Molecular Cloning of 11S Globulin and 2S Albumin, the Two Major Seed Storage Proteins in Sesame†

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Insoluble 11S globulin and soluble 2S albumin, conventionally termed α-globulin and β-globulin, are the two major storage proteins and constitute 80−90% of total seed proteins in sesame. Two full-length cDNA clones were sequenced and deduced to encode sesame 11S globulin and 2S albumin precursors, respectively. Deduced amino acid composition reveals that 2S albumin, but not 11S globulin, is a sulfur-rich protein. Three abundant polypeptides of 50−60 kDa were resolved on SDS−PAGE when seed-purified 11S globulin was prepared in nonreducing conditions. Immunological analysis suggests that these three polypeptides are encoded by homologous genes. Immunodetection on the overexpressed protein of the 11S globulin clone in *Escherichia coli* indicates that this clone encodes the precursor protein of one of the three purified 11S globulin polypeptides.

Keywords: *11S globulin; 2S albumin; seed; sesame; sulfur-rich protein*

INTRODUCTION

Plant seeds accumulate massive storage proteins in discrete vesicles termed protein bodies as a source of amino acids for use during germination and postgerminative growth of seedlings. Extensive research has been carried out at the molecular level in the past two decades on seed storage proteins, including studies of their structures, control of synthesis, mechanisms of targeting and deposition, functional properties, and application in genetic engineering (Shewry, 1995; Vasil and Anderson, 1997). On the basis of their solubility in various extraction solvents, seed storage proteins can be classified into four groups: soluble in water (albumins), dilute saline (globulins), alcohol/water mixtures (prolamins), and dilute acid or alkali (glutelins) (Osborne, 1924). The globulins are further divided into two subgroups according to their sedimentation coefficients: 7S vicilin-type and 11S legumin-type globulins (Danielsson, 1949).

The mature 11S globulins with estimated molecular masses ranging from 320 to 450 kDa in diverse species are assembled in a similar structure composed of six subunit pairs that interact noncovalently (Casey et al., 1986). The hexamers contain a random combination of different subunit pairs (Derbyshire et al., 1976). Each of the subunit pairs consists of an acidic subunit (30−40 kDa) and a basic subunit (20−25 kDa) linked by a single disulfide bond. These subunit pairs can be dissociated into individual acidic and basic subunits in the presence of a reducing agent, such as β-mercaptoethanol. The individual acidic and basic subunits of 11S globulins always occur as specific pairs instead of random combinations. This specific pairing results from their mode of synthesis: the two subunits being encoded by a single gene producing a precursor protein of 60 kDa which is cleaved post-translationally only after the formation of an intrachain disulfide bond between the N-terminal acidic subunit and the C-terminal basic subunit (Jung et al., 1997).

Sesame (*Sesamum indicum* L.) has been an important oil crop in Asian countries since ancient times. Sesame seed contains approximately 50% oil and 20% protein and is used for production of edible oil as well as for direct consumption mostly as an additive in food because of its high quality of nutritious content (methionine-rich) and unique flavor associated with the oil. The major protein of sesame is the insoluble 11S globulin, which constitutes 60−70% of the total seed proteins and is conventionally termed α-globulin (Hasegawa et al., 1978; Nishimura et al., 1979; Prakash and Narasinga Rao, 1990). The major soluble protein, 2S albumin, which constitutes approximately 25% of the total sesame proteins is named β-globulin (Rajendran and Prakash, 1988). Though elementary biochemical properties of sesame 11S globulin and 2S albumin have been investigated, no genes encoding these storage proteins have been reported at the present time.

To examine whether the abundant 11S globulin and/or 2S albumin account for the high quality of nutritious content in sesame, we cloned the corresponding cDNA sequences of 11S globulin and 2S albumin precursors. Deduced amino acid sequences indicate that 2S albumin, but not 11S globulin, is a sulfur-rich protein. While three homologous 11S globulin polypeptides were purified from sesame seed, the 11S globulin clone was identified via immunodetection to encode one of the three homologous 11S globulin precursors.

MATERIALS AND METHODS

Plant Materials. Mature and fresh maturing sesame (*Sesamum indicum* L., Tainan1) seeds were gifts from the Crop Improvement Department, Tainan District Agricultural Improvement Station. The mature seeds were soaked in water for 10 min prior to subcellular fractionation of seed proteins.

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† The nucleotide sequence data reported will appear in the GenBank Database under accession numbers AF091841 (2S albumin) and AF091842 (11S globulin).

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The fresh maturing seeds 24 days after flowering were harvested for the construction of a cDNA library.

Subcellular Fractionation of Total Seed Proteins. Mature sesame seeds were extracted with a medium containing 0.6 M sucrose and 10 mM sodium phosphate buffer, pH 7.5 (Peng and Tzen, 1998). The extract was separated into three fractions (pellet, supernatant, and oil bodies) by centrifugation at 10000*g* for 15 min. The pellet was saved for subsequent analysis and the supernatant was centrifuged at 100000*g* for 90 min to yield a 100000*g* pellet and a 100000*g* supernatant. The oil bodies were subjected to further purification according to the protocol developed by Tzen et al. (1997).

Analyses of Protein Contents in SDS−PAGE. To perform a better resolution, proteins in the various fractions of sesame seeds were resolved by glycine SDS−PAGE (Laemmli, 1970) or Tricine SDS−PAGE (Schagger and VonJagow, 1987) using 10% polyacrylamide. The samples were extracted with the sample buffer containing 62.5 mM Tris−HCl, pH 6.8, 2% SDS, 0.02% bromophenol blue, and 10% glycerol with (for glycine SDS−PAGE) or without (for Tricine SDS−PAGE) 5% β-mercaptoethanol according to the Bio-Rad instruction manual. Following electrophoresis, the gels were stained with Coomassie Blue R-250.

