (w/v), 10% glycerol (v/v), 5% β-mercaptoethanol) and boiled for 5 min. Samples were clarified by centrifugation for 5 min and the supernatant was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

### Electrophoresis

Soybean seed proteins were resolved by SDS-PAGE with the discontinuous buffer system of Laemmli (1970). The slab gel (10×8×0.75 cm) was made up of either a 12.5% or 8% separation gel and a 4% stacking gel (30%T 2.7%$C_{bis}$). Electrophoresis was carried out at a 20 mA constant current at room temperature. Equal volumes (10 μl/lane) of total seed proteins were loaded per lane. Each gel contained 10 lanes. Resolved proteins were visualized by staining with Coomassie Brilliant Blue.

### Western blot analysis and quantification of storage proteins

Protein blot analysis was performed as described earlier (Krishnan, 1999). Proteins resolved by SDS-PAGE were electrophoretically transferred to a nitrocellulose membrane. Following this, the nitrocellulose membrane was incubated with polyclonal antibodies specific for the β-subunit of β-conglycinin (Krishnan *et al.*, 2000), rice acidic and basic glutelin subunits (Krishnan and Okita, 1986), and soybean lipoxygenase (obtained from J. Polacco, University of Missouri) that were diluted 1:500 in Tris buffered saline (TBS, 10 mM Tris-HCl, pH 7.5, 500 mM NaCl) containing 5% (w/v) non-fat dried milk. Immunologically reactive proteins were identified by the horseradish peroxidase color development procedure recommended by the manufacturer (Bio-Rad Laboratories, Inc., Richmond, CA).

For quantification, the gels or nitrocellulose membranes were scanned using a UMAX PowerLook III scanner with UMAX MagicScan 4.2 Photoshop plug-in (Eastman Kodak). Coomassie blue stained gels were scanned in transmissive mode and the nitrocellulose membranes in reflective mode as 8-bit grayscale images. Average gray scale value was measured using MetaMorph Version 4.6.3 (Universal Imaging Corporation, Westchester, PA). The average grayscale value of each protein band was calculated and this value was used to calculate the percent change relative to the values obtained from protein bands at 0 DAI.

### Embedment of soybean seeds in paraffin

Germinating soybean seeds were spilt into two cotyledonary halves and immediately fixed in 50% ethyl alcohol, 5% glacial acetic acid and 10% formaldehyde (FAA) for 24 h at 4°C. The FAA-fixed soybean cotyledons were dehydrated in a graded ethanol series followed by a graded series of xylene. Tissue was infiltrated with several changes of paraffin at 60°C over a 3-day time period and embedded in Paraplast Plus Tissue Embedding Medium (Oxford Labware, St. Louis, MO). The paraplast embedded tissues were sectioned (10 μm thick) with a microtome and collected on poly-L-lysine-coated slides. The paraffin from the sections was removed with xylene and sections were stained with hematoxylin and eosin (Sheehan and Hrapchak, 1980).

### Immunostaining of paraffin sections

Deparaffinized and rehydrated sections were treated with methanol-hydrogen peroxide to inactivate endoge-

nous peroxidase activity. Sections were incubated for 1 h in 5% goat serum, 1% bovine serum albumin (BSA) and 0.03% triton in phosphate buffered saline (PBS). Antibodies raised against the β-subunit of β-conglycinin were used in 1:500 dilution in 2% goat serum, 1% BSA, and 0.03% triton in PBS for 20 min. Following this, the sections were treated sequentially with biotinylated linker, streptavidin conjugated to horseradish peroxidase, and substrate-chromogen solution according to the manufacturers recommendation (DAKO Corporation, Carpinteria, CA). The sections were counterstained with hematoxylin and examined with bright field optics.

### Light and electron microscopy

Soybean cotyledons harvested at different DAI were dissected into 2 to 3 mm pieces with a razor blade and fixed immediately in 2.5% glutaraldehyde buffered at pH 7.2 with 50 mM sodium phosphate buffer. The seed tissue was fixed at room temperature for 4 h and washed extensively with several changes of phosphate buffer. Following this, the tissue was post-fixed with 2% aqueous osmium tetroxide for 1 h. After several rinses in distilled water, samples were dehydrated in a graded acetone series and infiltrated with Spurrs (1969) resin essentially as described by Krishnan *et al.* (1986). The resin was cured at 65°C for 24 h. Thick sections were cut with a glass knife and stained with 1% toluidine blue for 2 min and examined with bright field optics. Thin sections were cut with a diamond knife on an ultramicrotome and mounted on uncoated 200-mesh nickel grids and stained with 0.5% aqueous uranyl acetate and 0.4% aqueous lead citrate (Hayat, 1972). The sections were examined with a JEOL 1200 EX (Tokyo, Japan) transmission electron microscope at 80 kV.

