An 8 kDa Methionine-Rich Protein from Soybean (*Glycine max*) Cotyledon: Identification, Purification, and N-Terminal Sequence

M. Jamela Revilleza,† Alfredo F. Galvez, Deanne C. Krenz, and Benito O. de Lumen*

Department of Nutritional Sciences and Graduate Group in Agricultural and Environmental Chemistry, University of California, Berkeley, California 94720-3104

The genes encoding methionine-rich proteins (MRP) in seeds are candidates for overexpression to enhance the nutritional quality of legume proteins, which are relatively deficient in methionine. Our previous work on soybean seed localized the MRP in the albumin fraction, and the present work further localized them in the low molecular weight fraction (LMW) of albumin. Enrichment of the albumin fraction for LMW proteins by heparin−Sepharose affinity chromatography before resolution on 2D SDS−PAGE and labeling of methionine-containing proteins with [1-^{14}C]iodoacetate resulted in identification of three methionine-rich 8 kDa proteins with different p*I* values. The most acidic of the three (2D-1) was recovered for amino acid analysis and partial protein sequencing. 2D-1 had 8.6% methionine, 11.4% lysine, and high amounts of glutamic acid and aspartic acid. This work lays the groundwork for cloning of the gene encoding 2D-1, its eventual overexpression in soybean seed, and elucidation of a possible biological role for 2D-1 and related proteins.

Keywords: *Methionine-rich protein; soybean (Glycine max)*

INTRODUCTION

The utilization of plant proteins has received increased attention due to the expanding demand for dietary proteins. Legumes are the richest sources of proteins among plant foods, but their full utilization is restricted by nutritionally limiting amounts of the essential amino acid methionine. Cysteine, although not an essential amino acid, is grouped with methionine because it is synthesized by humans and monogastric animals from methionine and has a sparing effect on methionine when present in the diet. Genetic engineering strategies to enhance the methionine content of legumes are classified into three basic categories (de Lumen, 1990): (a) modification of genes encoding seed storage proteins by adding methionine codons or inserting sequences coding for methionine-rich peptides (Hoffman et al., 1988; De Clerq et al., 1990); (b) transfer of genes encoding methionine-rich proteins (MRP) from other species to the target plant (Altenbach and Simpson, 1990); and (c) enhancing the level of endogenous, nonabundant MRP in the target plant. With soybean, the single largest source of protein in animal feed and widely used for human food, as our target, our laboratory has chosen the third strategy for the following reasons: (a) increasing the level of an endogenous protein is unlikely to be deleterious to the seed compared with an exogenous protein; (b) the use of a soybean gene encoding MRP could result in a more stable integration into the soybean genome and a higher expression compared with a foreign gene; and (c) the enhanced MRP would most likely be nonallergenic since there are extremely few cases of actual soybean allergenicity in spite of widespread use of soybean for human foods. Our strategy requires the identification and isolation of MRP from soybean in sufficient amounts for confirmatory amino acid analysis and protein sequencing.

The salt-soluble globulins, which include the major storage proteins in legumes, have been extensively studied, and although low in the sulfur amino acids (Gupta, 1983), they provide carbon and nitrogen sources to the germinating seedling. On the other hand, the water-soluble albumins are more diverse and in some instances possess biological activities such as lectins, allergens, and inhibitors of protease and α-amylase. Combining the powerful resolution obtainable with 2D SDS−PAGE and an *in vitro* labeling method developed in our laboratory for detecting methionine-containing proteins (de Lumen and Kho, 1987), we localized MRP in the soybean albumin fraction (Kho and de Lumen, 1988) and consequently reported a 10.8 kDa MRP from soybean with 12.1% methionine (George and de Lumen, 1991). Since the biological role of MRP in the seed is unknown, its enhancement requires a clear understanding of its possible biological role.

In a continuing investigation of MRP in soybean, we report here the identification and purification of three 8 kDa proteins from mature soybean cotyledons by heparin−Sepharose affinity chromatography and high-resolution 2D SDS−PAGE combined with the *in vitro* labeling of methionine-containing proteins. Amino acid analysis of the major protein, which was also the most acidic, confirmed its high methionine content of 8.6%, its high content of glutamic and aspartic acids, and a high lysine content of 11.4%. This work lays the foundation to cloning the 8 kDa MRP gene, enhancing its expression in the seed, and elucidating the possible biological role of this MRP in the seed.