Isolation of Total RNA and Poly(A)$^+$ RNA. Total RNA was extracted from the maturing seeds 24 days after flowering ground in liquid nitrogen using the phenol/SDS method (Wilkins and Smart, 1996). Poly(A)$^+$ RNA was isolated with Dynabeads (Dynal) following the manufacturer's instructions. The isolated poly(A)$^+$ RNA was dissolved in DEPC-treated water and then quantitated as the absorbance at 260 nm with a spectrophotometer.

cDNA Library Construction, Screening, and Sequencing. cDNA was synthesized from poly(A)$^+$ RNA according to the protocol described in the manufacturer's instructions (cDNA synthesis, ZAP-cDNA synthesis, and ZAP-cDNA Gigapack III Gold Cloning kits purchased from Stratagene). The majority of cDNA reversely transcribed from the isolated poly(A)$^+$ RNA was found in the range of 1.6−2.2 kb and presumably derived from the highly expressed mRNA of 11S globulin genes. A cDNA library of approximately 10^6 plaques was constructed with 5 μg poly(A)$^+$ RNA. Twelve plaques were selected at random and subjected to in vivo excision of the pBluescript phagemid from the Uni-ZAPXR vector following the manufacturer's instructions. The phagemids were digested with *Xho*I and *EcoR*I. After digestion, the DNA fragments were resolved in a 1% agarose gel. Clones of approximately 1.7 (for 11S globulin) or 0.7 kb (for 2S albumin) were selected for sequenceing using a Sequence Version 2.0 DNA Sequence kit (USB). N-terminal signal sequence was predicted using SignalP program in the World Wide Web Prediction Server Center for Biological Sequence Analysis (Nielsen et al., 1997). Sequence comparisons were performed with the GenBank using the Blast program (Altschul et al., 1990).

Isoelectrofocusing of Sesame 11S Globulin. Isoelectrofocusing was performed in a Bio-Rad Rotofor Cell using the procedure modified by Chuang et al. (1996). The horizontal cylindrical focusing cell of 55 mL was divided vertically into 20 chambers by partitions in order to minimize diffusion during electrophoresis and disturbance of the gradient during fractionation. Each partition was made of monofilament polyester screen with a 6 μm × 6 μm pore size. Fractionation of the contents of each chamber was achieved simultaneously within 1 s by a vacuum harvesting system. The pH gradient, made from 1% ampholyte (0.5% Bio-Lyte 6−8 and 0.5% Bio-Lyte 3−10, both from Bio-Rad), was preformed by applying an electric field at constant power (12 W) at 400−500 V for 10 min. Sesame 11S globulin (the 10 000*g* pellet fractionated from seed extract) containing 200 μg of protein was suspended in 200 μL of 10 mM KCl with or without β-mercaptoethanol and then applied to the gradient at the low pH end (approximately pH 2) of the chamber. The same electric field was applied for 20 min to focus the sample. After electrofocusing, the proteins fractionated in the 20 chambers were harvested. The protein content and pH along the gradient in these 20 fractions were detected at 280 nm with a spectrophotometer and a pH meter, respectively.

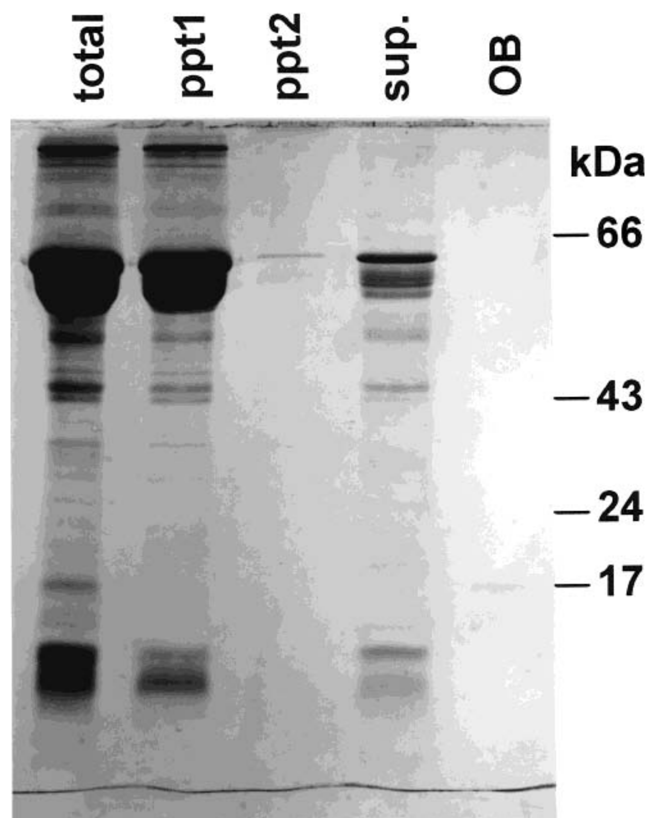

Figure 1. SDS−PAGE of sesame seed proteins fractionated by centrifugation. Total extract of sesame seed proteins was subjected to differential centrifugations to yield a 10000*g* pellet (ppt 1), a 100000*g* pellet (ppt 2), a 100000*g* supernatant (sup.), and an oil body fraction (OB). Total proteins of 20 μg separated into these four fractions were resolved by glycine SDS−PAGE using the sample buffer without β-mercaptoethanol. Labels on the right indicate the molecular masses of protein standards, BSA (66 kDa), ovalbumin (43 kDa), soybean olesoin (24 kDa), and sesame oleosin (17 kDa).

