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Differential Proteolysis of Glycinin and β-Conglycinin Polypeptides during Soybean Germination and Seedling Growth[1]

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ABSTRACT

The degradation of the major seed storage globulins of the soybean (*Glycine max* [L.] Merrill) was examined during the first 12 days of germination and seedling growth. The appearance of glycinin and β-conglycinin degradation products was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cotyledon extracts followed by electroblotting to nitrocellulose and immunostaining using glycinin and β-conglycinin specific antibodies. The three subunits of β-conglycinin were preferentially metabolized. Of the three subunits of β-conglycinin, the larger α and α′ subunits are rapidly degraded, generating new β-conglycinin cross-reactive polypeptides of 51,200 molecular weight soon after imbibition of the seed. After 6 days of growth the β-subunit is also hydrolyzed. At least six polypeptides, ranging from 33,100 to 24,000 molecular weight, appear as apparent degradation products of β-conglycinin. The metabolism of the glycinin acidic chains begins early in growth. The glycinin acidic chains present at day 3 have already been altered from the native form in the ungerminated seed, as evidenced by their higher mobility in an alkaline-urea polyacrylamide gel electrophoresis system. However, no change in the molecular weight of these chains is detectable by sodium dodecyl sulfate-polyarylamide gel electrophoresis. Examination of the glycinin polypeptide amino-termini by dansylation suggests that this initial modification of the acidic chains involves limited proteolysis at the carboxyl-termini, deamidation, or both. After 3 days of growth the acidic chains are rapidly hydrolyzed to a smaller (21,900 molecular weight) form. The basic polypeptides of glycinin appear to be unaltered during the first 8 days of growth, but are rapidly degraded thereafter to unidentified products. All of the original glycinin basic chains have been destroyed by day 10 of growth.

During the development of the dicot seed on the mother plant, storage molecules, particularly starches, proteins, and triglycerides, are laid down in the seed. These reserves are mobilized during germination and seedling growth to supply the energy and metabolic intermediates needed by the seedling prior to the establishment of photosynthetic autotrophism. In the legume seed a relatively large fraction of these reserves, on a weight basis, is composed of storage proteins.

The major legume storage proteins are the globulins legumin and vicilin. In the soybean (*Glycine max* [L.] Merrill) these proteins are called glycinin and β-conglycinin, respectively. Much work has been done on the structure, biosynthesis, and genetics of these soybean proteins (15, 18, 20), as well as on their degradation by mammalian trypsin (10–12). However, essentially no work has been done on their degradation *in vivo* during germination and seedling growth. We have previously demonstrated that the Bowman-Birk and Kunitz trypsin inhibitors in the soybean cotyledon are degraded during seedling growth (25), and that at least initially this degradation proceeds via a pathway of limited specific proteolysis with the accumulation of distinct intermediate species (9, 17). In this paper we demonstrate that the soybean glycinin and β-conglycinin are also subjected to limited specific proteolysis during germination with the production of identifiable intermediates.

MATERIALS AND METHODS

Reagents. Nitrocellulose membrane (0.2 μm porosity, BA83) was from Schleicher and Schuell, Keene, NH. Affinity-purified goat anti-rabbit IgG[2]-horseradish peroxidase conjugate and 4-chloro-1-naphthol were purchased as parts of the BioRad Immun-Blot Kit, while DEAE-Affigel Blue was from the same source. Coomassie Blue (Serva Blau G) and twice crystallized acrylamide were from Serva Fine Biochemicals, Inc. (Westbury, NY). Ultrapure urea was from Bethesda Research Laboratories (Gaithersburg, MD). SDS (95% w/w) and polyvinylpolypyrrolidone were from Sigma Chemical Co. Eupergit C oxirane-acrylic beads manufactured by Rohm Pharma GMBH, Weiterstadt, FRG, were obtained from Accurate Chemical and Scientific Corp., Westbury, NY.

All pH adjustments were made at room temperature (21 ± 1°C). Doubly-distilled H_2O was used throughout.

Plant Growth and Preparation of Extracts. Soybean seeds, *Glycine max* (L.) Merrill cv Amsoy 71, were obtained from May Seed and Nursery Co., Shenandoah, IA. The seeds were hand sorted to remove damaged seeds, and planted in moist vermiculite. Germination and growth was carried out in a 12 h, 25°C/12 h, 20°C light/dark cycle under fluorescent lighting. The plants were watered as required with distilled H_2O. After the desired period of growth the plants were rinsed free of vermiculite, and the cotyledons dissected from the axes and seed coats. The cotyledons were frozen at −20° C until needed.