### Immunocytochemical labeling

Immunogold localization of soybean seed proteins was carried out as described by Krishnan *et al.* (1986). Briefly, thin-sections of soybean cotyledons were collected on uncoated 200-mesh gold grids and blocked for 30 min with 1% BSA in TBS. The grids were incubated for 2 h in a 1:500 dilution of polyclonal antibodies raised against the β-subunit of β-conglycinin (Krishnan *et al.*, 2000) in TBS with 1% BSA. The grids were washed five times at 5 min intervals and incubated with 1:10 diluted protein A-gold particles (10 nm) in 10 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% Tween-20 (TBST) with 1% BSA. Grids were washed several times with TBST and water and stained with 0.5% aqueous uranyl acetate (Krishnan *et al.*, 1986).

## RESULTS

### Storage protein mobilization during seed germination

The mobilization of seed storage proteins during soybean seed germination was monitored by resolving the total seed proteins on a polyacrylamide gel (Fig. 1A and 1B). Quantification of protein concentration by a computer-assisted scanning procedure, indicated no major changes in protein profile during the first 24 h after seed imbibition (compare lanes 1and 2). The first visible change in the protein profile was evident at 2 DAI at which point the intensity of 7S stor-

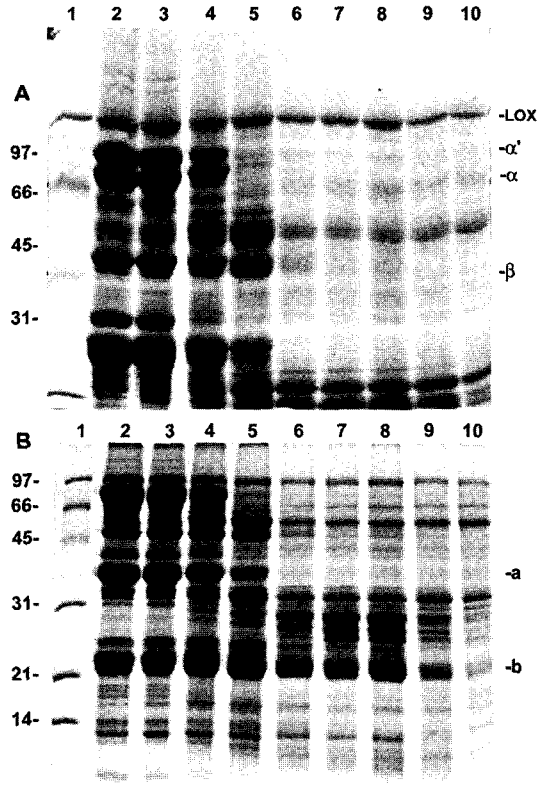



**Fig. 1.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of protein profiles of germinating soybean seeds. Total cotyledon seed proteins were resolved on a 8% (Panel A) and a 12.5% (Panel B) SDS polyacrylamide gel and stained with Coomassie brilliant blue. Lanes 2 to 9, represent equal volumes of protein extracts from cotyledons of seeds at 0, 1, 2, 3, 4, 5, 6, 7, and 8 days after seed imbibition. Panel A: LOX denotes lipoxygenase and α', α, and β represents the three subunits of β-conglycinin. The letters a and b in panel B represents the acidic and basic subunits of the glycinin. Lane 1 shows molecular weight markers in kilodaltons (from the top, down: phosphorylase b; bovine serum albumin; ovalbumin; carbonic anhydrase; soybean trypsin inhibitor, and lysozyme).

age protein decreased sharply (Fig. 1A, lane 3). At 2 DAI, the concentration of the α' and α-subunits of β-conglycinin were reduced by 68% and 52%, respectively when compared with 0 DAI. The α' and α-subunits of β-conglycinin were completely degraded by 3 DAI, while the β-subunit of β-conglycinin was still evident at 4 DAI (Fig. 1A). All three subunits of β-conglycinin, however, were degraded by day 5, and after that time these proteins were barely resolved. The acidic subunit of glycinin was degraded at the same time as the β-subunit of β-conglycinin. The onset of breakdown of the basic subunit was difficult to judge based on the Coomassie blue stained gel due to the presence of overlapping polypeptides in this region (Fig. 1B). Overall, greater than 80% of soybean seeds storage proteins were mobilized by 5 DAI. Concurrent with the disappearance of the major storage proteins, several new polypeptides were seen and some of them accumulated during seed germination (Fig. 1A and 1B).