Antibody Preparation and Western Blotting. Antibodies against each subunit of sesame 11S globulin were individually raised in chickens, and immunoglobulins were purified from egg yolks for the immunoassays (Polson, 1990). In the immunoassays, proteins in the Tricine SDS−PAGE gel were transferred onto nitrocellulose membrane in a Bio-Rad Trans-Blot system according to the manufacturer's instructions. The membrane was subjected to immunodetection using secondary antibodies conjugated with horseradish peroxidase (Sigma, St. Louis, MO) and then incubated with 4-chloro-1-naphthol containing H_2O_2 for color development as described by Chen et al. (1998).

Overexpression of the Sesame 11S Globulin Clone in *Escherichia coli*. The full-length cDNA clone of sesame 11S globulin precursor was constructed in the fusion expression vector, pET30b(+) (Novagen), using a *Nde*I site at the initial methionine position and a *Xho*I site in the polylinker of the vectors. The recombinant fusion protein was comprised of an N-terminal appendix of 50 amino acid residues. The recombinant plasmid was used to transform *E. coli* strain BL21 (DE3). Overexpression was induced by 1 mM IPTG in a bacteriophage T7 RNA polymerase/promoter system. Three hours after induction, the *E. coli* cells were harvested, lysed by sonication in 10 mM Tris−HCl, pH 8.0, and then subjected to Tricine SDS−PAGE and Western blot analyses.

RESULTS

Fractionation of Sesame Seed Extract. Sesame seed extract was separated into four fractions (10000*g*

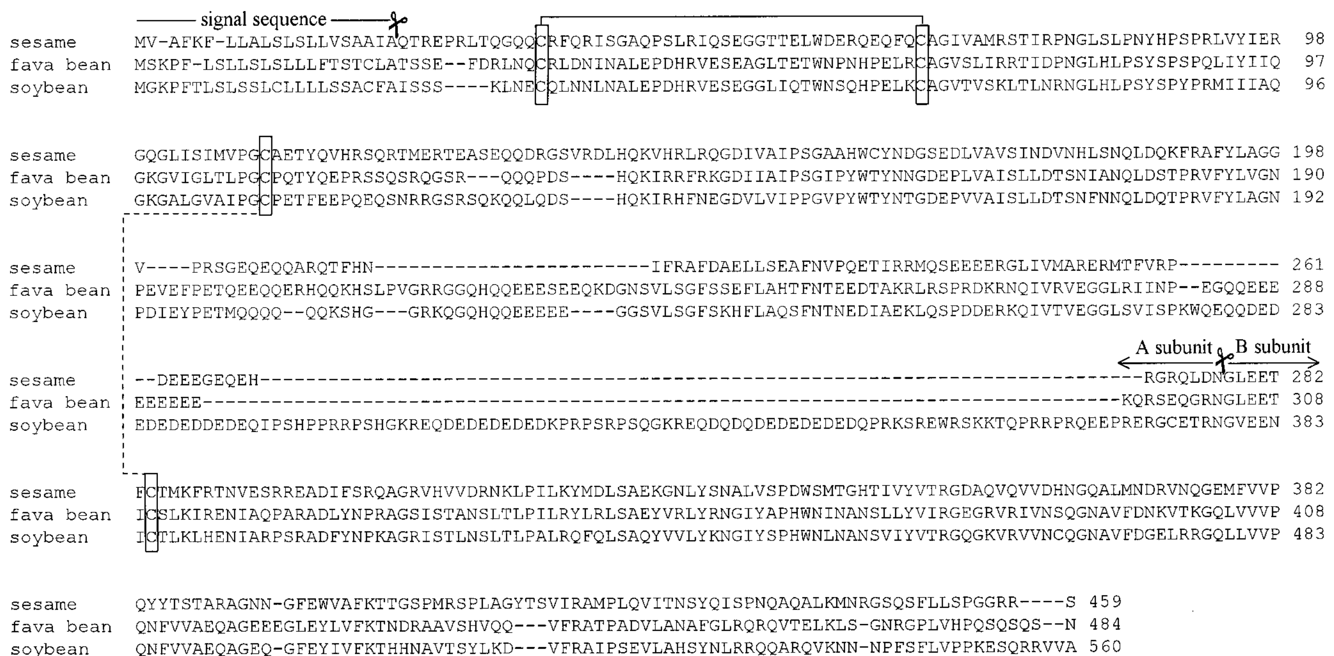

Figure 2. Sequence alignment of precursor proteins of sesame 11S globulin, fava bean legumin (Horstmann, 1983), and soybean glycinin (Staswick et al., 1984). The amino acid number for the last residue in each line is listed on the right for each species. Broken lines in the sequences represent gaps introduced for best alignment. The cleavage site of the putative N-terminal signal sequence and the consensus asparaginyl cleavage site that splits these aligned polypeptides into A (acidic) and B (basic) subunits are indicated by scissor symbols, individually. Four conserved cysteine residues are boxed and predicted to pair as two disulfide bonds; the intrachain disulfide bond in A subunit is linked by a solid line, while the interchain disulfide bridge between A and B subunits is linked by a broken line.