Extracts were prepared from partially thawed cotyledons. Cotyledons, 5 g, were homogenized with 5 g insoluble polyvinylpolypyrrolidone (previously hydrated and washed with distilled H_2O) and 25 ml of 50 mM Na phosphate + 0.25 M NaCl + 0.3 mM phenylmethylsulfonyl fluoride + 0.5 mM Na iodoacetate (pH 7.0). The mixture was filtered through cheesecloth, and centrifuged at 34,800*g* for 1 h. The supernatant was collected by decanting through glass wool to trap the floating liquid, and immediately frozen in aliquots at −20°C.

Electrophoresis. SDS-PAGE was carried out by the method of Laemmli (16) in 1.5 mm thick gels with 12.5% (w/v) separating

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[2] Abbreviations: IgG, immunoglobulin; PE-, *S*-pyridylethylated.

gels and 4% (w/v) stacking gels. Gels were stained for protein with Coomassie blue G (0.1% (w/v) in 50% (v/v) methanol + 10% (v/v) acetic acid and destained by leaching in 12% (v/v) 2-propanol + 10% (v/v) acetic acid.

The alkaline-urea gel system of Kitamura and Shibasaki (13) was used to separate the acidic chains of PE-glycinin. The separation of the basic chains of PE-glycinin was carried out in the acidic-urea gel system described by Kitamura *et al.* (14) with the exception that the gel was not pre-electrophoresed prior to loading of the samples. In both cases the gels were stained as described above.

For quantitation, gels stained with Coomassie blue were scanned using a Hoefer model GS300 scanning densitometer. Samples of purified glycinin and β-conglycinin were used to establish the range in which staining intensity was linearly proportional to protein content, and sample sizes adjusted to be in this range.

Electrophoretic Blotting and Immunostaining. The transfer of separated protein bands from SDS-PAGE gels to nitrocellulose membrane was carried out as described by Towbin *et al.* (28). The blots were stained for protein with amido black. Alternatively, duplicate blots were subjected to immunostaining using purified specific rabbit IgGs, goat anti-rabbit IgG-horseradish peroxidase conjugate, and 4-chloro-1-naphthol as the chromogenic substrate. The instructions of the Bio-Rad Immun-Blot kit (27) were followed with the exception that Tween-20 was omitted from the wash buffers.

Purification of Glycinin and β-Conglycinin. Defatted soybean meal was prepared by extracting 50 g of freshly ground meal with four 100 ml portions of petroleum ether at room temperature, followed by air drying overnight. The defatted meal was extracted with cold 0.1 M Na phosphate + 0.4 M NaCl + 10 mM 2-mercaptoethanol (pH 7.0) (7.5 ml/g meal). After stirring for 30 min at 4°C, the mixture was filtered through cheesecloth and centrifuged at 35,000*g* for 20 min at 4°C. The clarified extract was dialyzed against 35 mM Na phosphate + 0.2 M NaCl + 10 mM 2-mercaptoethanol (pH 7.0), and then recentrifuged. Solid $(NH_4)_2SO_4$ was added to 85% saturation, and the mixture stirred at 0°C for 1 h.

The precipitated protein was collected by centrifugation as above, redissolved in a minimal volume of 50 mM Na phosphate + 0.2 M NaCl + 10 mM 2-mercaptoethanol (pH 8.0), and extensively dialyzed against the same buffer. The resulting crude storage protein preparation was then subjected to zonal isoelectric precipitation chromatography as described by Derbyshire and Boulter (4). The elution of the column was monitored by SDS-PAGE and A_{280}. The first peak eluting from this column consisted of a mixture of β-conglycinin and some glycinin, as well as some Kunitz soybean trypsin inhibitor which eluted at the tailing edge of this peak. The second major peak consisted of glycinin which was judged to be free of other proteins (including the β subunit of β-conglycinin) by SDS-PAGE. The purified glycinin was dialyzed against 5 mM ammonium bicarbonate, lyophilized and stored as a powder at −20°C. To obtain purified β-conglycinin, the material from the first zonal isoelectric precipitation peak was applied to a 2.5 × 35 cm HA-Ultrogel column equilibrated to 30 mM K-phosphate (pH 7.6) at 4°C. The column was eluted at 25 ml/h with a linear gradient of 400 ml of 30 mM K-phosphate (pH 7.6) and 400 ml of 1 M K-phosphate (pH 7.6). The purified β-conglycinin peak (as determined by SDS-PAGE) was dialyzed against 5 mM ammonium bicarbonate, lyophilized, and stored as a dry powder at −20°C.

