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Identification of Soybean Glycinin Precursor *In Vivo*

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대두 세포내에서 Glycinin 전구체의 존재 확인

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ABSTRACT

Glycinin is the major storage protein in soybean. It has been known that a molecule of glycinin is composed of 6 subunits, each of which consists of two different kinds of polypeptides, acidic (A) and basic (B) one (MW 39K and 19K, respectively). To study the molecular origin and the relationship of glycinin subunit polypeptides, antibodies against A- and B-polypeptide were obtained by immunizing rabbits with either of the antigens purified by gel filtration and preparative electrophoresis. Each antibody was not only specific for its own antigen polypeptide in soybean but also recognized the precursor which was synthesized *in vivo* and *in vitro*. The polyadenylated mRNAs were isolated from immature seeds and leaves and were translated *in vitro* using wheat germ extract. One of the seed-specific translation products, MW 60K, was identified to be the precursor of glycinin subunit by immunoprecipitation with antibodies against glycinin A- and B-polypeptide. Mature A- and B-polypeptides were not detected in the translate *in vitro*. These results suggest that the precursor polypeptide is synthesized from the mRNA and is cleaved to yield A- and B-polypeptides which form a glycinin subunit in the cell. Glycinin genes were expressed with the maturation of soybean seeds in a tissue-specific and developmental stage-specific manner.

Abbreviations: A-polypeptide, acidic polypeptide of glycinin subunit; B-polypeptide, basic polypeptide of glycinin subunit; MW, molecular weight; K, 1,000 in molecular weight; SDS-PAGE, SDS-containing polyacrylamide gel electrophoresis.

INTRODUCTION

Most of the proteins in soybean seeds can be extracted with a buffered saline solution, which is the globulin fraction (Pusztai and Stewart, 1980). When this protein is separated by density gradient centrifugation, it is resolved into three major fractions with sedimentation coefficients

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of 2S, 7S and 11S (Catsimpoolas *et al.*, 1967; Derbyshire *et al.*, 1976; Hill and Breidenbach, 1974). The 2S band contains a mixture of proteins, including several enzymes and protease inhibitor. The 7S and 11S fractions correspond to the major storage proteins, β-conglycinin and glycinin, respectively. The 11S glycinin fraction is usually the most abundant, and it accounts for 50–60% of the total proteins (Catsimpoolas *et al.*, 1967; Hill and Breidenbach, 1974; Koshiyama, 1969). Neither the 7S nor the 11S fractions, however, are homogeneous.

SDS-PAGE shows that both the 7S and 11S proteins are made up of multiple subunits. β-conglycinin, which is the major form of 7S fraction, contains 3 polypeptides of MW 76K, 72K, and 53K. Each 7S molecule is composed of each copy of these polypeptides, but the combination is variable (Bray and Beachy, 1985; Coates *et al.*, 1985; Doyle *et al.*, 1986; Koshiyama and Fukushima, 1976; Schuler *et al.*, 1982a, 1982b; Thanh and Shibasaki, 1978). The 11S protein glycinin is MW 360K and composed of 6 subunits of average MW 60K (Bradley *et al.*, 1975). Each subunit is composed of a A-polypeptide of MW 37K-45K, whose pI is acidic, and a B-polypeptide of average MW 20K, whose pI is basic. They are linked by a disulfide bond (Catsimpoolas *et al.*, 1972; Kitamura and Shibasaki, 1975; Iyengar and Ravenstein, 1981; Moreira *et al.*, 1979, 1981; Staswick *et al.*, 1981, 1984a, 1984b). These A- and B-polypeptides are heterogeneous in size and amino acid composition. A-polypeptides are designated as A_{1a}, A_{1b}, A_2, A_3, A_4, A_5, and A_6 and B-polypeptides are as B_{1a}, B_{1b}, B_2, B_3, and B_4. Each subunit is composed of a specific combination of each of A- and B-polypeptides such as $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a} (group I), A_3B_4, A_4B_3, A_5B_3 (group II) (Ereken-Tumer *et al.*, 1982; Kitamura *et al.*, 1980; Moreira *et al.*, 1979, 1981; Staswick *et al.*, 1981, 1984a). Within each group there is 85–90% amino acid homology but between groups there is only about 50% homology (Iyengar and Ravenstein, 1981; Moreira *et al.*, 1979, 1981; Staswick *et al.*, 1981).

Although these subunits are synthesized from their own mRNA, the number of mRNA species encoding glycinin subunits may be fewer than that of their subunits. The microheterogeneity of these subunits may result from different post-translational modification, which is quite common in plants. Post-translational modification makes it difficult to identify each subunit and their polypeptides (Fukazawa *et al.*, 1985; Hirano *et al.*, 1984; Marco *et al.*, 1984).

To study the molecular origin and the relationship of glycinin subunit polypeptides, biosynthesis and post-translational modification of glycinin were examined *in vivo* and *in vitro* with antibodies against them in this report. Antibodies made against the polypeptides in seeds make it possible to track down the post-translational modification of the molecules. A primary translation product is synthesized and cleaved to yield A- and B-polypeptide which make a glycinin subunit.