## Immunological detection of storage protein degradation and their intermediates

Western blot analysis using protein extracts from developing soybean seeds and antibodies raised against the β-subunit of β-conglycinin confirmed the differential mobilization of the α'-, α-, and the β-subunits during seed germination. The antibody reacted very strongly with the 52 kD β-subunit and to a lesser extent with the 72 kD a'-subunit and 70 kD a-subunit of β-conglycinin (Fig. 2A). Immunoreactive polypeptides corresponding to the α'-and α-subunits of β-conglycinin were detected up to 3 DAI, while the β-subunit was seen up to 4 DAI (Fig. 2A). Several immunoreactive polypeptides (68, 50, 48, 28, and 26 kD), presumably the proteolytic breakdown products of the 7S globulin, were visible by 2 DAI (Fig. 2A). The 28 and 26 kD intermediate polypeptides increased in intensity starting from 2 DAI and showed the highest accumulation at 6 DAI (Fig. 2A). After 6 DAI, the accumulation of 28 and 26 kD polypeptides was greatly

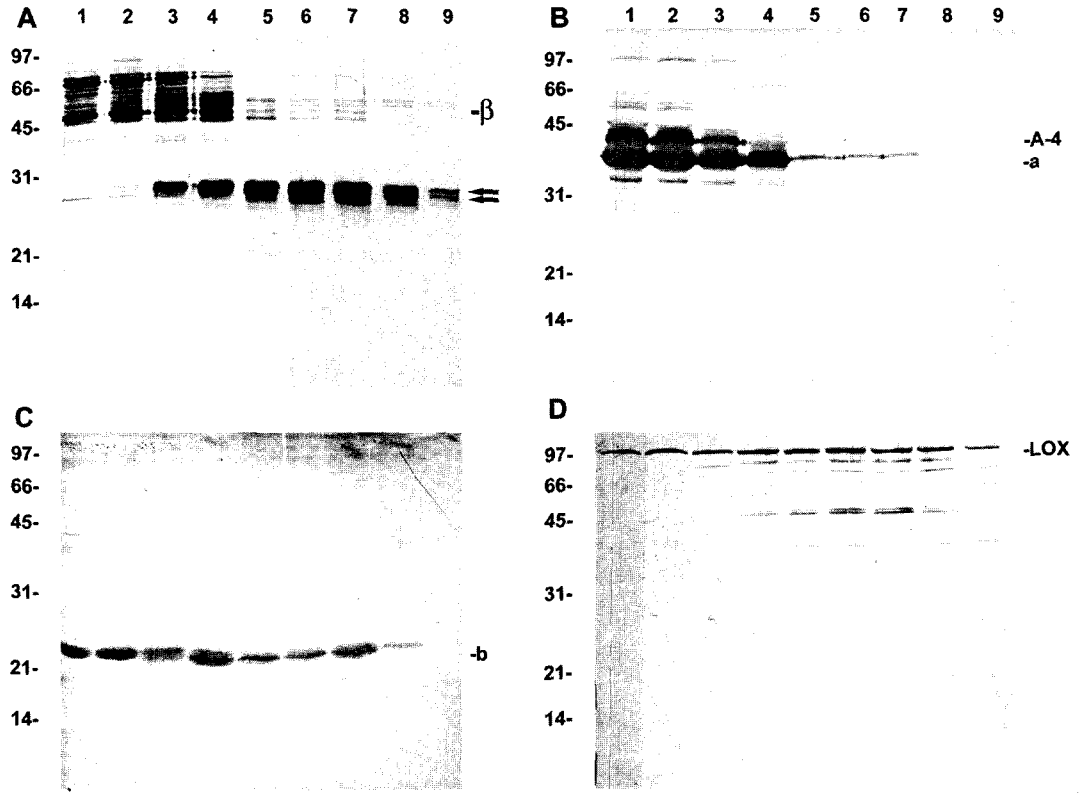
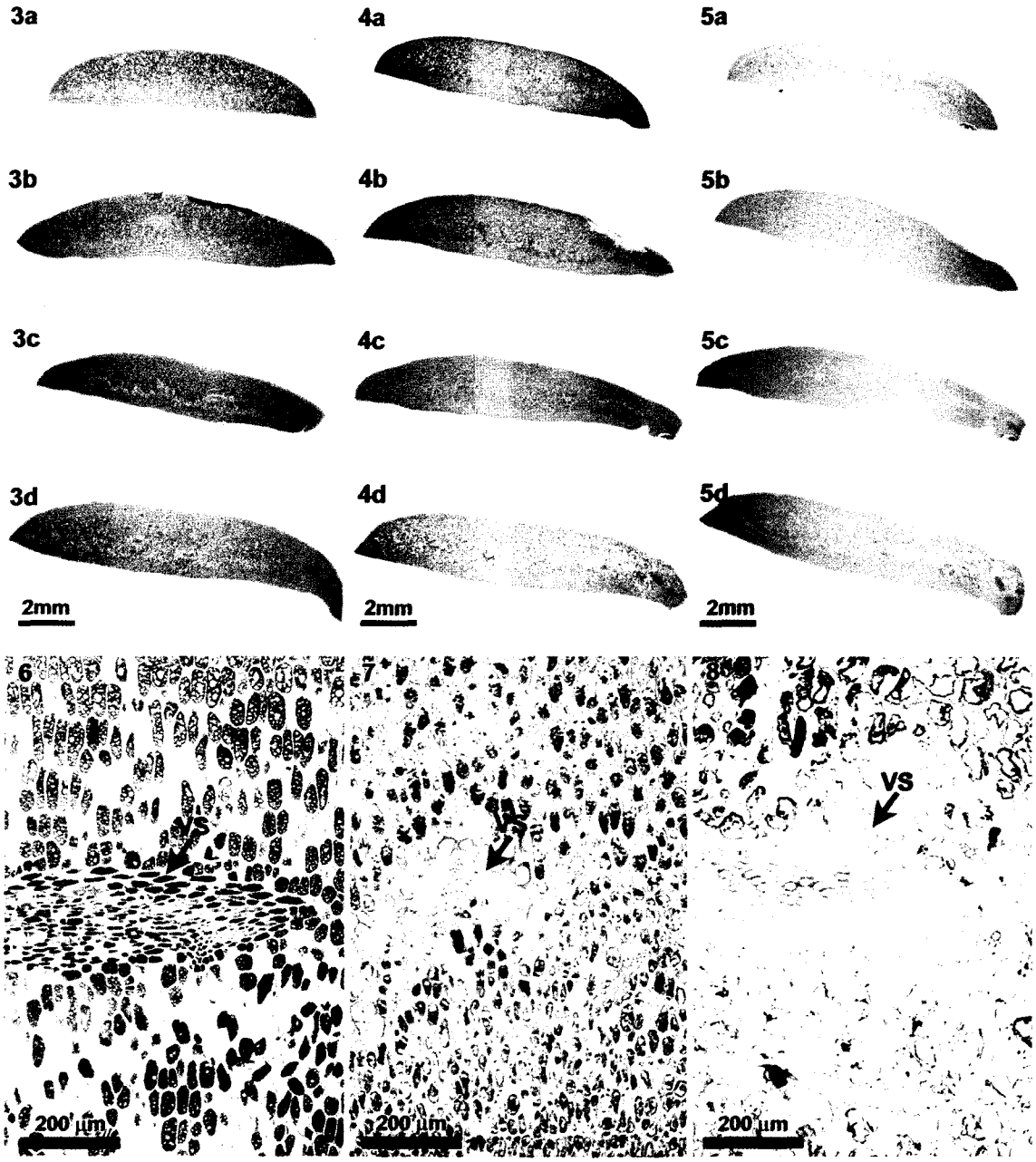