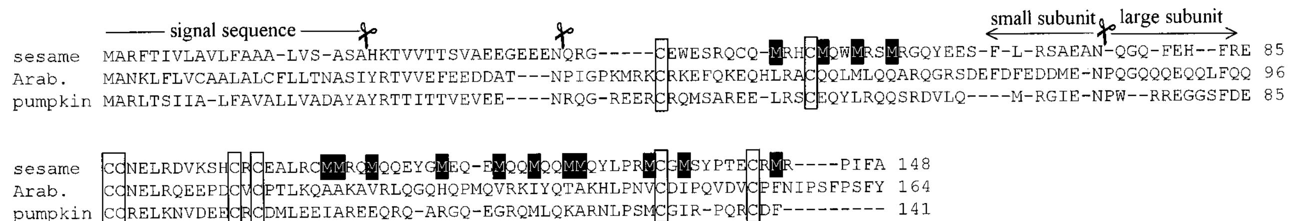

Figure 3. Sequence alignment of 2S albumin precursor proteins of sesame, Arabidopsis (D'Hondt et al., 1993), and pumpkin (Hara-Hishimura et al., 1993). The amino acid number for the last residue in each line is listed on the right for each species. Broken lines in the sequences represent gaps introduced for best alignment. The cleavage site of the putative N-terminal signal sequence and two asparaginyl cleavage sites that split these aligned polypeptides into small and large subunits are indicated by scissor symbols. Eight conserved cysteine residues, which probably form intrachain and interchain disulfide bonds, are boxed. The 15 methionine residues in the predicted mature protein of sesame 2S albumin are highlighted.

pellet, 100000*g* pellet, supernatant, and oil bodies). The proteins in these four fractions were resolved on glycine SDS−PAGE without β-mercaptoethanol (Figure 1). Most proteins were located in the 10 000*g* pellet (ppt1), with its major polypeptides ranging from 50 to 60 kDa. These proteins represented 60−70% of the total sesame seed proteins, in agreement with the 11S globulin (α-globulin) of sesame seeds reported previously (Rajendran and Prakash, 1988). Relatively few proteins were found in the 100000*g* pellet (ppt2). Upon concentration of this latter solution, it was found that the majority of its proteins were also the 11S globulin (data not shown), which probably represented aggregated polypeptides of smaller sizes or contaminants from fractionation. Substantial 11S globulin in a soluble form was also present in the supernatant fraction (sup.). In addition, a relatively abundant protein of molecular mass 13 kDa was co-fractionated in the supernatant. This co-fractionated protein is the soluble 2S albumin (β-globulin), which can be cleaved into two subunits of 9 and 4 kDa when treated with β-mercaptoethanol (data not shown). The two visible proteins of 17 and 15 kDa in the oil body fraction are two isoforms of a unique oil-body protein termed oleosin (Peng and Tzen, 1998).

Sequencing of 11S Globulin and 2S Albumin cDNA Clones from Maturing Sesame Seeds. A full-length cDNA clone (accession no. AF091842) with an open reading frame encoding an 11S storage globulin precursor was obtained and sequenced. The cDNA fragment comprises 1576 nucleotides, consisting of a 14-nucleotide 5′ untranslated region, an open reading frame of 1380 nucleotides, and a 182-nucleotide 3′ untranslated region. The deduced sequence comprises 459 amino acid residues including a putative N-terminal signal sequence of 21 residues (Figure 2). This polypeptide seems to be precisely cleaved at a consensus site by an asparaginyl endopeptidase (Jung et al., 1998) and is split into two subunits of 256 and 182 residues. The deduced molecular masses of the two subunits are 29 389 and 20 300 Da, and their calculated isoelectric points are pH 6.0 and 9.7, respectively. In comparison with two well-studied 11S globulins, legumin of fava bean (Horstmann, 1983) and glycinin of soybean (Staswick et al., 1984), an intrachain disulfide bond