MATERIALS AND METHODS

Materials. Oligo(dT)-cellulose was purchased from Collaborative Research, Inc. and nitrocellulose paper was from Schleicher & Schuell, Inc. Protein A-agarose was purchased from

Bochringer Mannheim, GmbH. [4, 5-^{3}H] leucine was from Amersham.

Isolation of mRNA. Soybean mRNA was isolated by phenol extraction, LiC1 precipitation and chromatography on oligo(dT)-cellulose (Aviv and Leder, 1972). About 2 g of either seeds or leaves of *Glycine max* cv. Paldal 30 days after flowering were ground with 10 ml extraction buffer (50 mM Tris-HC1, pH 8.0; 5 mM EDTA; 100 mM NaCl; 1% β-mercaptoethanol; 2% SDS) and 2 g sea sand in pre-chilled mortar for 2 min. The supernatant was treated with proteinase K (250 μg/ml) for 10 min at 65℃ and was extracted 3 times with equal volume of saturated phenol and once with chloroform. The aqueous phase was adjusted to 0.1 M LiCl, and 2 volumes of EtOH was added. It was placed at -20℃ for 24 h to precipitate nucleic acid. After centrifuge for 10 min at 13,000 *g*, the precipitate was resuspended in 2 ml of buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA), adjusted to 2 M LiCl and RNA was precipitated for 12 h at 4℃. The precipitate was resuspended in 2 ml elution buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA; 0.05% SDS), adjusted to 0.5% SDS, heated for 5 min at 65℃ and quenched. Adjusted to 0.5 M LiCl, it was applied to oligo(dT)-cellulose column. After washing the column with 10 ml binding buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA; 0.5% SDS; 0.5 M LiCl), the mRNA retained by the oligo(dT)-cellulose was eluted with 1 ml elution buffer. Adjusted to 1% SDS, this eluted fraction was heated for 10 min at 65℃, quenched and repeated the oligo(dT)-cellulose chromatography. The mRNA fraction was precipitated with 200 mM NaCl and 2 volumes of EtOH at -20℃ overnight. After centrifuge, the mRNA was resuspended in H_2O to 1 mg/ml and stored at -20℃. By this protocol, 16 μg and 33.5 μg of mRNA from 2 g of leaves and seeds were isolated, respectively.

Translaion *in vitro*. *In vitro* translation was carried out in wheat germ extract. Wheat germ extract was prepared by the method of Roberts and Paterson (1973). *In vitro* translation was carried out in 25 μ1 translation mixture containing wheat germ extract, [4, 5-^{3}H] leucine and the other amino acids for 90 min at 30℃. Proteins synthesized *in vitro* were separated by 12.5% SDS-PAGE. Gels were stained with Coomassie blue and impregnated with 2, 5-diphenyloxazole and fluorography was performed with X-ray film at -70℃ (Laskey and Mills, 1975).

Preparation of antibodies. Purified glycinin A- and B-polypeptides were mixed with equal volume of Freund's adjuvant and injected into rabbits (New Zealand White), separately, 3 times at 2 week interval. Immunized rabbits were bled 2 weeks after the 3rd injection and the serum was processed by the standard method (Garvey *et al.*, 1980).

Immunoprecipitation and immunoblot. Immunoprecipitation was carried out according to the procedure described previously (Choi and Dreyfuss, 1984a). Translation mixture was diluted with 200 μ1 immunoprecipitation buffer (50 mM Tris-HCl, pH 8.0; 100 mM NaCl; 0.5% Triton X-100), 2 μl antibody and 25 μ1 of protein A-agarose. After incubation for 2 h at 4℃, the antigen-antibody and protein A-agarose complex was washed 5 times with immunnoprecipitation buffer. This complex was mixed with 25 μ1 of SDS-PAGE sample buffer and analyzed by SDS-PAGE and fluorography with PPO.

Blotting of proteins from SDS-PAGE gels onto nitrocellulose paper was carrried out by electrotransfer at 0.15 A for 12 h in 50 mM Tris-glycine (pH 9.1) containing 20% methanol (Choi and Dreyfuss, 1984b). The nitrocellulose blot was incubated first with anti-glycinin antibody (1: 50 dilution) and then with [^{125}I] labeled goat anti-ribbit 2nd antibody. It was visualized by autoradiography.