MATERIALS AND METHODS

Materials. Soybean plants (*Glycine max* cv. Hodgson 78) were grown in the greenhouse, and seeds were collected at maturation stage (60 days after flowering) and stored at −80

* Author to whom correspondence should be addressed at Department of Nutritional Sciences, 238 Morgan Hall, University of California, Berkeley, CA 94720-3104 [telephone (510) 642-8144; fax (510) 642-0535; e-mail nitto@nature.berkeley.edu].

† Present address: Institute of Chemistry, University of the Philippines at Los Banos College, Laguna, Philippines.

S0021-8561(96)00063-5 CCC: $12.00 © 1996 American Chemical Society

°C until use. Ampholytes, Hi-Trap heparin, and protein standards were from Pharmacia, Serva Blue G was from Serva (Westbury, NY), and urea, tricine, and all other reagents were from Fisher Scientific (Fair Lawn, NJ). [1-^{14}C]Iodoacetic acid was from ICN Radiochemicals (Irvine, CA), and PVDF membranes (Immobilon) were from Millipore (Bedford, MA).

Protein Extraction. Seeds were dehulled and the cotyledons separated and ground in liquid nitrogen to a fine powder. Soluble proteins were extracted from 10 g of seeds with 100 mL of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM PMSF, and 0.05 M β-mercaptoethanol using a polytron for 3 min at high speed. The slurry was filtered through 12 layers of cheesecloth and centrifuged at 12 000 rpm (SS 34 rotor, Sorvall) at 4 °C for 1 h. The supernatant was passed through a glass wool column to get rid of the oil film and subsequently dialyzed against 10 mM phosphate buffer (pH 6.8) for 48 h at 4 °C. The albumin fraction was collected after centrifugation at 14 000 rpm for 1 h, and traces of the oil film were removed by again passing the supernatant through a glass wool column. This was lyophilized and stored at −20 °C until use.

Heparin−Sepharose Affinity Chromatography. The lyophilized albumin fraction was dissolved in a minimum amount of water and filtered through a 0.2 μm acrodisc (Gelman Sciences). The extract containing 15 mg of protein was loaded onto a 5 mL Hi-Trap heparin column previously equilibrated with 50 mM sodium phosphate buffer (pH 7.0) until protein was detected in the eluate with the Bio-Rad dye-binding reagent. The unbound protein was washed off with 50 mM sodium phosphate buffer until the absence of the protein in the eluate was established. The bound protein was then eluted with a NaCl gradient of 0−1.5 M in sodium phosphate buffer in a total volume of 80 mL, and 5 mL fractions were collected. The protein in each tube was monitored using the dye-binding assay and the composition of each determined through SDS−PAGE (Schagger and Von Jagow, 1987). Fractions containing the LMW proteins were dialyzed overnight against 10 mM sodium phosphate buffer (pH 7.0) with 1 mM PMSF and kept at −20 °C.

Five fractions from different runs were pooled and reloaded onto a 5 mL Hi-Trap heparin column and processed as above for further enrichment of LMW proteins. Fractions were lyophilized after dialysis and stored at −20 °C.

One-Dimensional SDS−PAGE. Electrophoresis was carried out on a high-resolution 10% T, 3% C, 13% glycerol, tricine−SDS−PAGE (Schagger and Von Jagow, 1987). The 0.75 × 20 × 20 cm^3 resolving gel was overlaid with 2 cm 4% T, 3% C stacking gel. Both gels were degassed prior to polymerization with the addition of 50 μL of 10% ammonium persulfate solution and 5 μL of TEMED/10 mL, polymerizing the resolving gel first. The discontinuous buffer system used was composed of 0.2 M Tris (pH 8.9) (anode buffer) and 0.1 M Tris, 0.1 M Tricine, and 0.1 M SDS (pH 8.25) (cathode buffer).

The protein samples and molecular markers were each incubated for 30 min at 40 °C in 4% SDS, 12% glycerol (w/v), 50 mM Tris, 2% β-mercaptoethanol (v/v), and 0.01% Serva Blue G adjusted with HCl to pH 6.8. Sixty microliters of the reacted samples (0.5−2 μg/band) was loaded onto each well of the vertical slab gel using a syringe. Electrophoresis was done at room temperature on a Hoeffer SE 400 system for 20−24 h, at 40 mA and constant voltage.

