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Short Communication

Degradation of β-Conglycinin in Early Stages of Soybean Embryogenesis[1]

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DONNA M. SHUTTUCK-EIDENS AND ROGER N. BEACHY*
Plant Biology Program, Department of Biology, Washington University, St. Louis, Missouri 63130

ABSTRACT

This study focuses on the role of protein turnover in the accumulation of the storage protein β-conglycinin (7S protein) during soybean embryogenesis. The results of pulse:chase experiments using ^{3}H-leucine indicate that the turnover of the subunits of β-conglycinin by proteolysis is more rapid in early stages of cotyledon maturation than in later stages.

The seeds of soybean, *Glycine max*, contain approximately 45% protein at maturity, of which about 70% is in the form of the storage proteins, β-conglycinin (7S protein) and glycinin (11S protein). This study focuses on the turnover of β-conglycinin during embryogenesis. β-Conglycinin is composed of three glycosylated subunits, α′, α, and β of apparent mol wt of 76, 72, and 53 kD, respectively. These subunits have been shown to accumulate to high levels during a discrete period in seed maturation (7). The expression of the genes encoding these subunits has been proposed as a model system for the study of the developmental regulation of a gene family (3, 4, 7).

Previous studies have shown that the accumulation of 7S storage proteins follows the appearance of large amounts of messenger RNA (mRNA) in the cytoplasm of cotyledon cells (7). However, we made the observation that translatable mRNA was present in immature embryos 2 to 3 d before the protein was first detected by immunological methods. The inability to detect protein concurrent with the appearance of mRNA could be due to a lag in the translation of the mRNA or to the instability of the protein product. Madison *et al.* (5) reported no measurable degradation of soybean storage proteins in experiments using soybean cotyledons cultured as described by Thompson *et al.* (9). In those experiments, Madison *et al.* monitored the distribution of ^{3}H-glycine labeled proteins after fractionating seed extracts on sucrose density gradients. Here we present the results of pulse:chase experiments using cotyledons taken from seeds at different stages of maturation. Our analysis of immunoprecipitated proteins by SDS-PAGE indicates that the turnover of 7S storage protein is more rapid in early stages of cotyledon maturation than in later stages. We propose that the lability observed *in vitro* reflects the fact that this storage protein becomes increasingly stable during seed maturation. By using SDS-PAGE of the labeled and immunoprecipitated proteins we were able to detect degradation of storage proteins. Madison *et al.* (5) analyzed fractions from sucrose density gradients and were unable to detect degradation. The role of this delayed stability in storage protein accumulation is discussed.

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MATERIALS AND METHODS

Plant Material. Soybean plants, *Glycine max* cv Provar, were grown in the greenhouse as described by Meinke *et al.* (7). Embryos of the appropriate size were selected by looking through developing pods held up to a bright light source.

Cotyledon Culture and *in Vitro* Labeling. Embryos were removed from pods by axenic techniques and size selected according to the length of the seed. Table I, abbreviated from Meinke *et al.* (7), summarizes the developmental stages considered in this study. After 24 h in culture medium, separated cotyledons without the embryonic axis were placed flat side down on 0.2 ml solution of ^{3}H-leucine (2.5 mCi/ml, 15–30 Ci/mmol) in growth media (9) and incubated in a controlled environment at 24°C. After the labeling period the cotyledons were rinsed in distilled water, blotted dry, and placed in 1.5 ml microfuge tubes to be weighed and quick frozen in liquid N_2. Samples from which the radioactivity was to be chased were transferred to 10 ml of growth media containing 4 mM leucine in a 50-ml flask. The flask was placed on a rotary shaker set at 100 rpm. Cotyledons removed after the chase period were also blotted dry and placed in microfuge tubes to be weighed and frozen. When large cotyledons were weighed, the part of the tissue that did not come in direct contact with the label was removed and discarded so that only a 2 to 3 mm slice was further processed.

Extraction of Seed Globulin Fractions. Proteins were extracted by grinding single thawed cotyledons in five parts (w:v) of 0.035 M K-phosphate buffer (pH 7.6), 0.4 M NaCl, and 0.01 M 2-mercaptoethanol. The extract was clarified by a 10-min spin in

Table I. *Timetable for the Early Stages of Seed Development in Glycine max cv Provar*

Each stage is defined by a specific range in seed lengths. Seeds mature approximately 60 d after anthesis.

Stage	Seed Length	Seed Wt	Time after Anthesis
	mm	*mg*	*d*
G	4.6–5.4	10–25	14–16
H	5.6–6.4	25–45	16–18
I	6.6–7.4	45–65	17–19
J	7.6–8.4	65–85	18–20
K	8.6–9.4	85–120	19–21
L	9.6–10.4	120–160	20–22
M	10.6–11.4	160–200	21–23
N	11.6–12.4	200–250	>24
O	11.6–12.4	250–300	>28

a microfuge. The protein concentration of the sample was determined by the method of Bradford (1).