Gel electrophoresis. Protein samples were analyzed by discontinuous SDS-PAGE (Choi and Dreyfuss, 1984a). The separating gel was prepared from a stock of 33.5% acrylamide and 0.3% N, N'-bisacrylamide to a final concentration of 12.5% acrylamide. The separating gel buffer contained 0.38 M Tris-HCl (pH 9.1). The stacking gel was prepared from a stock of 30% acrylamide and 0.4% N, N'-bisacrylamide to a final acrylamide concentration of 4% in 0.125 M Tris-HCl (pH 6.8). Both gels contained 0.1% SDS and were polymerized with ammonium persulfate and N, N, N', N'-tetramethylenediamine. The electrode tank buffer was 25 mM Tris-192 mM glycine containing 0.1% SDS. Samples were prepared by boiling for 3 min in a 0.125 M Tris-HCl (pH 6.8) buffer containing 1% SDS, 5% mercaptoethanol, 10% glycerol, and bromophenol blue.

RESULTS

Purification of glycinin polypeptides. To study the molecular structure of glycinin, it was purified by Sepharose 4B gel filtration and preparative electrophoresis from dry soybean seed crude extract. Two grams of defatted soybean meal were extracted with the extraction buffer (50 mM Tris-HCl, pH 7.5; 0.5 M NaCl) for 30 min with agitation and crude extract was passed through Sepharose 4B column (Fig. 1). Every other fraction was analyzed by SDS-PAGE. Fractions corresponding to MW 360K, which contains mostly glycinin, were

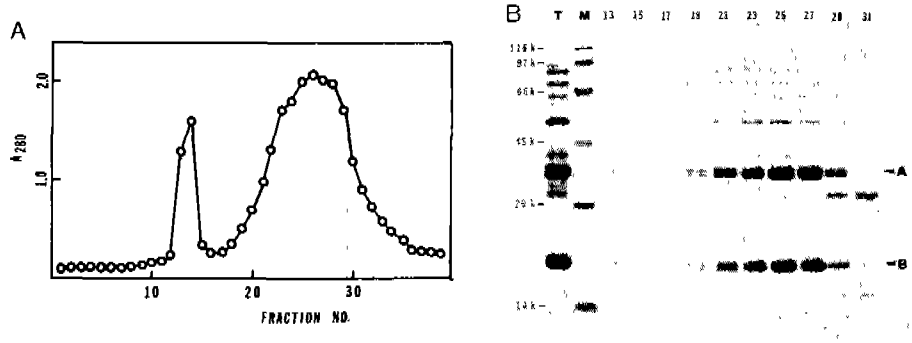
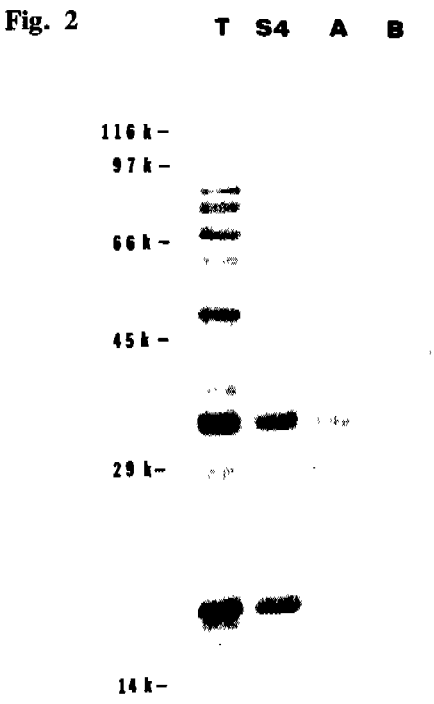


Fig. 1. Gel filtration chromatogram of soybean total extract on Sepharose 4B column. Column dimension was 1.5cm × 40cm and fraction volume was 2 ml each. Fractions indicated were analyzed by SDS-PAGE. Glycinin A- and B-polypeptides are indicated.
Panel A, Absorbance of the gel filtrates at 280nm; panel B, SDS-PAGE analysis of the gel filtrates; lane T, soybean crude extract; lane M, molecular weight markers.

pooled and purified further by preparative SDS-PAGE and band isolation. Excised bands from the gel were extracted and pure A- and B-polypeptide of MW 39K and 19K, respectively, were obtained (Fig. 2). Although there are many kinds of proteins in soybean seed extract, relatively pure glycinin polypeptides were obtained by Sepharose 4B gel filtration (Fig. 2, lane T vs lane S4). The presence of A- and B-polypeptide of much smaller size rather than 360K suggests the multimeric structure of glycinin in soybean seeds (Fig. 1).