PE-glycinin was prepared from purified glycinin by the method of Moreira *et al.* (19), as were the individual acidic chains of PE-glycinin. The nomenclature of Moreira *et al.* (19) was used for the acidic and basic chains of glycinin, and the nomenclature of Thanh and Shibasaki (26) was used for the subunits of β-conglycinin. The F2 acidic component was found to be very minor in Amsoy 71, comprising only 3% of the acidic chains (based on A_{280}), while A1, A2, A3, and A4 corresponded to 31, 25, 13, and 27%, respectively, of the total acidic chains in this cultivar.

Production of Specific Antibodies. Solutions of glycinin, PE-glycinin, and β-conglycinin were prepared in 1.5 mM K-phosphate + 0.15 M NaCl (pH 7.4). Aliquots of these solutions were mixed with Freund's complete adjuvant in a ratio of 2:1 (v/v) and injected subcutaneously in the back and thigh of New Zealand White rabbits. This initial injection contained 1.8 mg glycinin or 0.7 mg of PE-glycinin or 2.5 mg of β-conglycinin. Ten d later the rabbits received a second injection in the thighs of the same protein dosage but with Freund's incomplete adjuvant. The rabbits were bled and the serum tested for the presence of the appropriate antibodies. When positive, the rabbit was bled from a marginal ear vein on 3 consecutive d. If the initial test for antibodies was negative, the rabbit was again injected in the thigh with the protein and incomplete adjuvant 10 d after the second injection. These three injections were the maximum needed to elicit an immune response in the rabbits used. The rabbits were boosted every 3 months in the same manner and bled 1 week later. Antisera from rabbits immunized with glycinin or PE-glycinin were negative for reaction with β-conglycinin by Ouchterlony double-diffusion analysis. Antisera from rabbits immunized with β-conglycinin were negative for reaction with glycinin or PE-glycinin. For immunostaining of blots the appropriate IgG fractions of the antisera for PE-glycinin or β-conglycinin were produced by $(NH_4)_2SO_4$ fractionation (8) followed by DEAE-Affigel Blue (Bio-Rad) chromatography according to the manufacturer's instructions.

The β-conglycinin-specific antibodies were further fractionated by affinity chromatography. A preparation of α + α′ subunits free of the β subunit was prepared by the method of Thanh and Shibasaki (26), and covalently linked to Eupergit C beads according to the manufacturer's instructions. This affinity matrix was used to prepare α + α' specific antibodies.

RESULTS

Changes in Storage Protein Polypeptides. Examination of extracts of germinating soybeans readily demonstrates the degradation of the polypeptides of the storage proteins glycinin and β-conglycinin during germination and seedling growth (Figs. 1 and 2). The catabolism of glycinin can be discerned in the disappearance of its acidic and basic chains. The acidic chains decrease after d 3, with less than 40% of the initial level of acidic chains remaining by d 6 (Fig. 2). In contrast, there is no observable decrease in the basic chains of glycinin until after d 8. A similar differential metabolism of the polypeptides of β-conglycinin is observed (Figs. 1 and 2). Both the α′ and α subunits have largely disappeared by d 6, while no decline in the β subunit is noted before d 6.

At the same time that the polypeptides of the native storage globulins are disappearing a number of polypeptides appear and/or increase in intensity (Fig. 1; Table I). Most notable are bands 9–12, which appear and rapidly increase after d 4, band 13, which appears on d 3 and peaks in abundance on d 6, and band 4, which increases and peaks on d 4. The appearance of a number of new polypeptides concomitant with the disappearance of the polypeptides of the native storage globulins suggest that the former represent degradation products of the latter. However, it must be borne in mind that the cotyledons of the soybean become enlarged and deep green in color, implying that they become active photosynthetic organs. Newly appearing polypeptides might thus represent parts of this new photosynthetic apparatus rather than any storage globulin-derived degradation products. We therefore utilized the specificity of antibodies to allow the