Detection of Methionine-Rich Proteins. After electrophoresis, the proteins were electroblotted onto Immobilon membrane by using a semidry electroblotter as described previously (George and de Lumen, 1991) except that the transfer buffer used was 25 mM Tris, 20% methanol, and 0.1 M tricine (pH 8). Detection of methionine-containing proteins on the 2D SDS−PAGE blot was carried out as reported previously (de Lumen and Kho, 1987; George and de Lumen, 1991). The method is based on radioactive tagging of methionine residues in proteins by specific alkylation of the thioether moiety with [1-^{14}C]iodoacetate at pH 2.0 (de Lumen and Kho, 1987). Autoradiograph signals were quantified using Bioscan. For each protein band, the ratio of the autoradiograph signal to that of the membrane stained for protein with Coomassie blue, termed the methionine index, is a measure of the methionine content.

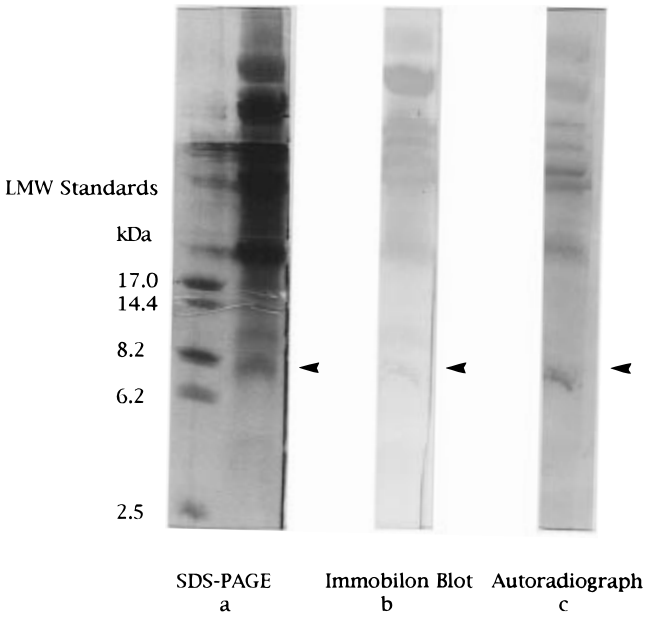

Figure 1. Identification of methionine-containing 8 kDa protein band on 1D SDS−PAGE. Total soluble proteins extracted from mature soybean cotyledon were resolved on high-resolution SDS−PAGE gel (Schagger and Von Jagow, 1987) and stained with Coomassie blue (a), electroblotted onto Immobilon membrane (Millipore) and stained with Coomassie blue (b), and then reacted with [1-^{14}C]iodoacetate at pH 2.0 to alkylate methionine residues (de Lumen and Kho, 1987) and exposed to X-ray film to obtain the autoradiograph (c). The well in (a) contained 100 μg of total soluble protein. For each protein band, the ratio of the autoradiograph signal to that of the membrane stained with Coomassie blue is an index of methionine content. Arrows point to the 8 kDa MRP.

Two-Dimensional SDS−PAGE. Proteins were separated in the first dimension by isoelectrofocusing (IEF) as previously described (George and de Lumen, 1991; O'Farrell, 1975) with some procedural modifications as outlined below and by SDS−PAGE in the second dimension.

Ten milligrams of the lyophilized LMW proteins resolved on the heparin column was dissolved in 20 μL of water and mixed with 12 mg of urea and 20 μL of lysis buffer. The mixture was incubated for 30 min at 40 °C and loaded on the IEF tube gel with a 50 μL syringe. Ten microliters of carbamylated carbonic anhydrase standard with an apparent MW of 30 kDa and p*I* range of 4.8−6.7 was layered on top of the sample. IEF was conducted at 500 V until the reading in milliamps approaches zero within 5−6 h. The IEF gels were carefully released from the tubes and incubated with mild shaking three times for 10 min in equilibration buffer (10% glycerol, 4.9% DTT, 2% SDS, 0.004% Bromphenol Blue, and 125 mM Tris, pH 6.8) in preparation for SDS−PAGE.

SDS−PAGE was carried out on a 1.5 cm × 20 cm × 9 cm gel. Twenty microliters of the equilibrated LMW standards was mixed with 20 μL of melted 2% agarose and allowed to solidify. Half was layered on the SDS−PAGE gel, 1 cm from the IEF equilibrated tube gel.

Amino Acid Analysis and N-Terminal Sequencing. The major spot (2D-1) was excised from three identical 2D SDS−PAGE blots and submitted for amino acid analysis and N-terminal sequencing using an ABI 470A protein sequencer and Model 120A online HPLC system at the Protein Structure Laboratory at University of California, Davis.