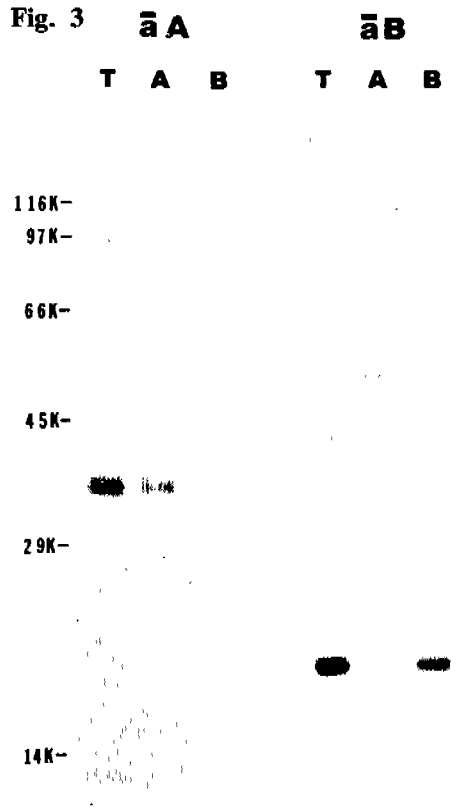


Fig. 2. SDS-PAGE analysis of purified glycinin A- and B-polypeptide. Soybean seed total extract was subjected to Sepharose 4B gel filtration, preparative SDS-PAGE and purified samples were analyzed by 12.5% SDS-PAGE. Each polypeptide was assigned according to the Fontes *et al.*(1984).
Lane T, soybean seed total extract; lane S4, Sepharose 4B gel filtrate; lanes A and B, band isolates from preparative SDS-PAGE.

Fig. 3. Immunoblot analysis of antibodies to glycinin A- and B-polypeptide. Protein samples were separated by 12.5% SDS-PAGE, transferred onto nitrocellulose paper and probed with antibodies indicated.
Panel aA, anti-A polypeptide antibody; panel aB, anti-B polypeptide antibody; lane T, soybean seed total extract; lane A, purified glycinin A-polypeptide; lane B, purified glycinin B-polypeptide.

Fig. 4

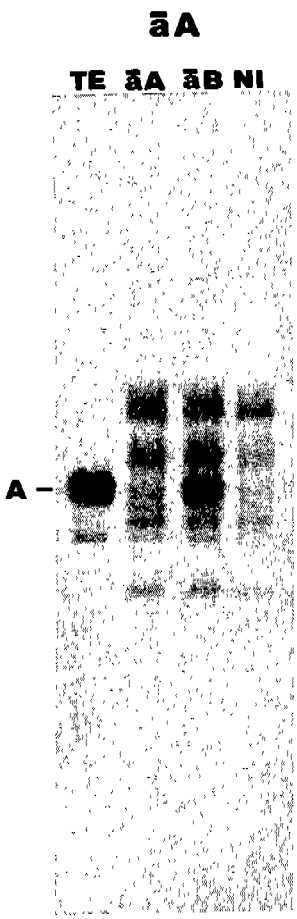


Fig. 5

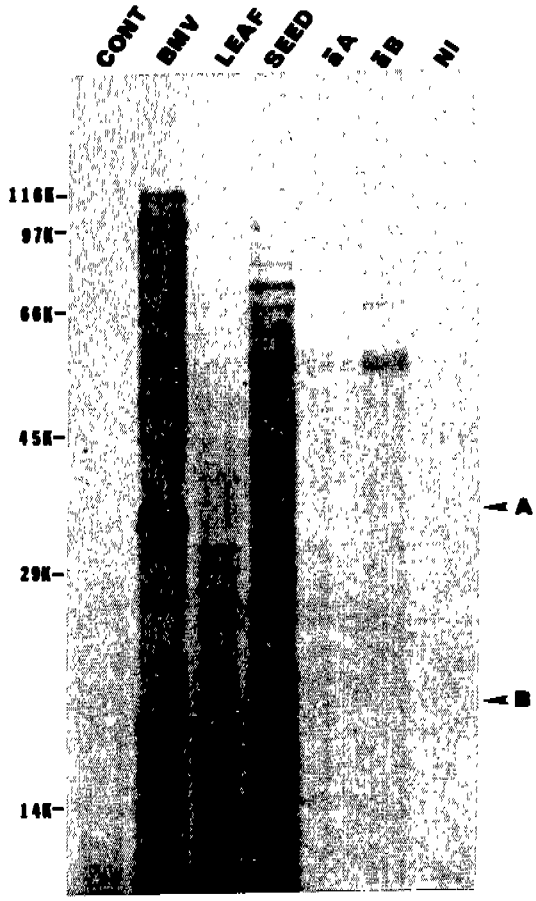


Fig. 4. Immunoblot analysis of immunoprecipitated soybean glycinin. Glycinin was immunoprecipitated with anti-B polypeptide antibody from soybean seed total extract and was analyzed by immunoblot with anti-A polypeptide antibody.
Lane T, soybean seed total extract; lane aA, immunoprecipitate with anti-A polypeptide antibody; lane aB, immunoprecipitate with anti-B polypeptide antibody; lane NI, immunoprecipitate with non-immune rabbit serum.

Fig. 5. *In vitro* translates of mRNAs isolated from soybean. mRNA was isolated by phenol extraction and oligo(dT)-cellulose chromatography and *in vitro* translation was carried out in wheat germ extract. They were separated by 12.5% SDS-PAGE and visualized by fluorography.
[4,5-^{3}H]leucine was used as a label.
The positions of glycinin A- and B-polypeptides are shown by arrow heads.
Lane CONT, no exogeneous mRNA was added; lane BMV, Bromo mosaic virus RNA was added; lane LEAF, soybean seed mRNA was added; lane aA: immunoprecipitation with anti-A antibody; lane aB, immunoprecipitation with anti-B antibody; lane NI, immunoprecipitation with non-immune serum.