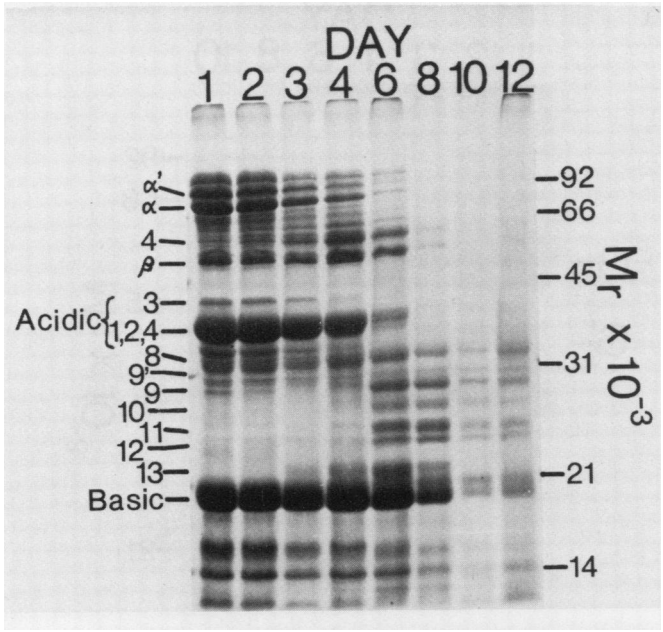

FIG. 1. SDS-PAGE of extracts of the cotyledons from germinating soybeans. Electrophoresis in a 12.5% slab gel was carried out in the Laemmli system and stained for protein with Coomassie blue G. The α', α, and B subunits of β-conglycinin and the acidic basic polypeptides of glycinin are indicated, as are those peptides which appear or increase during seedling growth (4, 8, 9′, 9, 10, 11, 12, and 13). Samples equivalent to 0.008 cotyledons (d 1–4, or 0.006 cotyledons (d 6, 8, 10, 12) were applied.

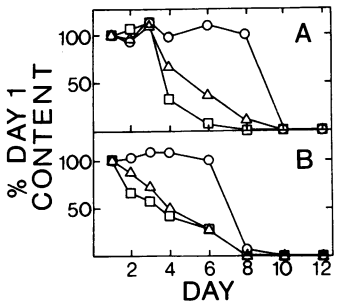

FIG. 2. Variation of the glycinin and β-conglycinin polypeptides during seedling growth. Gels such as that in Figure 1 were scanned as described in the text. The reported values have been corrected for the varying amounts of extract applied to the gel and have been normalized to the content of each polypeptide at d 1 equal to 100%. Frame A, glycinin chains; (○), basic chains; (△), A1 + A2 + A4 acidic chains; (□), A3 acidic chain. Frame B, β-conglycinin chains: (○), β; (△), α; (□), α'.

identification of the latter.

Characterization of β-Congylcinin Degradation. The separated polypeptides from SDS-PAGE gels were electrophoretically transferred to nitrocellulose membranes to allow immunochemical characterization of the newly appearing polypeptides. Immunostaining with anti-β-conglycinin IgG followed by the horseradish peroxidase conjugate of goat antirabbit IgG (Fig. 3) revealed the intact α', α, and β subunits in the d 1 extract. Five species of mol wt intermediate to the α and β subunits were also weakly stained, as was a band with a slightly higher mol wt than band 8. Very weak staining occurred at the position of the glycinin basic chain band. With growth and germination there is a gradual decrease in the immunostaining of the α' and α subunits first with these subunits disappearing by d 8. The β subunit appears to persist relatively unchanged to at least d 6, after which it rapidly disappears also. The immunochemical

Table I. *Major Polypeptides that Appear or Increase in Prevalence during Seedling Growth*

Gel Band[a]	Mol Wt	Immunological Cross-Reaction[b]	
		Glycinin	Conglycinin
4	51,200	±	++
8	33,100	−	+++
9′	31,300	+	+
9	29,800	+	++
10	27,500	±	+
11	25,700	−	+
12	24,000	−	±
13	21,900	+++	±

[a] Nomenclature as in Figure 1. [b] Relative scale of reaction by immunostaining with anti-glycinin and anti-β-conglycinin antibodies. +++, Very strongly reactive; ++, strongly reactive; +, reactive; ±, weakly reactive; −, not reactive.