RESULTS

Electrophoretic Analysis of Soy Proteins. Analysis of soy total soluble proteins on SDS−PAGE after Coomassie blue staining showed that the majority of the proteins ranged in size from 30 to 70 kDa and a minority was found in the 4−14 kDa range (Figure 1a). The high

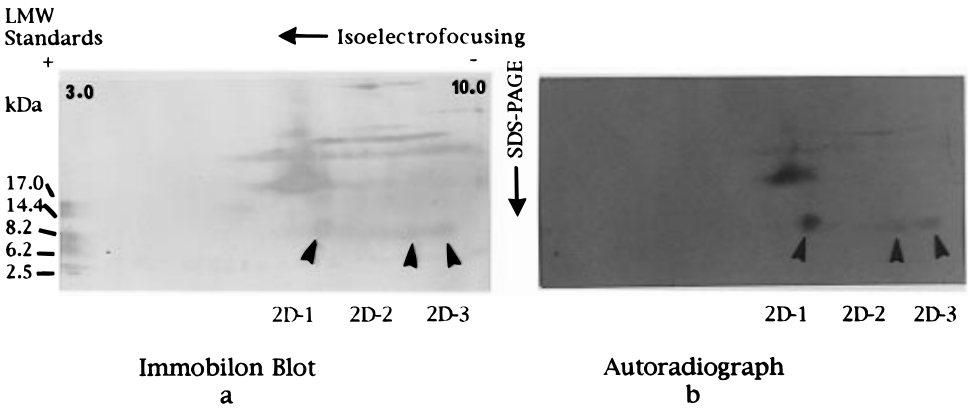

Figure 2. Resolution of total soluble protein extract from soybean cotyledon on 2D SDS−PAGE. Isoelectrofocusing at p*I* 3−10 was carried out on the protein extract before MW separation by SDS−PAGE. Three 8 kDa proteins (2D-1, 2D-2, and 2D-3) with different isoelectric points were revealed in a Coomassie blue stained Immobilon membrane (Millipore) to which the 2D gel had been electroblotted (a). Reaction of the membrane with [1-^{14}C]iodoacetate (6) and autoradiography (b) resulted in the three 8 kDa proteins showing methionine signals, with 2D-1 showing the most distinct signal (shown by arrows).

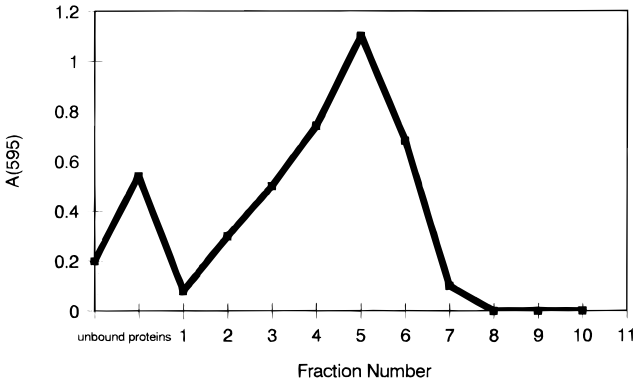

Figure 3. Heparin−Sepharose affinity chromatography of albumin fraction from soybean cotyledon. The albumin fraction was prepared from the total protein extract of soybean cotyledon and then loaded onto 5 mL of Hi-Trap heparin. After the unbound protein was washed off with the equilibration buffer, the bound protein was eluted with a 0−1.5 M NaCl gradient in equilibration buffer using a total volume of 80 mL and collecting 5 mL fractions. Protein concentration was monitored with the dye-binding assay (Bio-Rad).

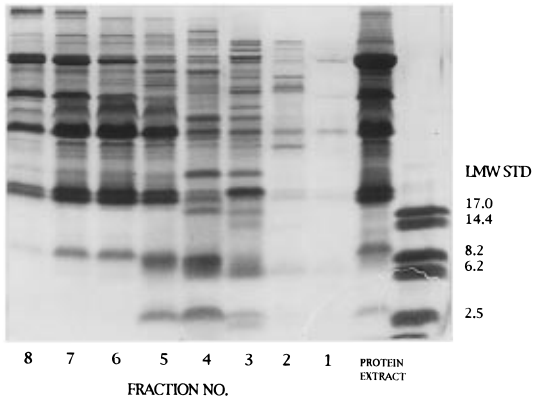

Figure 4. 1D SDS−PAGE profiles of bound protein fractions eluted from heparin−Sepharose affinity chromatography. The numbers correspond to the fractions eluted from the column shown in Figure 3. Note the enrichment of fractions 3−5 with the LMW proteins compared with the total protein extract.