SDS-PAGE analysis of the purified glycinin A- and B-polypeptides are shown in Fig. 2 (lanes A and B). These A- and B-polypeptide bands, however, are composed of several tightly spaced bands reflecting the microheterogeneity of the glycinin subunits. According to Fontes *et al.* (1984), A_{1a}, A_{1b}, A_2 and A_4 polypeptides migrate at about the same rate in SDS-PAGE but A_3 migrate slower and A_5 faster than the others suggesting differences in size. A_3 and A_5 polypeptides, therefore, were excluded during the preparative electrophoresis and band isolation. B-polypeptides are so tightly spaced in the gel that they were recovered all together.

Preparation of anti-glycinin antibodies. As molecular probes for glycinin, anti-glycinin antibodies were obtained by immunizing rabbits with either purified A- or B-polypeptide of glycinin separately. Specificity of the anti-glycinin antibodies were demonstrated by immunoblot analysis. Anti-A (Fig. 3, panel A) and anti-B polypeptide antibodies (Fig. 3, panel B) recognize only their own antigen, glycinin A- or B-polypeptide, out of total seed protein extract, respectively. No cross specificity was observed between A- and B-polypeptides, suggesting that they are not related in structure. When the samples were heavily overloaded as in Fig. 6, however, anti-B polypeptide antibody recognizes the A-polypeptide very weakly. These antibodies also recognize the precursor molecule synthesized *in vivo* and *in vitro* as shown in Figs. 5 and 6.

Structure of glycinin subunit. To test the identity of these A- and B-polypeptides of glycinin subunit, glycinin was immunoprecipitated with anti-B polypeptide antibody from crude extract of mature soybean seeds. It was separated by SDS-PAGE and immunoblotted with anti-A polypeptide antibody. As shown in Fig. 4, A-polypeptide was present in the antigen-antibody complex immunoprecipitated by anti-B polypeptide antibody from seed extract. It shows that these A- and B-polypeptides exist in the same glycinin molecule present in seeds. Taken together with gel filtration result of Fig. 1, this result suggests further that glycinin is composed of multiple copies of A- and B-polypeptides and the antibodies made in this experiment are indeed against the glycinin polypeptides. There were some backgrounds common to all 3 immunoprecipitates including control immunoprecipitate with non-immune antibody. This might come from low levels of non-specific antibody in those serums. But the signal above the background was specific and strong enough to show the difference. The anti-A antibody, however, could not immunoprecipitate the glycinin molecule in seed under non-denaturing condition. Although it is not clear at this moment, it seems to result from the effect of tertiary and quarternary structure of glycinin in seeds.

Translation *in vitro* of soybean mRNA. To pursue the molecular origin of A- and B-polypeptide of glycinin, total polyadenylated RNA was isolated from immature soybean seeds by phenol extaction and oligo(dT) chromatography and translation *in vitro* was carried out with it in wheat germ extract. In eukaryotic cells including plant and animal cells, mRNA has been known to have about 200 nucleotides of adenylic acid at 3' end. Though some precursors of mRNA have the poly(A) tail, their relative amounts are very little. The RNA, therefore, isolated in this experiment could be considered as mRNA. The presence and the species of the

mRNA encoding glycinin was examined by translation *in vitro* in wheat germ extract. Fig. 5 shows polypeptides translated *in vitro* in wheat germ extract. They show the synthesis of numerous kinds of polypeptides in the range of MW upto 100K. Background translation by endogeneous mRNA in wheat germ extract was almost none and the translation was quite specific for exogeneous mRNA added. The patterns of proteins synthesized *in vitro* are quite distinct in two different tissue types. The mRNAs encoding the polypeptides of MW 13.5K, 18K, 21.5K, 48K, 59K, 65K, 86K and 90K are present only in seeds, but not in leaves, and of 14K and 30K in leaves *vice versa*. This demonstrates that many genes are expressed in tissue-specific manner.

Neither A- nor B-polypeptide (MW 39K and 19K) was found in the translate. However, those major polypeptides synthesized *in vitro* (Fig. 5, lanes SEED and LEAF) may not be the major proteins present in seeds and leaves. The relative intensity of these bands is related with both the content of [4, 5-^{3}H] leucine in polypeptide used for label during translation *in vitro* and the relative amount of the mRNAs corresponding to those proteins. Even the major proteins synthesized *in vitro* can not be compared to the known proteins in seeds directly on the basis of the mobility in SDS-PAGE. In plant, a lot of proetins are known to be modified post-translationally. These reactions can not be expected to occur identically in translation *in vitro* in wheat germ extract. To overcome these problems, anti-glycinin antibodies were used to characterize the glycinin polypeptide translated *in vitro*.