SDS-PAGE. Proteins were heated in boiling water in the presence of (cracking buffer) (0.5 M Tris-HCl [pH 6.8], 2% SDS, 20% glycerol, and 1% 2-mercaptoethanol) before electrophoresis in a discontinuous polyacrylamide slab gel system. The gels were 5% acrylamide in the stacking gel and 10% acrylamide in the separating gel (6).

Immunoprecipitation of 7S Protein. Extract containing 15 to 20 μg of protein was added to 0.9 ml of solution A (150 mM NaCl, 10 mM Tris [pH 8.3], 10 mM Na_2EDTA, and 1% Triton X-100). To this, 5.5 μl of aprotinin (26 trypsin inhibitor units/ml) and 5.5 μl of a polyclonal anti-7S antibody (7) were added. Incubation was carried out at room temperature for 12 h, then at 4°C for 16 to 18 h. One hundred μl of a 10% w/v cell suspension of *Staphylococcus aureus* cell walls (IgGsorb, Enzyme Center, Inc., Boston, MA) was then added and incubated at room temperature for 2 h with occasional mixing. The immunoprecipitate was collected by a 2- to 3-min spin in the microfuge. The pellet was washed three times in solution A and heated in the presence of 2-mercaptoethanol and SDS before electrophoresis through polyacrylamide.

Fluorography. Fluorography of polyacrylamide gels containing ^{3}H-labeled proteins was carried out using the procedure described by Jen and Thach (2).

RESULTS AND DISCUSSION

The accumulation of storage proteins in developing soybean seed cv Provar has previously been described (7). We extended that description by labeling the proteins in cultured cotyledons. Using the criteria described by Meinke *et al.*, developing embryos from stages H to O were aseptically dissected from the pod and the cotyledons were placed in culture media containing ^{3}H-leucine. After 6 h of labeling, the salt soluble proteins were extracted and subjected to electrophoresis with or without prior immunoprecipitation with antiserum against β-conglycinin. Figure 1 shows the protein bands detected after fluorography. The predominant immunoprecipitable proteins at all stages of seed development are the α′, α, and β subunits (76, 72, and 53 kD, respectively). The precursor of the α′ and/or α subunit is also prominent, migrating in this gel between the α′ and α subunits at an apparent mol wt of 74 kD. Other polypeptides, with apparent mol wt between 31 and 66 kD also immunoprecipitated from protein extracts of stage H and I embryos, but not from extracts of embryos in late stages of development.

Figure 2 shows proteins labeled in a pulse chase experiment. Only the results of experiments with cotyledons in stage I and stage L are shown for simplicity. Labeling with ^{3}H-leucine was carried out for 1 h followed by chases of 2, 4, 6, and 12 h. The label in the precursor polypeptide appears to move primarily into the α subunit during the chase period. The time required for this processing is less than 2 h for cotyledons at both stages of development. The low mol wt polypeptides (<50 kD) described in Figure 1 are also observed at both stage I and L. However, the timing of the appearance of these low mol wt products is different at the two stages of development. At stage I these polypeptides can be detected within 2 h after labeling. These products are not readily apparent in the stage L cotyledon until 4 to 6 h after labeling. The distribution of radioactivity in these gels, as depicted in the original autoradiograms shown in Figure 2 were analyzed on a Joyce-Loeb densitometer. The polypeptides of apparent mol wt <50 kD represented 40 and 56% of the label in the immunoprecipitated 7S protein in stage I cotyledons after 6 and 12 h of chase, respectively. In stage L cotyledons, however, these polypeptides represented only 5 and 36% of the 7S protein at 6 and 12 h of chase, respectively. The appearance of these low mol wt polypeptides does not directly coincide with the loss of precursor polypeptide and therefore we assume that they are derived from the mature α′ and α subunits. Since the cotyledons used in this experiment were maintained in