**Fig. 2.** Western blot analysis of degradation of soybean seed proteins. Total seed proteins were resolved on a 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were transferred to a nitrocellulose membrane. The membrane was reacted with antiserum specific for the β-subunit of β-conglycinin (Panel A), rice glutelin acidic subunit (Panel B), rice glutelin basic subunit (Panel C) and soybean seed lipoxygenase (Panel D) and goat anti-rabbit immunoglobulin-horseradish peroxidase. Lanes 1 to 9 contain protein extracts from cotyledons of seeds at 0, 1, 2, 3, 4, 5, 6, 7, and 8 days after seed imbibition. The symbol 'β' at the night side of the figure 2A points to the β-subunit β-conglycinin and the two arrows refer to the two major degradation intermediates of the β-subunit of β-conglycinin. Letters 'a', 'b', and LOX refers to the acidic and basic glycinin subunits and lipoxygenase, respectively. The numbers on left side of the figure indicates the position of the molecular weight markers in kilodaltons.

reduced. The antibody against the β-subunit of β-conglycinin did not react with any other low molecular weight proteins (Fig. 2A).

Legume globulins share antigenic homology to rice glutelins (Okita *et al.*, 1988). Therefore antibodies raised against the rice acidic and basic glutelin subunits were used to monitor mobilization of glycinin in the cotyledons during seed germination. The rice acidic glutelin antibody cross-reacted



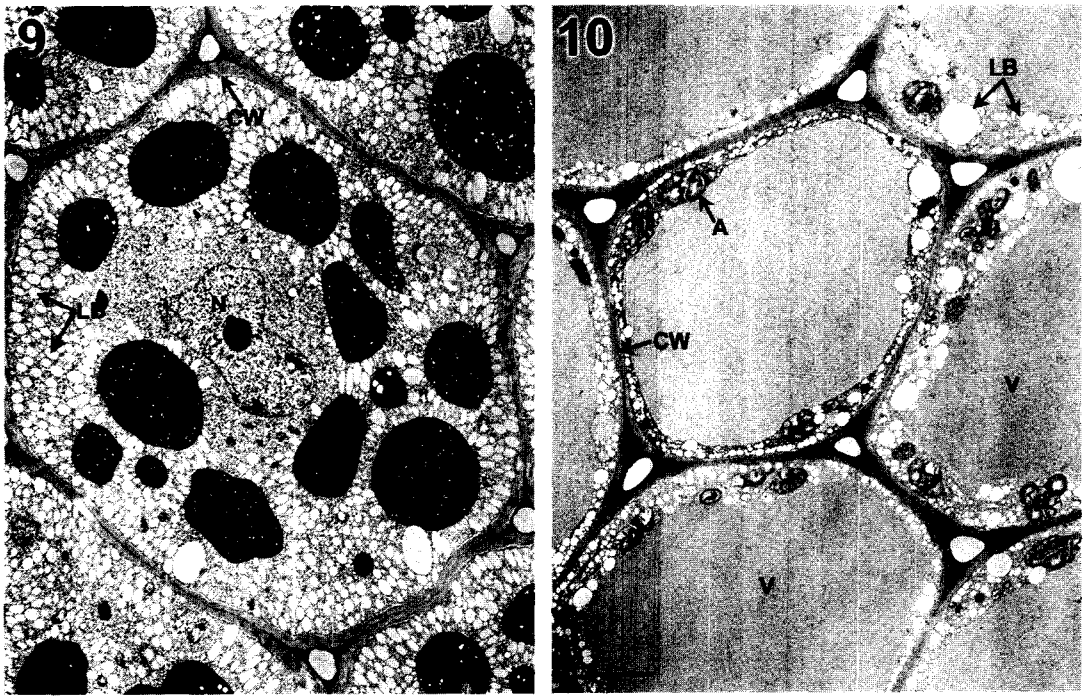

**Figs. 3-8.** Immunohistochemical analysis of temporal and spatial degradation of the β-subunit of β-conglycinin during soybean seed germination. Photomicrographs of germinating soybean seeds stained with hematoxylin and eosin (Figs. 3a-3d), β-subunit of β-conglycinin specific antiserum (Figs. 4a-4d) and pre-immune serum (Figs. 5a-5d). Note the progressive decline in the intensity of immunostaining in Figs. 4a to 4d. a, b, c, and d: soybean seeds at 2 days after seed imbibition (DAI), 4 DAI, 6 DAI, and 8 DAI, respectively. Figs. 6-8: Degradation of the β-subunit of β-conglycinin during soybean seed germination is initiated in the vascular strand (VS). Note the staining of β-subunit of β-conglycinin of the VS is drastically reduced by 4 DAI (Fig. 7) and is completely absent at latter stages of seed germination (Fig. 8). Figs. 6, 7, and 8: soybean seeds at 2 DAI, 4 DAI, and 8 DAI, respectively. Bar = 200 µm.

against both the A-4 glycinin and the abundant acidic glycinin subunit. About 90% of A-4 glycinin was mobilized by 3 DAI and 85% of the glycinin acidic subunit were depleted by 4 DAI (Fig. 2 B). The basic subunit was also subjected to protelysis early during seed germination. After 2 DAI, the basic glycinin subunit in the cotyledons underwent limited proteolysis yielding an intermediate product approximately 2 kD smaller than the native basic subunit (Fig. 2C). This intermediate product is recognizable up to 7 DAI. Western blot analysis revealed no other proteolytic intermediates. In contrast to the acidic subunit, the basic subunit was mobilized relatively slowly and about 50% of this truncated subunit still could be detected at 5 DAI (Fig. 2C). Unlike the major storage proteins, the soybean lipoxygenase was detected even after 8 DAI (Fig. 2D). There are several lipoxygenases in soybean, some of which are synthesized during seed development, some during germination and others in vegetative tissues. The antibody used in this study does not discriminate the different lipoxygenases. Thus the immunoreactive polypeptides detected during seed germination may represent lipoxygenases that are synthesized during seed germination. However, there appears to be some degradation during seed germination since some proteolytic intermediate products of lipoxygenase can be seen as early as 2 DAI (Fig. 2D).