The presence of the mRNA which codes for glycinin was demonstrated by immunoprecipitation of *in vitro* translates with anti-glycinin antibodies. Both of the antibodies to A- and B-polypeptide of glycinin immunoprecipitate neither A- nor B-polypeptide but the protein of MW 59K at the same time (Fig. 5, lanes A and B). It was not detected by control immunoprecipitation with non-immune serum (Fig. 5, lanes NI). It was immunoprecipitated only from seed mRNA translate but not from leaf mRNA translate (data not shown). The MW 59K polypeptide is much larger than their antigens, A and B polypeptide of MW 39K and 19K, respectively (Fig. 5, lane A and B). It suggests that glycinin subunit is synthesized as a precursor polypeptide of MW 59K containing both A- and B-polypeptide in a single moleucle contiguously. It is converted into the A-polypeptide of MW 39K and the B-polypeptide of MW 19K by endoproteolytic cleavage (Fig. 2). Post-translational processing, however, did not occur in the wheat germ extract system *in vitro*.

The polypeptide of MW 67K is also recognized by anti-B polypeptide antibody (Fig. 5, lane B). It might be the precursor for glycinin subunit A_3B_4. The A_3 polypeptide is known to be larger than the other A polypeptides, which was not included in the antigen preparation. However, the B_4 polypeptide was included in the antigen for anti-B polypeptide antibody, which could immunoprecipitate the precursor for A_3B_4 subunit (Ereken-Tumer, 1982; Fontes *et al.*, 1984).

Glycinin biosynthesis in soybean seeds. The processing pathway of the glycinin subunit was examined in developing soybean seeds. Total soluble seed proteins were extracted with

SDS-PAGE sample buffer from immature seeds at different stages of development and analyzed by immunoblot. Developing soybean seeds were collected and divided into 5 groups by their size assuming that they are at different stages of development. Embryo size was about 5 mm (average 23 mg per seed) for stage I, 8 mm (62 mg) for stage II, 11 mm (110 mg) for stage III, 13 mm (210 mg) for stage IV, and 15 mm (367 mg) for stage V. The seeds at stage V were yellow-green and at their maximum size. The amount of the A- and B-polypeptide in unit weight of seed increases abruptly with the development of seeds, suggesting that the level

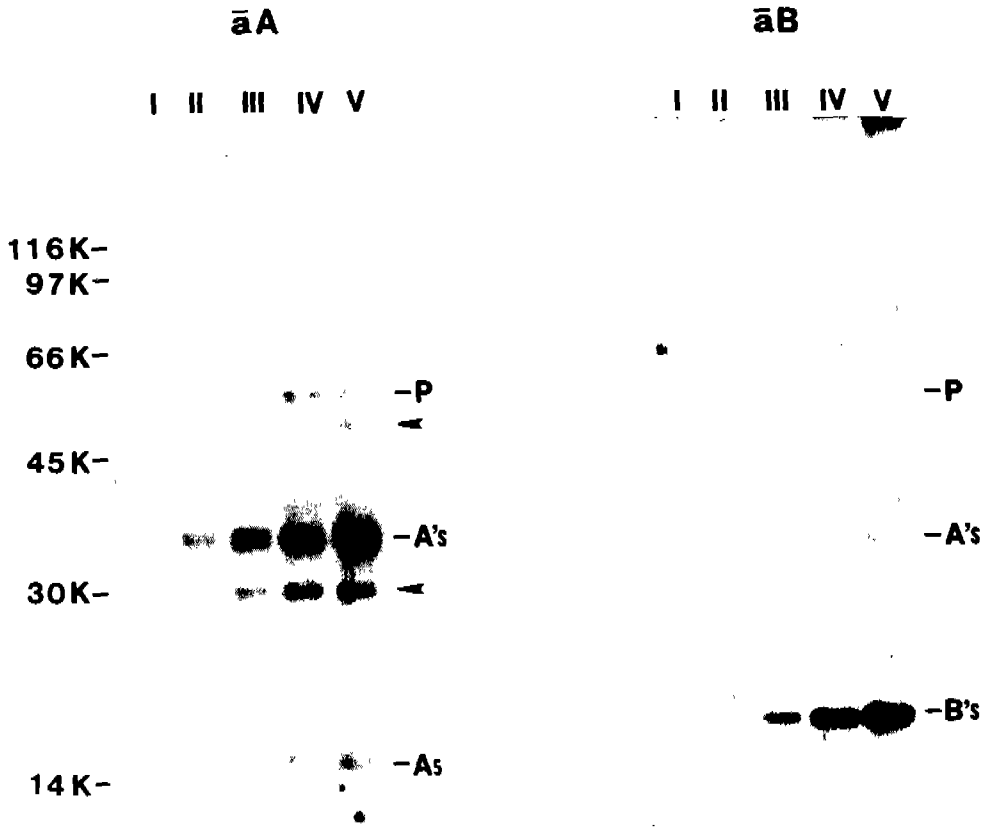


Fig. 6. Immunoblot analysis of glycinin in developing soybean seed. Immature soybean seeds were collected and divided into 5 groups by size. Crude extract was analyzed by immunoblot with anti-glycinin A or anti-glycinin B polypeptide antibody.
Panel aA, anti-A polypeptide antibody; panel aB, anti-B polypeptide antibody; lane I, extract from seed at stage I; lane II, extract from seed at stage II; lane III, extract from seed at stage III; lane IV, extract from seed at stage IV; lane V, extract from seed at stage V.