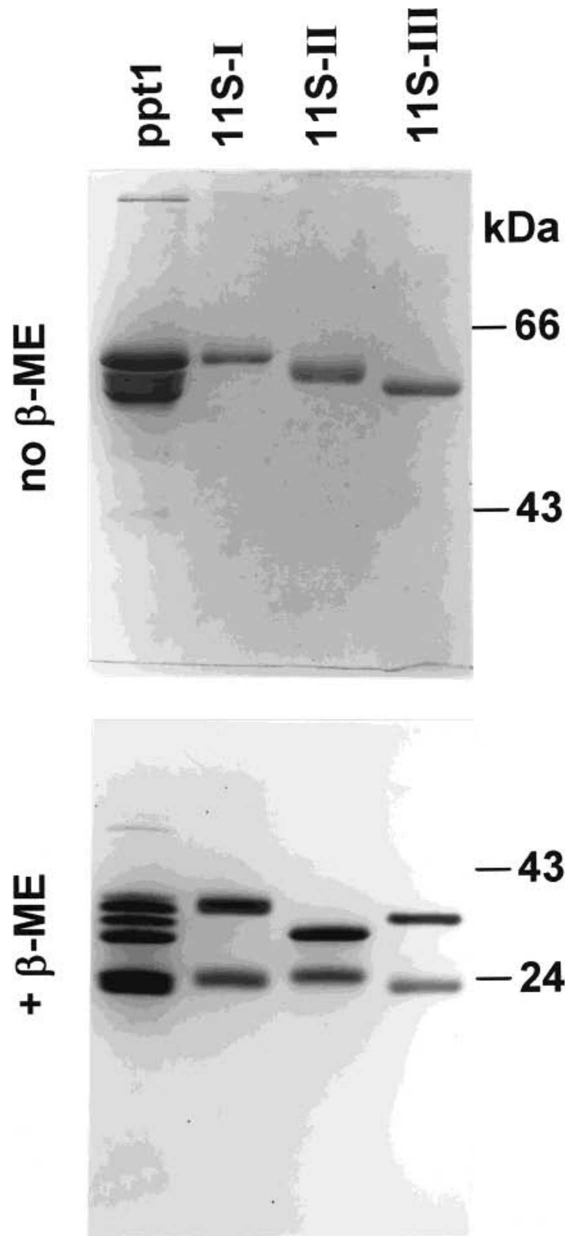

Figure 4. SDS−PAGE of sesame 11S globulin isoforms extracted from the 10000*g* pellet treated with or without β-mercaptoethanol. (upper panel) The 10000*g* pellet (mainly 11S globulin) fractionated from sesame seed extract as well as three purified polypeptides of 11S globulin (11S−I, 11S−II, and 11S−III) were extracted with the sample buffer according to the Bio-Rad instruction manual in the absence of β-mercaptoethanol and then resolved by Glycine SDS−PAGE. In this analysis, 10 μg of ppt1 proteins and 3 μg of each 11S polypeptide were separately loaded in the gel. (lower panel) Duplicated samples (except using 5 μg instead 3 μg of each 11S polypeptide) as described in the upper panel were extracted with the same sample buffer except for the addition of β-mercaptoethanol and then resolved by Tricine SDS−PAGE.

in the acidic subunit and an interchain disulfide bridge between the acidic and the basic subunits are predicted.

A full-length cDNA clone (accession no. AF091841) with an open reading frame encoding a 2S storage albumin precursor was obtained and sequenced. The cDNA fragment comprises 679 nucleotides, consisting of a 42-nucleotide 5′ untranslated region, an open reading frame of 447 nucleotides, and a 190-nucleotide

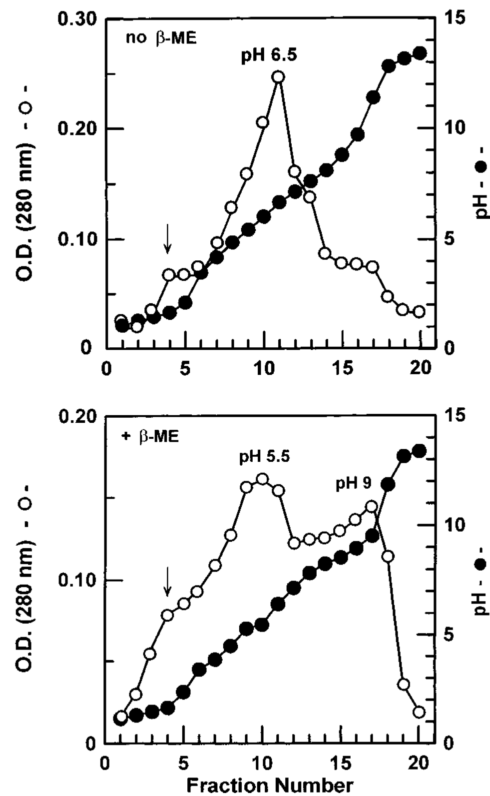

Figure 5. Isoelectrofocusing of sesame 11S globulin in the absence (upper panel) and presence (lower panel) of β-mercaptoethanol. Isoelectrofocusing was performed in a horizontal Bio-Rad Rotofor Cell. The pH gradient was preformed before loading the 10000*g* pellet (mainly 11S globulin) suspended in 10 mM KCl with or without β-mercaptoethanol. The position where the sample was loaded is indicated by an arrow. After electrofocusing, the proteins in the pH gradient were fractionated into 20 tubes. The distribution of proteins detected at OD 280 nm (○) and the pH gradient (●) was recorded for each tube.

3′ untranslated region. The deduced sequence comprises 148 amino acid residues rich in sulfur-containing amino acids; 15 methionine and 10 cysteine residues are found in the predicted mature protein of 110 residues (Figure 3). In comparison with two well-studied 2S albumin precursors in Arabidopsis (D'Hondt et al., 1993) and pumpkin (Hara-Hishimura et al., 1993), the sesame 2S albumin precursor is proposed to possess an N-terminal signal sequence of 21 residues and is split into two subunits of 38 and 72 residues in its mature protein, probably cleaved by the same asparaginyl endopeptidase described in the 11S globulin processing. Eight conserved cysteine residues are present in 2S albumin and may be involved in formation of disulfide linkage.

Multiple 11S Globulin Isoforms Found in Sesame Seed. Under the experimental conditions, three abundant polypeptides of molecular masses 60, 55, and 50 kDa (termed 11S−I, 11S−II, and 11S−III, respectively) were resolved in the 10 000*g* pellet and separately purified to homogeneity (Figure 4). Each of the three polypeptides was cleaved into two subunits (termed A and B) when treated with β-mercaptoethanol, in accord with the acidic subunit (30−35 kDa) and the basic subunit (20−25 kDa) linked by a disulfide bond ubiquitously present in the known 11S globulins of diverse species (Jung et al., 1997).

Isoelectrofocusing of the 11S Globulin. To examine the isoelectric points of subunits of the 11S globulin in sesame seeds, the 10 000*g* pellet fraction of the seed

extract was suspended in 5% β-mercaptoethanol and 10 mM KCl and then subjected to isoelectrofocusing (Figure 5). After isoelectrofocusing, two peaks of protein absorbance were detected at pH 5.5 and 9, respectively. These results confirm the presence of acidic and basic subunits in 11S globulin in sesame. Meanwhile, a single peak of protein absorbance was isoelectrofocused at pH 6.5 when the 10000*g* pellet fraction was suspended in the same buffer without β-mercaptoethanol. The result indicates that the disulfide-bonded polypeptide (11S−I, 11S−II, or 11S−III) of one acidic and one basic subunit in the 11S globulin of sesame displays a resultant isoelectric point lower than pH 7; thus, the protein bodies in sesame seeds are probably negatively charged in their physiological environments.