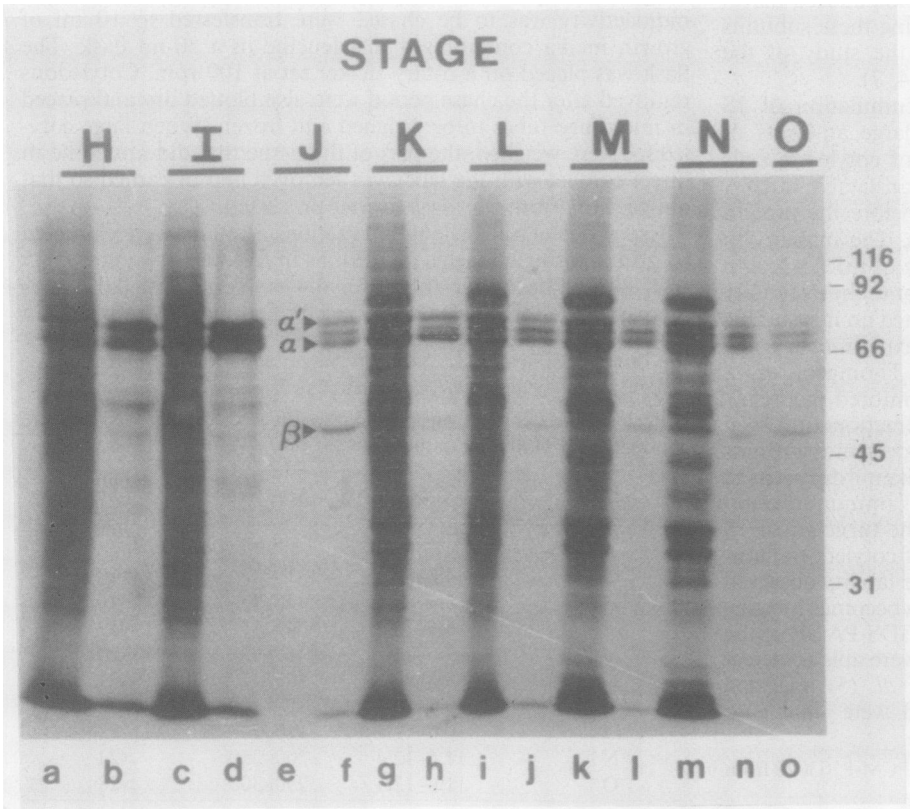

FIG. 1. Proteins labeled with ^{3}H-leucine for 6 h in cotyledons from seeds of increasing maturity. Seeds were developmentally staged as described in Table I. Using the procedures described in "Materials and Methods" labeled proteins were extracted, reacted with antiserum against 7S protein, and immunoprecipitated proteins were resolved by SDS-PAGE. The figure shows the fluorogram of that gel. The letters H–O indicate the maturation stage of the selected cotyledons. Into lanes a, c, g, i, k, and m total protein extracts were loaded. Immunoprecipitated protein was loaded into lanes b, d, f, h, j, l, n, and o. Lane e contained no sample. Mol wt standards in kilodaltons are indicated to the right.