of glycinin is controlled in a developmental stage-specific manner (Fig. 6). Furthermore, glycinin showed a tissue-specific expression pattern since no glycinin has been found in leaves (data not shown).

The polypeptide of MW 60K was detected in immature seeds, not in dry mature seeds, by antibodies against glycinin A- and B-polypeptides. These results are consistent with the observation made in translation *in vitro* (Fig. 5). They suggest that the polypeptide of MW 60K is the precursor of A- and B-polypeptides. Post-translational endopeptidic cleavage yields 39K and 19K (A- and B-) polypeptides from which a glycinin subunit is made. The amount of the precursor was maximum at mid-maturation stage Ⅳ and decreased in mature seeds at stage Ⅴ reflecting relative rate of protein synthesis and processing with maturation. The biosynthesis rate of storage protein might be slowed down at later stage of development but post-translational processing rate might be relatively constant, which results in the decrease of the amount of the precursor.

There were unknown bands of MW 57K and 36K just below the precursor band and A-polypeptide band, respectively, detected by anti-A polypeptide antibody (Fig. 6, arrow-heads). They were not detected in mature seed extract (Fig. 3). Those might be proteolytic degradation products generated during the extraction from developing immature seeds, in which biological activities were more active than in mature dry seeds.

DISCUSSION

Glycinin is one of the most abundant storage proteins present in soybean protein body, and represents about 50–60% of the total protein (Catsimpoolas *et al.*, 1967; Hill and Breidenbach, 1974; Koshiyama, 1969). Glycinin protein is 360K in MW and composed of 6 subunits. At least 5 differnt kinds of subunits have been known on the basis of differences in size and amino acid composition. Each subunit is composed of an A- and B-polypeptides (Ereken-Tumer *et al.*, 1982; Kitamura *et al.*, 1980; Moreira *et al.*, 1797, 1981; Staswick *et al.*, 1981, 1984a).

To study the biosynthesis mechanism and the origin of subunit polypeptides of glycinin, antibodies against the acidic (A)- and basic (B)-polypeptides were made by immunizing rabbits with either of the purified antigens, separately. Antibody has been recognized as a very useful tool in protein chemistry to study the structure of the antigens and the identification of the derivatives. The post-translational modification and the heterogeneity occur in general in plant storage proteins (Fukazawa *et al.*, 1985; Argos *et al.*, 1982; Hagen annd Rubenstein, 1980; Marks and Larkins, 1982). The heterogeneity appears in various kinds of way such as size of polypeptide, amino acid composition and charge by proteolytic modification or glycosylation. It is not easy, therefore, to identify the translation product *in vivo* and *in vitro* from the mature proteins in the cell from which they are derived.

Antibodies against A- and B-polypeptides of glycinin subunit were highly specific for their own antigens, suggesting that there is little structural similarities each other (Fig. 3). The

presence of A- and B-polypeptides in the same glycinin molecule is shown by immunoprecipitation of glycinin molecules with anti-B polypeptide antibody. Immunoblotting of the immunoprecipitates with anti-A polypeptide shows the presence of A- and B-polypeptide in the same molecule. This also demonstrates the identity of each subunit and thus antibodies against them. These polypeptides, however, are synthesized as a single precursor molecule and subsequent endopeptidic cleavage results in the A- and B-polypeptides.

The presence of A- and B-polypeptide contiguously in the same precursor polypeptide was demonstrated *in vitro* and *in vivo*. mRNA isolated from developing soybean seeds were translated *in vitro* in wheat germ extract. The precursor molecule of MW 60K was immunoprecipitated from the *in vitro* translate with either anti-A or anti-B polypeptide antibodies rather than A- or B-polypeptides of MW 39K and 19K, respectively (Fig. 5). It suggests that A- and B-polypeptide of glycinin subunit is synthesized as a precursor containing them contiguously and post-translational endopeptidic cleavage results in each polypeptide in the soybean seeds. These results were also observed *in vivo*. The precursor of MW 60K was present in immature developing soybean seeds rather than in mature dry seeds (Fig. 3 vs. Fig. 6). However, it was processed into A- and B-polypeptides *in vivo*. It suggests that such a endopeptidic cleavage needs a specific peptidase and/or cellular component such as microsomes which are not present in wheat germ extract. Similar results have been observed in rabbit reticulocyte lysate and *Xenopus* oocyte. Glycinin mRNA was translated in these systems but no endopeptidic cleavage has been observed (Ereken-Tumer *et al.*, 1982).