## Temporal and spatial pattern of β-conglycinin mobilization

In order to observe the temporal and spatial pattern of β-conglycinin mobilization during soybean seed germination, thick sections of cotyledons at various stages of germination were subjected to an immunohistochemical analysis. Figs 3a 3d revealed the anatomy of soybean cotyledons at different germination periods. When these sections were reacted with antibodies raised against the β-subunit of β-conglycinin, the entire cotyledon was stained (Fig. 4a). During later stages of seed germination, when the storage proteins are rapidly mobilized, the intensity of the immunological stain decreased steadily and only a faint staining was evident in 8 DAI cotyledon (Compare Figs. 4a and 4d). The staining with the antibodies was very specific to β-conglycinin, since under similar experimental conditions, no immunoreactive staining was observed with pre-immune serum (Figs. 5a-5d).

During the early phases of seed germination, most of the cotyledonary mesophyll cells were stained with β-conglycinin antibodies including vascular strands (Fig. 6). The initial degradation of β-conglycinin was first observed in the vascular strands. The immunoreactive proteins that stained prominently in the vascular strand,



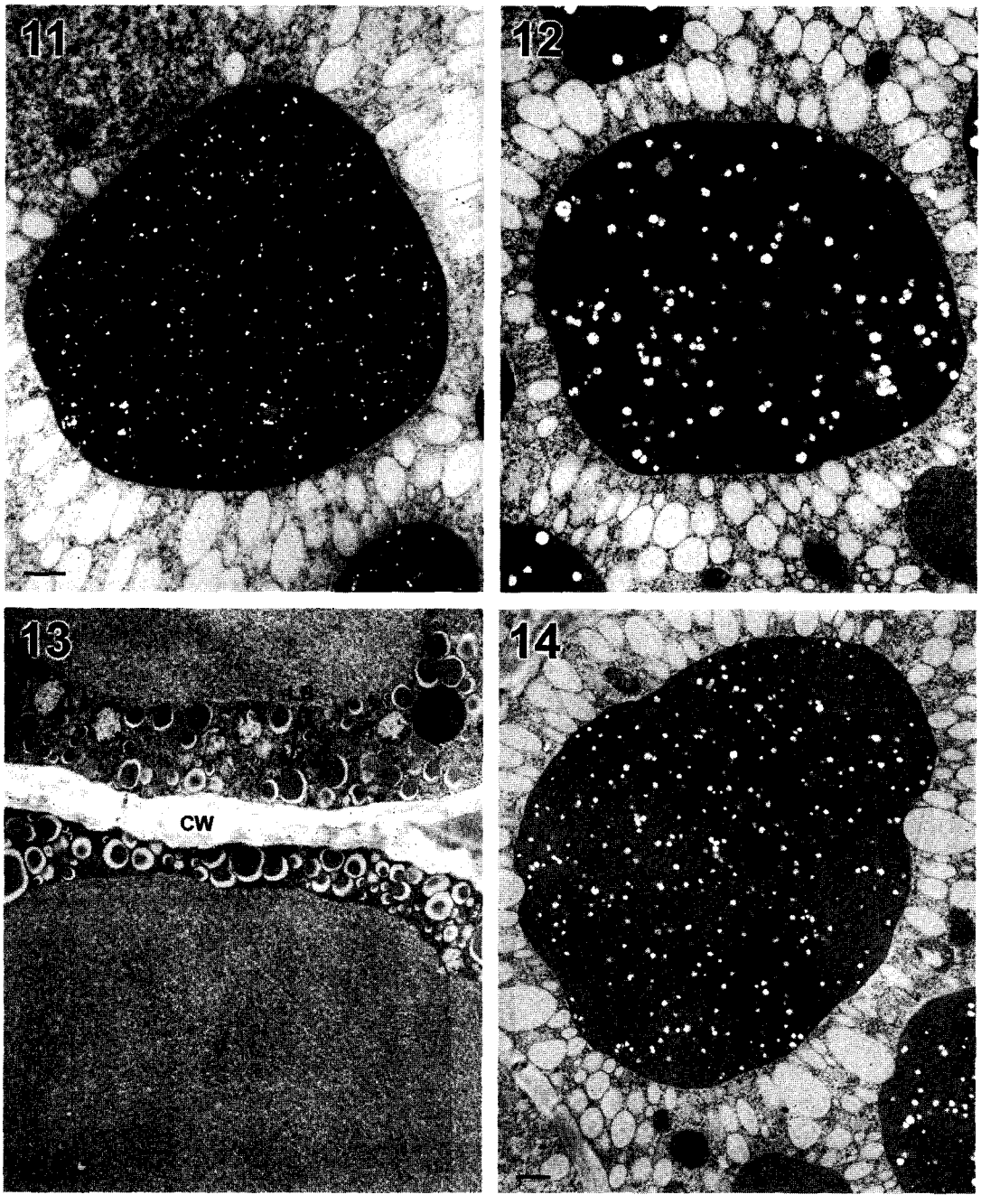

**Figs. 9-10.** Ultrastructural changes in germinating soybean cotyledon cells. Soybean cotyledons at 12 h after seed imbibition (Fig. 9) contain several darkly staining protein bodies and numerous lipid bodies. By 8 DAI (Fig. 10), the protein bodies were converted into central vacuoles. The lipid bodies and amyloplasts have been pushed against the cell wall. PB = protein bodies; LB = lipid bodies; N = nucleus; CW = cell wall; A = amyloplast; V = vacuole. Bars = 2 μm.

however, disappeared rapidly during subsequent stages of seed development (Figs. 7-8).

The β-conglycinin mobilization appears to be initiated at the vascular strand and proceed outwards towards the epidermis.

## Immunocytochemical observation of β-conglycinin mobilization

Electron micrographs of storage parenchyma cells of soybean cotyledons 12 h after imbibition showed cells that were




**Figs. 11-14.** Immunogold localization of β-subunit of β-conglycinin on thin sections of germinating soybean cotyledons. Thin sections obtained from 1 day after seed imbibition (DAI) soybean cotyledons when reacted with antibodies specific for the β-subunit of β-conglycinin and protein A gold particles reveal heavy labeling over protein bodies (Fig. 11). No specific labeling on lipid bodies was observed. By 3 DAI, the protein A-gold labeling intensity has not drastically decreased (Fig. 12), but by 8 DAI, the labeling intensity is drastically reduced (Fig. 13). Thin-sections incubated with pre-immune serum and protein A gold revealed no specific labeling on the protein bodies (Fig. 14). PB = protein bodies, LB = lipid bodies. Bars = 0.5 μm (Figs. 11, 12, and 14), 1 μm (Fig. 13).