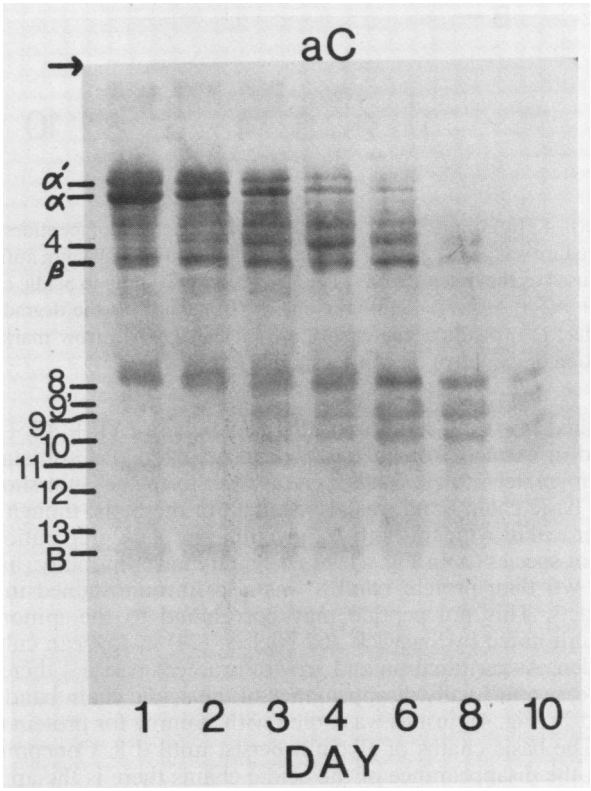

FIG. 3. Immunostaining of β-conglycinin cross-reactive polypeptides. SDS-PAGE was carried out as in Figure 1, and the separated polypeptides transferred to nitrocellulose paper. The 'Western' blot was then stained using rabbit anti-β-conglycinin antibody as the first antibody. The α', α, and β subunits of β-conglycinin are indicated, as are the immunoreactive degradation products, bands 4, 8, 9′, 9, 10, 11, 12, and 13. The arrow marks the position of the top of the separating gel.

results thus correlate well with the protein staining results (Figs. 1 and 2). Conglycinin-specific immunostaining corresponding to polypeptide band 4 increases after d 1, peaking in intensity on d 4. Subsequent to the appearance of this species anti-conglycinin staining appears and increases corresponding to bands 8, 9′, 9, and 10, and to a lesser extent 11 and 12. By d 8 species 8, 9′, 9, and 10 are the major β-conglycinin cross-reactive material remaining in the cotyledon extract.

Characterization of Glycinin Degradation. Staining of blots with anti-PE-glycinin IgG (Fig. 4) as the first antibody followed by horseradish peroxidase conjugated to goat anti-rabbit IgG

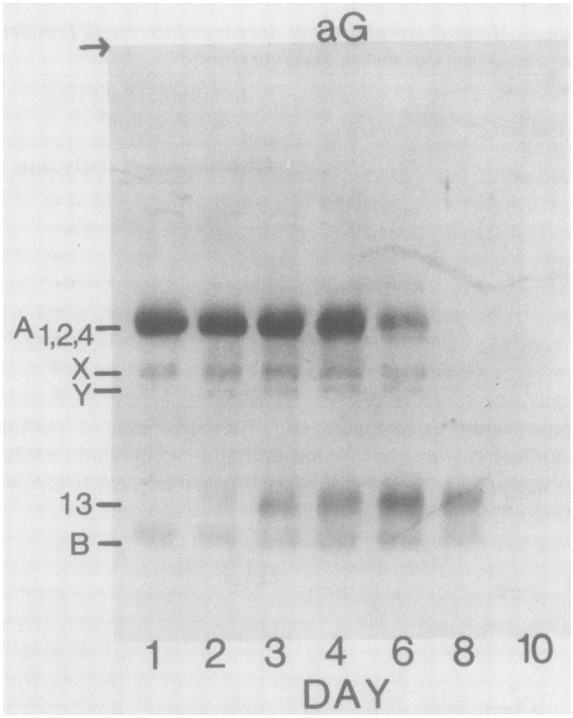

FIG. 4. Immunostaining of glycinin cross-reactive polypeptides. The procedure was as in Figure 3, except that rabbit anti-PE-glycinin antibody was used as the first antibody. The bands corresponding to acidic chains A1 + A2 + A4 (A_{124}) and basic chains (B), as well as the degradation product (13) of the acidic chains, are indicated. The arrow marks the position of the top of the separating gel.