On the basis of cell fractionation studies in pea (*Pisum sativum*), mRNAs directing the synthesis of both 7S and 11S proteins are translated on rough endoplasmic reticulum (RER). Entering the RER, these are transported to vacuolar membranes where they aggregate and form the dense masses called protein bodies (Chrispeels *et al.*, 1982). It is not until the 11S protein reaches the vacuole where cleavage of the 11S acidic-basic polypeptide complexes takes place, and presumably these polypeptides are associated into an 11S molecule (Chrispeels *et al.*, 1982). Eventually, 11S proteins end up in the protein body (Koshiyama, 1972a, 1972b). Considering the structural similarities to pea storage proteins, soybean glycinin might follow the same pathway in the cells.

cDNA clones for the glycinin subunit A_2B_{1a}, $A_{1a}B_x$, A_3B_4 and $A_5A_4B_3$ have been isolated (Negoro *et al.*, 1985; Fukazawa *et al.*, 1985; Marco *et al.*, 1984; Momma *et al.*, 1985). Structural feature of the glycinin subunit precursor was revealed by the comparison of the deduced amino acid sequences from the nucleotide sequences of these clones to the amino acid sequences of the glycinin subunit polypeptide. Glycinin is synthesized as a precursor of average MW 60K and undergoes post-translational modification by protease to form A- and B-polypeptides. The precursor contains a short signal peptide (24 amino acids for A_3B_4). A-polypeptide is located at the NH_2-terminus of the precursor protein and B-polypeptide is at the COOH-terminus. Between two polypeptide domains, there are linker amino acids (4 amino acids for A_2B_{1a}) (Fukazawa *et al.*, 1985; Marco *et al.*, 1984). But A_5 subunits are known to be formed by further

proteolysis of A4 polypeptide (Iyengar ad Ravenstein, 1981; Staswick and Nielsen, 1983). Therefore, A- and B-polypeptides which form the glycinin subunit are synthesized from the same mRNA and thus a specific combination of subunit polypeptide by a disulfide bond is maintained (Barton *et al.*, 1982; Croy *et al.*, 1980; Erenken-Tumer *et al.*, 1982; Moreira *et al.*, 1981; Staswick *et al.*, 1981, 1984a, 1984b; Tumer *et al.*, 1981).

Glycinin is synthesized predominantly during cell expansion phase of seed development for short period, but not synthesized in other tissues (Figs 5 and 6). The other storage proteins including β-conglycinin are also synthesized in the cotyledons of the developing embryo (Derbyshire *et al.*, 1976; Goldberg *et al.*, 1981a, 1981b; Kuhlemeier *et al.*, 1987; Meinke *et al.*, 1981). The tissue-specific and/or development stage-specific expression patterns have been reported for several cases. The soybean Kunitz trypsin inhibitor, one of the soybean storage proteins, is known to be expressed only in seed but not in leaf. In the case of potato and tomato, the proteinase inhibitors are known to be expressed at very low level in leaves in normal condition. But when their leaves are wounded by insects or other mechanical forces, the proteinase inhibitors are expressed at high level (Lee, 1987). Studying the mechanism of the developmental and tissue-specific expression of glycinin might provide an opportunity to understand the gene expression regulation in eukaryotic cells, especially in plant cells, at molecular level.

적 요

Glycinin은 대두의 주요 저장단백질이다. Glycinin 분자는 6개의 subunit로 이루어져 있으며 각 subunit는 산성(분자량 39K) 및 염기성(분자량 19K) polypeptide로 구성되어 있다. 이러한 subunit polypeptide의 유래와 상호관계를 밝히기 위하여 순수분리한 산성 및 염기성 polypeptide를 각각 항원으로 사용하여 항체를 제조하였다. 각각의 항체는 항원으로 사용한 산성 혹은 염기성 polypeptide에 대하여 특이적으로 반응할 뿐만 아니라 체내 혹은 기내에서 합성된 전구체 분자와도 특이적으로 반응하였다. 대두 잎과 미성숙 종자에서 mRNA를 분리한 후, 밀기울 추출액을 사용하여 기내에서 단백질 합성을 실시하였다. 합성된 단백질에서 대두에 존재하는 산성 및 염기성 polypeptide는 검출되지 않는 반면에 면역침전법에 의하여 분자량이 60K인 전구체가 존재하고 있음을 확인하였다. 이와 같이 glycinin subunit는 mRNA에서 전구체의 형태로 생합성된 후 내부절단에 의하여 산성 및 염기성 polypeptide로 생성되고 이들이 결합하여 subunit를 구성하는 것임을 알 수 있었다. 또한 glycinin유전자는 대두 종자의 성숙과 함께 발현이 되는 것으로 봐 조직 및 분화 특이성 발현형태를 나타내는 것을 알 수 있었다.

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