densely packed with lipid and protein bodies (Fig. 9). Few starch grains were found. The protein bodies, which varied in size from less than 1 μm to greater than 10 μm in cross-section, were prominent and contained electron dense material (Fig. 9). Embedded within the protein bodies were numerous small inclusions, presumably phytate particles (Fig. 9). As reported earlier by Melroy and Herman (1991), the large protein bodies in the storage parenchyma cells were converted into prominent central vacuoles during the later stages of seed germination (Fig. 10).

The fate of β-conglycinin during seed germination was followed by incubating thin sections of soybean seeds with antibodies raised against the β-subunit of β-conglycinin. Thin sections of soybean cotyledons at 24 h after imbibition reacted strongly with β-conglycinin antibodies as evident by the intense protein A-gold labeling on the protein bodies (Fig. 11). The intensity of labeling on protein bodies was marginally decreased by 3 DAI (Fig. 12). By 8 DAI, the protein bodies, which have been converted into a large central vacuole, revealed drastically lower amounts of protein A-gold particles (Fig. 13). When pre-immune serum was substituted for the β-conglycinin antibodies, no specific labeling was detected on the protein bodies (Fig. 14). Protein A-gold localization studies utilizing rice acidic glutelin antibodies also revealed similar mobilization patterns as observed for the β-subunit of β-conglycinin (data not shown).

## DISCUSSION

An examination of the protein profile of soybean seeds at different stages of seed germination revealed that the three subunits of β-conglycinin are differentially degraded. A visual observation of the Commassie-stained gel revealed that the α'- and α-subunits of β-conglycinin are degraded much faster than the β-subunit. The results from the Western blot analysis clearly establish that the degradation of the β-subunit of β-conglycinin started as early as 2 DAI. This result indicated that the protease responsible for the degradation of the β-subunit of β-conglycinin is active at early stages of seed germination, but degrades the β-subunit at a much slower rate than the protease that is active on the α'- and α-subunits.