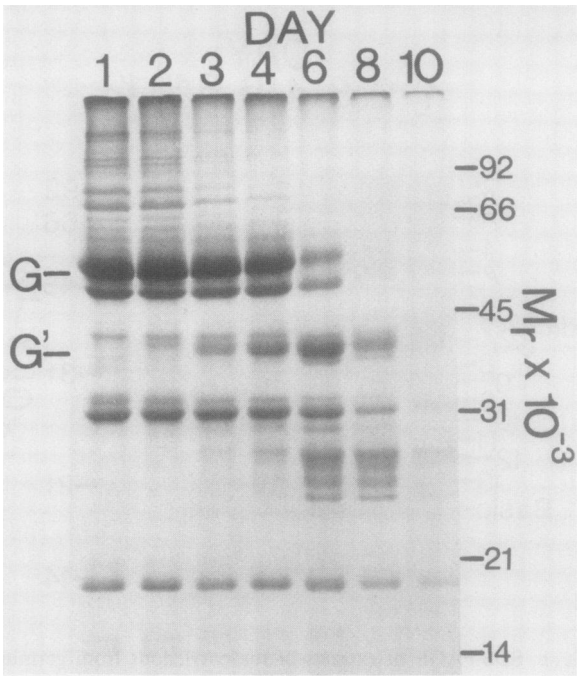

FIG. 5. SDS-PAGE of extracts from soybean seedling cotyledons under nonreducing conditions. Electrophoresis was identical to that in Figure 1, with the exception that the samples were heated in sample buffer without 2-mercaptoethanol before electrophoresis. G, glycinin acidic chain-basic chain disulfide-linked pairs; G', glycinin cross-reactive species appearing during seedling growth.

resulted in strong staining of the acidic chain (A1 + A2 + A4) band in extracts from d 1 to 4. The A3 band was also stained when greater protein loads were applied to the gel (not shown). The basic chain band was also stained in the blots, though to a lesser extent with this anti-PE-glycinin antibody preparation. A minor species (X in Fig. 4) with a slightly lower mobility (higher mol wt) than protein band 8 was also immunostained in d 1 extracts. This polypeptide may correspond to the minor A6 subunit noted by Staswick and Nielsen (24) in soybean cultivar Raiden. As germination and growth proceeds past d 4 there is a decrease and finally disappearance of the acidic chain band and band X (Fig. 4), just as was noted with staining for protein (Fig. 1). The basic chains of glycinin persist until d 8. Concomitant with the disappearance of the acidic chains there is the appearance and increase of the glycinin-specific immunostaining of band 13. A very minor species (Y in Fig. 4) with a mol wt somewhat less than band 8 was also observed to appear after d 1 and to peak in abundance at approximately d 4.

The data presented above suggest that the first observable degradation of glycinin occurs by the proteolysis of the acidic chains to yield the band 13 polypeptide(s), with no observable degradation of the basic chains. Assuming for the moment that band 13 and the basic chain are disulfide-linked, as the native acidic and basic chains are to form the native glycinin subunit, one would predict the appearance of a partially degraded subunit with a mol wt of approximately 42,000 (21,900 mol wt band 13 + 19,500 mol wt basic chain). The following experiments indicate that this is indeed the case.

SDS-PAGE of the extracts was carried out under nonreducing conditions (Fig. 5). The major protein band at d 1 that was found to be reactive with anti-glycinin antibodies migrated with an apparent mol wt of 50,500 (band G). This is comparable to that expected for the native subunits of glycinin, the acidic chain-basic chain disulfide-bonded complexes (*e.g.* A2B1a, A1aB2, and A3B4 [20]). A second lesser band, mol wt approximately 30,000, also immunostained for glycinin. This probably corresponds to the A5B3 disulfide-bonded complex (20). As growth progresses both G and 30,000 mol wt species decline, and largely disappear by d 6. At the same time, a new immunochemically cross-reactive species, G', with an apparent mol wt of 40,000 appears and increases until d 6. The only other immunologically cross-reactive species present at this time are the remaining traces of G and the 30,000 mol wt species. Two-dimensional SDS-PAGE (not shown) was carried out under nonreducing conditions in the first dimension (cylindrical gel), and under reducing conditions in the second (slab gel) dimension. Under these conditions glycinin (G) is resolved, as expected, into the acidic and basic chains. G' is resolved into the basic chain and band 13 after the second reducing dimension. The hypothesis that an early degradation product of glycinin is the disulfide-linked basic chain-band 13 subunit is thus confirmed.