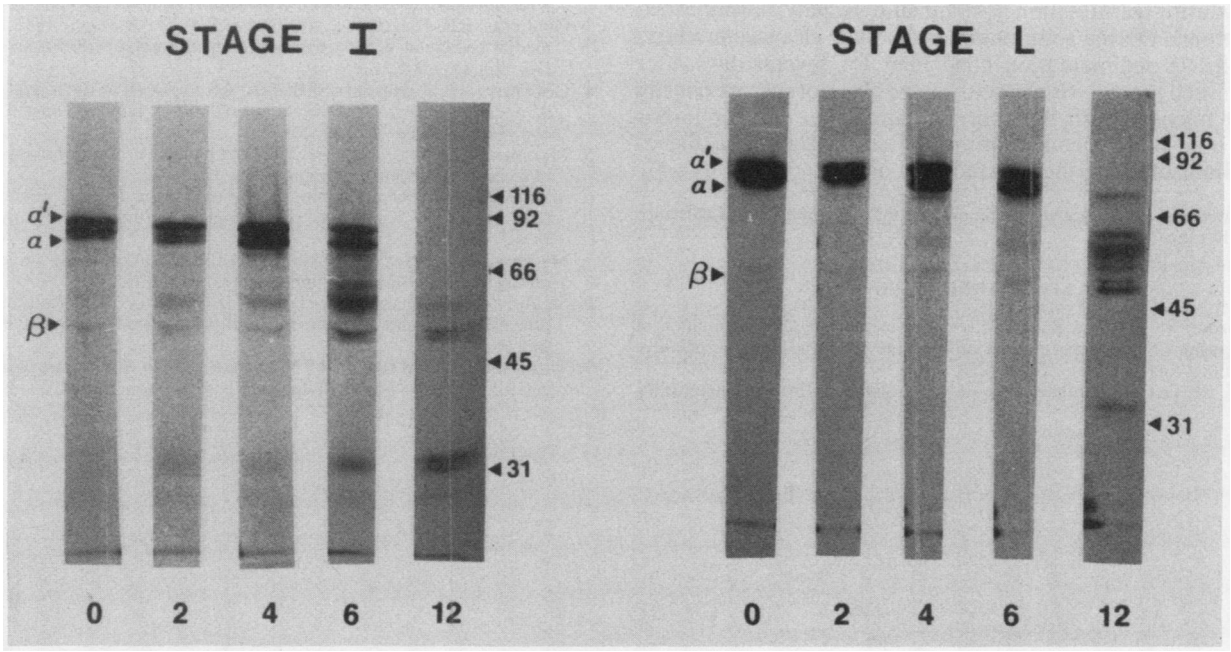

FIG. 2. Cotyledons were pulse labeled with 3H-leucine and chased for different periods of time as described in "Materials and Methods". The numbers at the bottom of each lane indicate the number of hours the cotyledons were in chase medium before the proteins were extracted and reacted with antiserum against 7S protein. The immunoprecipitated proteins were then subjected to SDS-PAGE. The fluorograms of gels with protein from stage I and stage L cotyledons are presented. Mol wt standards are shown in kilodaltons on the right.

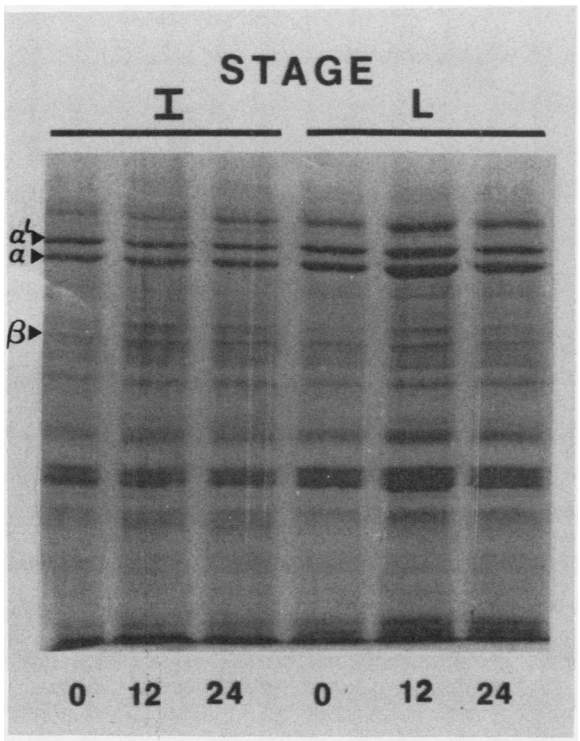

FIG. 3. Total protein extracted from cotyledons held in culture and subjected to electrophoresis in SDS-PAGE and stained with Coomassie blue. Extracts of cotyledons at maturation stages I and L are presented. The numbers at the bottom of each lane refer to the number of hours the cotyledons were held in culture before the proteins were extracted.

growth media for 24 h before the labeling period, we do not believe that this degradation of the storage protein is the result of protein turnover induced by the culture condition. This premise is supported by Figure 3, which shows that the pattern of Coomassie blue-stained proteins on SDS-polyacrylamide gel is essentially constant during the course of the experiment. The degradation does vary with the pH of the media, however, and is enhanced at lower pH (data not shown).

Meinke *et al.* (7) have shown that the mRNA encoding the 7S subunits can be detected at developmental stage G, several days before the protein accumulates in the cotyledon at stage J. Western blot analysis of accumulated proteins does not detect the α' and α subunits of β-conglycinin until stage I (data not shown). We have shown in Figure 1 that the subunits can readily be labeled *in vitro* at stage H indicating that active translation of these mRNAs occurred before accumulation of the protein was detected. These mRNAs can also be found on the polyribosomes at stage H, further indicating that the messages are translated at that developmental stage (data not shown). The degradation we detected in the pulse chase experiments would delay the detection of the subunits of the protein and is consistent with the observed delay between appearance of transcript and the accumulation of protein product. Whether this degradation is the result of the action of a specific protease and whether it is dependent on the subcellular localization of the protein is being further investigated. In any event, these data indicate that the 7S storage protein subunits are not intrinsically stable, and that their stability increases during seed maturation. The previous report on the turnover of soybean storage protein used sucrose density gradient centrifugation to fractionate proteins in an effort to detect movement of radioactivity out of the protein in the 11S, 7S, and 2S fractions (5). Because no loss of label from the protein sedimenting at 7S was detected they assumed that no degradation had occurred. The degradation we report here may occur while β-conglycinin remains in a 7S configuration and would thus not have been detected by this method of analysis. This situation may be analogous to the reported degradation of the 7S storage

protein during germination (Bryant and Beachy, unpublished observations). During germination proteolytic cleavage products retain the 7S sedimentation coefficient for several days after initial limited proteolysis has ocurred. Analysis of sucrose density gradient fractions with SDS-polyacrylamide gel electrophoresis will be used to further determine the configuration of the β-conglycinin subunits when degradation occurs.

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