Immunological Cross-Recognition of the Acidic and Basic Subunits of the 11S Globulin. It has been known that the combined polypeptide of one acidic and one basic subunit in the 11S globulin is encoded by a single gene and post-translationally linked by a disulfide bond prior to enzymatic cleavage of the two subunits (Jung et al., 1997). Each of the three acidic and three basic subunits from the sesame 11S globulin were purified to homogeneity and subjected to antibody production in chickens (Figure 6). The antibodies were used to examine the cross-recognition among these six subunits in the 11S globulin of sesame. The results revealed that immunological cross-recognition was detected among the three acidic subunits or among the three basic subunits but not between these two types of subunits. The data suggest that these three polypeptides are encoded by homologous genes.

Immunodetection of Overexpressed 11S Globulin in *E. coli.* The cDNA clone of 11S globulin precursor was constructed in a fusion vector and then overexpressed in *E. coli*. The overexpressed 11S globulin precursor in *E. coli* was resolved by SDS−PAGE and further detected by immunoassaying using antibodies raised against each of the three acid subunits purified from sesame 11S globulin (Figure 7). The expressed fusion protein containing the 11S globulin precursor was strongly recognized by antibodies against 11S−II and weakly recognized by those against 11S−I or 11S−III (data not shown) in comparison with their recognition to original antigens. The results indicate that the 11S globulin clone encodes 11S−II precursor polypeptide.

DISCUSSION

In this study, we cloned two cDNA sequences encoding precursors of the two major storage proteins, 11S globulin (α-globulin) and 2S albumin (β-globulin), in sesame and found that 2S albumin is a sulfur-rich protein that presumably attributes to sesame nutrition. In the literature, two other homologous sulfur-rich 2S albumin genes were previously reported in Brazil nut (Altenbach et al., 1987) and sunflower (Kortt et al., 1991). These two sulfur-rich 2S albumin genes have been separately transformed into grain legumes (Saalbach et al., 1994) and lupin (Molvig et al., 1997) to improve methionine content in the target crops. However, some seed storage proteins, including the sulfur-rich 2S albumin of Brazil nuts, have been identified as allergens (Nordlee et al., 1996). Recently, a few sesame allergy cases were reported in Europe, in which cases the allergen(s) have not been verified (Kolopp-Sarda et al., 1997). It remains to be studied whether sesame 2S albumin is suitable to serve as a donor protein for

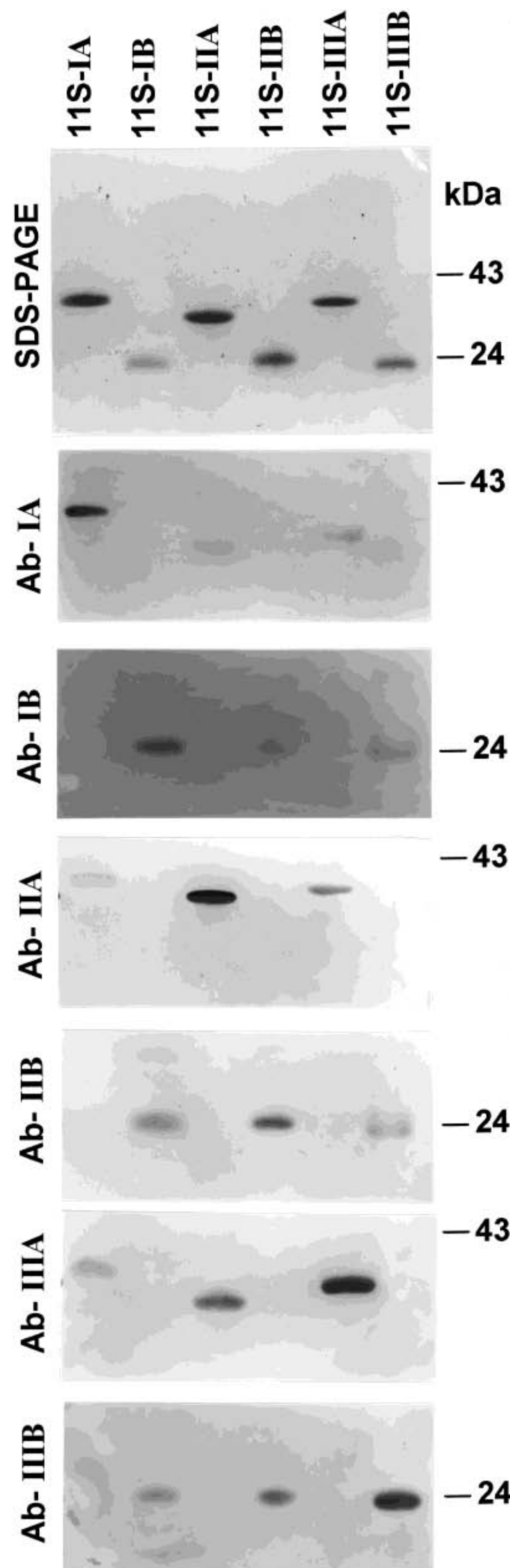

Figure 6. SDS−PAGE and immunological cross-recognition of the six 11S globulin subunits. Tricine SDS−PAGE was performed in the same conditions as those described in Figure 4. Each 11S globulin subunit of 3 μg was loaded in the gel. Replicate SDS−PAGE gels were transferred onto nitrocellulose membranes and then subjected to Western blots using antibodies against each of the three acidic (11S−IA, 11S−IIA, and 11S−IIIA) and the three basic (11S−IB, 11S−IIB, and 11S−IIIB) subunits. In each immunoassay, one-tenth of the protein content was loaded in the sample well containing the original antigen to avoid the interference of over-reaction.