The SDS-PAGE systems used above do not resolve the different types of glycinin acidic chains (except A3 from A1 + A2 + A4) from each other. Likewise the multiple basic chain forms are not differentiated. To examine the fate of individual types of acidic and basic chains during germination and growth we isolated the glycinin fraction from soybeans at 0, 3, and 6 d after the initiation of imbibition using the methods described in "Materials and Methods." After reduction and *S*-pyridylethylation the acidic chains were separated in the alkaline-urea electrophoresis system. Purified individual acidic chains from *S*-pyridylethylated native glycinin were used as standards. Densitometric scans of the gels are shown in Figure 6.

The A1 chain migrates as a doublet poorly resolved from A2. A3 and A4 are clearly separated from A1 and A2 and each other. While the glycinins from d 3 cotyledons appear identical in chain composition to the glycinin in ungerminated seeds in the SDS-PAGE system, they are clearly different in the alkaline-urea

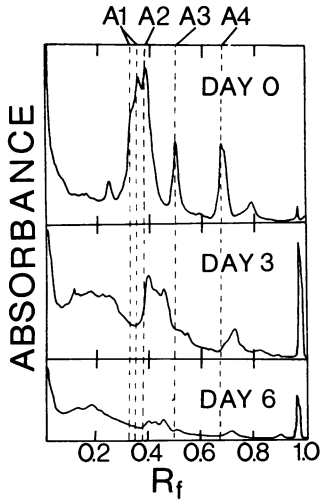

FIG. 6. Densitometric scans of alkaline-urea PAGE of the glycinin fractions isolated from cotyledons of soybeans grown for 0, 3, and 6 d. The gels were run as described in "Materials and Methods." The positions of the A1, A2, A3, and A4 acidic chains, as identified by purified standards, are indicated.

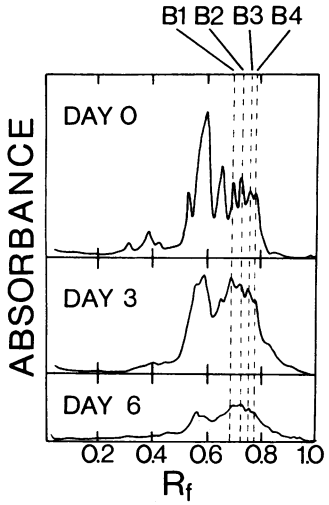

FIG. 7. Densitometric scans of acidic-urea PAGE of the glycinin fractions from cotyledons as in Figure 6. The positions of the B1, B2, B3, and B4 basic chains, as identified from purified standards, are indicated.

PAGE system. By d 3 all of the original acidic chains have disappeared. In their place at least four new acidic chain forms have appeared which have somewhat higher electrophoretic mobilities. That these species are indeed different from A1–A4 was unambiguously shown by mixing samples of the d 0 and d 3 PE-glycinin chains. In this case, the electropherogram showed the number of bands expected from the sum of the d 0 and day 3 bands (not shown). The acidic chains of d 6 PE-glycinin appeared to be identical to those of day 3, although they are recovered at lower levels from the cotyledons.

The basic chains were examined in a similar manner by acid-urea PAGE and the densitometric scans shown in Figure 7. Unlike the acidic chains, the S-pyridylethylated basic chains of glycinin show no obvious changes in electrophoretic mobility during the first 3 d of seedling growth, and indeed the same basic chain species appear to be present even at d 6 of growth.

Examination of the PE-glycinin from d 0, 3, and 6 by the dansylation technique of Gray (5) revealed only the amino-terminal residues expected for undegraded glycinin acidic chains A1–A4 and basic chains B1–B4 (19)—glycine, leucine, phenylalanine, isoleucine, and arginine.

DISCUSSION

The data presented above show that, at least initially, the proteolytic degradation of glycinin and β-conglycinin during germination proceeds via limited specific proteolysis (29). During this first phase of mobilization there is an accumulation of discrete modified forms of these storage proteins. The results also demonstrate that the different polypeptide chains of the two storage globulins are degraded at significantly different rates.