Cysteine proteinases (CPRs) are mainly responsible for globulin mobilization (Muntz, 1996). In vetch, the initial mobilization of globulin in the cotyledon and the embryonic axis is initiated by stored CPRs and the subsequent degradation of the globulins in the cotyledons is mediated by proteolytic enzymes formed *do-novo* (Schlereth *et al.*, 2001). Soybean seeds also contain several stored proteolytic enzymes, however, it is not known whether these stored proteinases play a role in the initial mobilization of globulins in the cotyledons during germination. Unlike in vetch, the mobilization of soybean storage proteins is initiated by a serine protease. The enzyme responsible for the degradation of the α'- and α-subunits of β-conglycinin has been purified from 12 day-old soybean seedlings (Qi *et al.*, 1992). It is a serine protease with a molecular weight of 70,000 and has an acidic pH optimum. This protease catalyzes the degradation of the α'- and α-subunit of β-conglycinin but not the β-subunit. The initial cleavage of the α'- and α-subunits occur near the amino terminus of the protein. The amino terminus sequences that are conserved between the α'- and α-subunits (Sebastiani *et al.*, 1990), are missing in the β-subunit (Tierney *et al.*, 1987). Recently, a cysteine endopeptidase, protease C2, was purified from the cotyledons of soybean seedlings (Seo *et al.*, 2001). This enzyme has been shown to be responsible for the cleavage of the β-subunit of β-conglycinin and the activity of this enzyme was evident only at 4 DAI (Seo *et al.*, 2001). Identification and characterization of all these proteinases is essential to fully understand the respective roles of the proteinases in the mobilization of the major soybean seed storage proteins.

Wilson *et al.* (1986) demonstrated that the degradation of β-conglycinin results in the accumulation of at least six intermediate polypeptides ranging from 31 to 24 kD. Later, it was demonstrated that the α'- and α-subunits are degraded by a serine protease into intermediates of 63, 61, 55, 53.5, 50 and 48 kD (Wilson *et al.*, 1986). The antibodies used in the previous study were more specific for the α'- and α-subunit of β-conglycinin and hence, the proteolytic products of the β-subunit of β-conglycinin were not identified. The antibodies employed in the present study, however, are specific to the β-subunit of the β-conglycinin. Western blot analysis demonstrated that the β-subunit first undergoes a proteolytic cleavage that removes about 4 kD of peptide from the mature protein. The resulting proteolytic intermediate is further degraded into two major products of 28 to 26 kD. Based on the Western blot analysis, a pathway for the degradation of the β-subunit degradation of β-conglycinin is proposed as follows: β-subunit (52 kD) →48 kD →28 kD→26 kD. Further degradation of the 28 and 26 kD intermediates is presumably mediated by non-specific proteases.

Earlier studies have demonstrated that the acidic and basic subunits of glycinin undergo differential proteolysis. During the initial stages of seed germination, the acidic subunit of glycinin is rapidly mobilized, while the basic subunit remains intact (Wilson *et al.*, 1988). In this earlier study antibodies raised against the total glycinin was employed to monitor glycinin proteolysis. This antibody, however, reacted very weakly against the basic subunit and showed preferential reactivity against the acidic subunit (Wilson *et*

*al.*, 1988). In the current study antibodies raised against individually purified rice glutelin acidic and basic subunits (Krishnan and Okita, 1986) were used to trace the mobilization of the glycinin subunits. The rice glutelin antibodies were highly specific for their respective subunits and showed very little cross-reaction with other seed proteins. Unlike the previous study, results from the current study reveal that the basic glycinin subunit is also subjected to proteolytic degradation as early as 2 DAI resulting in a truncated intermediate approximately 2 kD smaller than the native basic subunit. The truncated basic glycinin subunit is presumably degraded by proteinase that yields no recognizable intermediates.

Growth of seedlings can be grouped into two types, epigeal and hypogeal, based on the fate of the cotyledons following germination. In seedlings exhibiting epigeal growth (*Phaseolus vulgaris, G. max*), the cotyledons are raised above the soil and become foliate and photosynthetic. In seedlings showing hypogeal growth (*Vicia faba*), the cotyledons remain beneath the soil. The spatial pattern of storage protein mobilization has been examined in several legumes including *Phaseolus vulgaris* L. (Opik, 1966), *Phaseolus aureus* Roxb. (Harris and Chrispeels, 1975), *Pisum arvense* L. (Smith and Flinn, 1967), *Vicia faba* L. (Briarty *et al.*, 1970), *Vicia sativa* L. (Tiedemann *et al.*, 2000) and *Brassica napus* L. (Tiedemann *et al.*, 2000). There appears to be no relationship between the pattern of initiation of storage protein mobilization and the type of seedling growth (epigeal vs. hypogeal). For example, in *P. vulgaris* the break down of storage proteins starts throughout the cotyledons, but in cells furthest from the vascular bundles. This is in contrast to the situation seen in soybean. Immunohistochemical analysis presented in this study indicates that the breakdown of storage proteins was initiated near the vascular strand, similar to the situation encountered in *V. faba* (Briarty *et al.*, 1970). However, additional information on the spatial and temporal pattern of storage protein mobilization is required from different legumes, in order to establish correlation between the pattern of initiation of storage protein mobilization and the type of seedling growth.

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