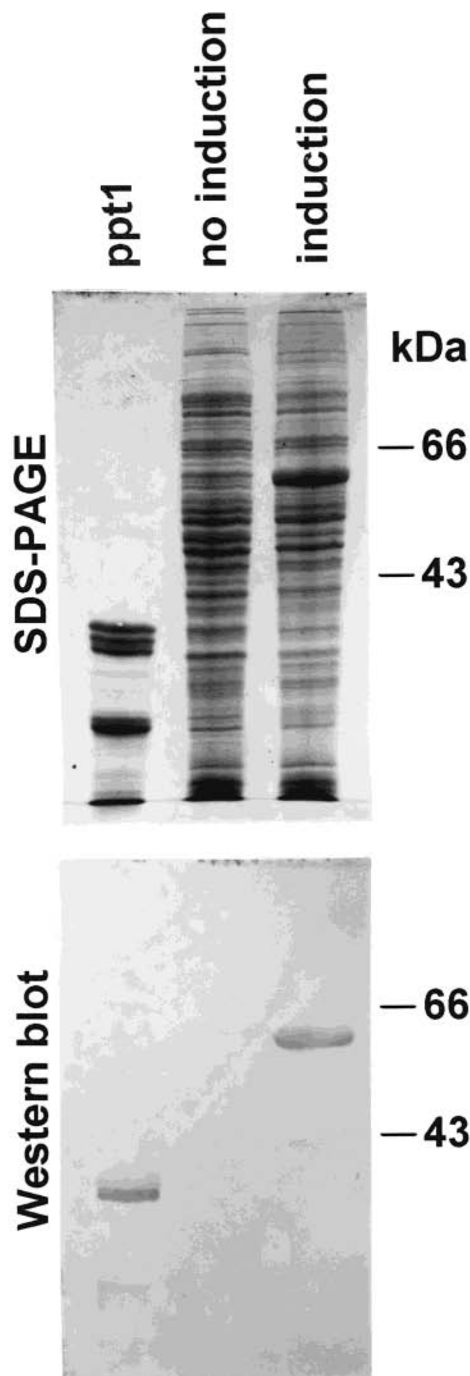

Figure 7. SDS–PAGE and Western blotting of the overexpressed fusion protein containing sesame 11S globulin precursor. (upper panel) Along with sesame-insoluble 11S globulin in ppt1 (5 μg), the total proteins (20 μg) of *E. coli* overexpressed in a fusion vector before or after IPTG induction were resolved in Tricine SDS–PAGE. (lower panel) A duplicate gel was transferred onto nitrocellulose membrane and then subjected to immunoblotting using antibodies against 11S IIA subunit. Labels on the right indicate the molecular masses of protein standards, BSA (66 kDa) and ovalbumin (43 kDa).

methionine enrichment in crops via genetic engineering and gene transformation.

Mature 11S globulin, which is encoded by a small family of genes, is synthesized and assembled through a complex process that involves a series of post-translational modifications as the proteins transported to and deposited within protein bodies (Heim et al., 1989). Though three major polypeptides were resolved on glycine SDS–PAGE under our experimental conditions (Figure 4), we are still not certain how many genes and how many post-translationally modified polypeptides are present in the 11S globulin family of sesame. Nevertheless, it is certain that most, if not all, polypeptides of 50–60 kDa in the 10000*g* pellet of sesame seed extract are constituents of 11S globulin since almost all these polypeptides can be cleaved into two subunits when treated with β-mercaptoethanol. According to their immunological cross-recognition, these three polypeptides appear to be encoded by three homologous genes, rather than modified from the product of the same gene.

The abundance of 11S globulin and 2S albumin in sesame seeds suggests that the 5′ untranslated regions of 11S globulin and 2S albumin precursor genes contain relatively strong promoters specifically expressed during the active synthesis of protein bodies in seed maturation. Characterizing promoters of the 11S globulin and 2S albumin genes may provide a useful clue for the investigation on gene regulation during seed maturation and potential application of transgenic sesame plants.

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LITERATURE CITED

Altenbach, S. B.; Pearson, K. W.; Sun, S. S. M. Cloning and sequence analysis of a cDNA encoding a Brazil nut protein exceptionally rich in methionine. *Plant Mol. Biol.* **1987**, *8,* 239–250.

Altschul, S. F.; Warren, G.; Webb, M.; Eugene, W. M.; David, J. L. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215,* 403–10.

Casey, R.; Domoney, C.; Smith, A. M. Legume storage proteins and their genes. *Oxford Surveys of Plant Molecular and Cell Biology*; Miflin, B. J., Ed.; Oxford University Press: Oxford, 1986; Vol. 3, pp 1–95.

Chen, E. C. F.; Tai, S. S. K.; Peng, C. C.; Tzen, J. T. C. Identification of three novel unique proteins in seed oil bodies of sesame. *Plant Cell Physiol.* **1998**, *39,* 935–941.

Chuang, R. L. C.; Chen, J. C. F.; Chu, J.; Tzen, J. T. C. Characterization of seed oil bodies and their surface oleosin isoforms from rice embryos. *J. Biochem.* **1996**, *120,* 74–81.

Danielsson, C. E. Seed globulins of the Gramineae and Leguminoseae. *Biochem. J.* **1949**, *44,* 387–400.