During the first 3 d after the initiation of imbibition the acidic chains of glycinin are rapidly modified, as reflected in their increase in mobility in the alkaline-urea PAGE system. In this same time period there is no obvious change in mol wt of these polypeptides as determined by SDS-PAGE. This suggests that if proteolysis has occurred, it must only involve the removal of one or a few amino acid residues from either the amino- or carboxyl-terminus (or both). However, the results of dansylation suggest that limited proteolysis at the amino-terminus is unlikely. However, hydrolysis of one or several residues from the carboxyl-terminus cannot be excluded. We have measured considerable carboxypeptidase activity (with carbobenzyloxy-phenylalanyl-alanine as substrate at pH 6.0) in the ungerminated soybean (KA Wilson, unpublished data) although we have not established the subcellular localization of this activity. An alternative explanation is a deamidation of one or more asparaginyl or glutaminyl residues in the glycinin acidic chains. Such a deamidation of storage proteins during germination has been suggested in a number of earlier studies (3, 23). No change in the electrophoretic mobility of the glycinin basic chains (in the acid-urea system) is noted for at least the first 6 d of growth.

After 3 d of growth overt proteolysis of the glycinin acid chains occurs, with the production of band 13 polypeptide. In this process the acidic chains are reduced in mol wt by approximatley 14,000. It is not clear which of the acidic chains band 13 is derived from. The degraded acidic chains (band 13) remain tightly bound to the apparently undegraded basic chains by disulfide bonding, as evidenced by SDS-PAGE under nonreducing conditions (Fig. 5). After d 8 the basic chains of glycinin are degraded, as are the band 13 polypeptides. No intermediates in this or subsequent phases of the glycinin degradation were detectable with the methodology used.

The pattern of overt proteolysis of glycinin in the soybean is similar to that found with legumin degradation in vetch (*Vicia sativa*) (21), *i.e.* the acidic chains of the globulin are initially degraded with no obvious attack on the basic chains. A similar pattern of degradation has been observed by Hara *et al.* (7) for the legumin-like storage protein of the pumpkin (*Cucurbita moschata*). It therefore seems likely that the preferential degradation of the acidic chains of legumin-like globulins is a generalized phenomenon in dicots utilizing this type of storage protein.

The degradation of β-conglycinin during storage protein mobilization exhibits a similar preferential proteolysis of some polypeptide chains over others. In this case, the α and α′ subunits, are first degraded. By d 6, the α and α′ subunits have largely disappeared. At the same time band 4, which may represent a degradation product of the α and/or α′ subunits (or both), increases in prominence. Between d 6 and 10 the band 4 polypeptide(s) are further degraded, apparently producing bands 8, 9′, 9, 10, and possibly 11 and 12. After d 6 the β subunit is also degraded (Fig. 2). It is not obvious what product polypeptides are produced from this subunit, although they may correspond to one or more of bands 8, 9′, 9, 10, and 11, as no new bands appear upon the degradation of the β subunit (*i.e.* after d 6). Since the anti-(α + α′ subunit) subpopulation of our anti-β-conglycinin antibodies cross-reacts with the β subunit, this prob-

lem cannot yet be resolved. We have not identified any additional distinct β-conglycinin-cross reactive degradation products past this point.

Autolysis of extracts of ungerminated soybean var Williams at pH 8.0 has been demonstrated by Bond and Bowles (1). They found that the α′ and α chains of β-conglycinin and the acidic chains of glycinin were rapidly destroyed, while the β subunit of β-conglycinin and the basic chains of glycinin were not apparently affected. The activity involved was identified as a neutral or alkaline metalloendopeptidase. We have unfortunately not been able to demonstrate any such degradation in the extracts of ungerminated soybean var Amsoy 71.

The initial preferential degradation of the acidic chains (compared to the basic chains) of glycinin noted above is similar to the pattern of polypeptide degradation in the germinating vetch and pumpkin seeds. In both of these systems sulfhydryl-dependent proteinases are responsible for the acidic chain degradation (2, 6, 22). We do already know that such sulfhydryl-dependent proteinases are active in protein degradation in the soybean cotyledon. At the same time that the proteolysis of the glycinin acidic chains occurs the Kunitz soybean trypsin inhibitor is also subjected to a specific proteolysis near its carboxyl-terminus (9). We have demonstrated that the proteinase involved is sulfhydryl dependent (P Hartl, KA Wilson, unpublished data).

Characterization of the enzymes involved, as well as their times of appearance during germination and seedling growth, will explain the patterns of proteolysis we have demonstrated here. This information will provide further insight into the control of the mobilization of protein reserves in the seed.

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