Derbyshire, E.; Wright, D. J.; Boulter, D. Legumin and vicilin storage proteins of legume seeds. *Phytochemistry* **1976**, *15,* 411–414.

D′Hondt, K.; Damme, J. V.; Bossche, C. V. D.; Leejeerajumnean, S.; Rycke, R. D.; Derksen, J.; Vandekerckhove, J.; Krebber, E. Study of the role of the propeptides of the *Arabidopsis thaliana* 2S albumin. *Plant Physiol.* **1993**, *102,* 425–433.

Hara-Hishimura, I.; Takeuchi, Y.; Inoue, K.; Nishimura, M. Vesicle transport and processing of the precursor to 2S albumin in pumpkin. *Plant J.* **1993**, *4,* 793–800.

Hasegawa, K.; Murata, M.; Fujino, S. Characterization of subunits and temperature-dependent dissociation of 13S globulin of sesame seed. *Agric. Biol. Chem.* **1978**, *42,* 2291–2297.

Heim, U.; Schubert, R.; Baumlein, H.; Wobus, U. The legumin gene family: Structure and evolutionary implications of *Vicia faba* B-type genes and pseudogenes. *Plant Mol. Biol.* **1989**, *13,* 653–663.

Horstmann, C. Specific subunit pairs of legumin from *Vicia faba*. *Phytochemistry* **1983**, *22,* 1861–1866.

Jung, R.; Nam, Y. W.; Saalbach, I.; Muntz, K.; Nielsen, N. C. Role of the sulflhydryl redox state and disulfide bonds in processing and assembly of 11S seed globulins. *Plant Cell* **1997**, *9,* 2037–2050.

Jung, R.; Scott, M. P.; Nam, Y. W.; Beaman, T. W.; Bassuner, R.; Saalbach, I.; Muntz, K.; Nielsen, N. C. The role of proteolysis in the processing and assembly of 11S seed globulins. *Plant Cell* **1998**, *10,* 343–357.

Kolopp-Sarda, M. N.; Moneret-Vautrin, D. A.; Gobert, B.; Kanny, G.; Brodschii, M.; Bene, M. C.; Faure, G. C. Specific humoral immune responses in 12 cases of food sensitization to sesame seed. *Clin. Exp. Allergy* **1997**, *27,* 1285–1291.

Kortt, A. A.; Caldwell, J. B.; Lilley, G. G.; Higgins, T. J. V. Amino acid and cDNA sequence of a methionine-rich 2S protein from sunflower seed (*Helianthus annuus* L.). *Eur. J. Biochem.* **1991**, *195,* 329–334.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227,* 680–685.

Molvig, L.; Tabe, L. M.; Eggum, B. O.; Moore, A. E.; Craig, S.; Spencer, D.; Higgins, T. J. V. Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94,* 8393–8398.

Nielsen, H.; Engelbrecht, J.; Brunak, S.; von Heijne, G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng.* **1997**, *10,* 1–6.

Nishimura, N.; Okubo, K.; Shibasaki, K. Chemical and physical properties of 13S globulin, the major protein in sesame seeds. *Cereal Chem.* **1979**, *56,* 239–242.

Nordlee, J. A.; Taylor, S. L.; Townsend, J. A.; Thomas, L. A.; Bush, R. K. Identification of a Brazil-nut allergen in transgenic soybeans. *N. Engl. J. Med.* **1996**, *334,* 688–92.

Osborne, T. B. *The vegetable proteins*; Longmans, Green & Co.: London, 1924.

Peng, C. C.; Tzen, J. T. C. Analysis of the three essential constituents of oil bodies in developing sesame seeds. *Plant Cell Physiol.* **1998**, *39,* 35–42.

Polson, A. Isolation of IgY from the yolks of eggs by a chloroform poly(ethylene glycol) procedure. *Immunol. Invest.* **1990**, *19,* 253–258.

Prakash, V.; Narasinga Rao, M. S. Hydrogen ion equilibria of α-globulin from *Sesamum indicum* L. *J. Agric. Food Chem.* **1990**, *38*, 612–615.

Rajendran, S.; Prakash, V. Isolation and characterization of β-globulin low molecular weight protein fraction from sesame seeds (*Sesamum indicum* L.) *J. Agric. Food Chem.* **1988**, *36*, 269–275.

Saalbach, I.; Pickardt, T.; Machemehl, F.; Saalbach, G.; Schieder, O.; Muntz, K. A chimeric gene encoding the methionine-rich 2S albumin of the Brazil nut (*Bertholletia excelsa* H. B. K.) is stably expressed and inherited in transgenic grain legumes. *Mol. Gen. Genet.* **1994**, *242,* 226–236.

Schagger, H.; VonJagow, G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **1987**, *166,* 368–379.

Shewry, P. R. Plant storage protein. *Biol. Rev.* **1995**, *70,* 375–426.

Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. Identification of the cystines which link the acidic and basic components of the glycinin subunits. *J. Biol. Chem.* **1984**, *259,* 13431–13435.

Tzen, J. T. C.; Peng, C. C.; Cheng, D. J.; Chen, E. C. F.; Chiu, J. M. H. A new method for seed oil body purification and examination of oil body integrity following germination. *J. Biochem.* **1997**, *121,* 762–768.

Vasil, I. K.; Anderson, O. D. Genetic engineering of wheat gluten. *Trends Plant Sci.* **1997**, *2,* 292–297.

Wilkins, T. A.; Smart, L. B. Isolation of RNA from plant tissue. In *A Laboratory Guide to RNA*; Krieg, P. A., Ed.; Wiley-Liss Inc.: New York, 1996; Vol. 2, pp 21–41.

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