molecular weight fraction corresponds to the acidic and α- and *β*-subunits of glycinin (George and de Lumen, 1991). Using the *in vitro* radioactive labeling technique and the appropriate low molecular weight standards, the MRP was found in the 8 kDa band (Figure 1). Further resolution using 2D SDS−PAGE revealed that the single band on 1D SDS−PAGE was actually a mixture of three 8 kDa proteins with different isoelectric points (Figure 2a). Radioactive labeling of the methionine residues on the 2D blot by the iodoacetate reaction suggested that all three were putatively methionine-rich (Figure 2b).

Isolation of the Soybean MRP. Although the 8 kDa albumins were adequately resolved on the 2D gel, the amounts were still insufficient for definitive amino acid analysis and partial sequencing, requiring a preconcentration step. Heparin−Sepharose affinity chromatography resolved the albumins into two peaks corresponding to the unbound and bound proteins (Figure 3). The 8 kDa proteins were found in the unbound (not shown in the gel) and mostly in the early eluting fractions 3−5 (Figure 4). Fraction 4 was especially enriched with the 8 kDa proteins with relatively less contamination with the HMW proteins. Fractions enriched with 8 kDa proteins were dialyzed and lyophilized prior to 2D SDS−PAGE. Resolution on 2D SDS−PAGE confirmed the enrichment of this fraction with respect to the three 8 kDa proteins, with all three proteins clearly prominent and practically all the HMW removed. (Compare Figure 5 with Figure 2a, noting that the orientation of the blots is reversed.) This allowed the recovery of the 2D-1 protein from multiple blots in sufficient amounts for confirmatory amino acid analysis and protein sequencing. However, not enough amounts were recovered for the other two 8 kDa proteins, 2D-2 and 2D-3. Future efforts will be done to obtain adequate amounts of these MRP to do definitive analysis and sequencing on them.

Amino Acid Composition. The amino acid composition of the 8 kDa protein band on 1D SDS−PAGE and 2D-1 isolated from sequential heparin−Sepharose affinity chromatography and 2D SDS−PAGE are given in Table 1, together with that of soymeal protein, Brazil nut MRP, and the FAO reference protein for comparison. As a band on 1D SDS−PAGE, the 8 kDa protein contained 7.7% methionine and 5.0% cysteine and was characterized by high contents of glutamic acid, aspartic acid, and arginine, a feature shared with other soybean proteins. 2D-1 had 8.6% methionine and a high amount of lysine (11.4%), which was almost twice that of defatted soymeal protein. The high aspartic and glutamic acids explain its acidic p*I* of 5.0 as deduced from the 2D gel. In comparison, Brazil nut MRP, the most commonly used protein for methionine enrichment in legumes, had twice as much methionine as 2D-1, but

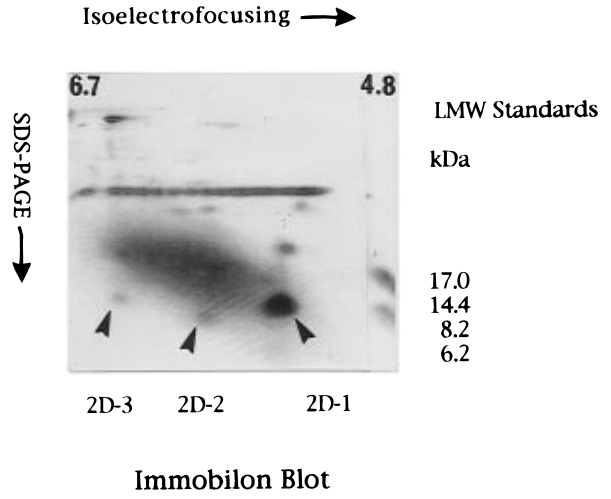

Figure 5. 2D SDS−PAGE profile of heparin−Sepharose affinity column fractions enriched with LMW proteins. Hi-Trap heparin column fractions enriched with LMW proteins were lyophilized after dialysis. The lyophilized LMW proteins were resolved on 2D SDS−PAGE as described in Figure 2. Ten microliters of carbamylated carbonic anhydrase with an apparent MW of 30 000 and p*I* range of 4.8−6.7 was used as an internal standard, shown as a continuous streak. Note that the orientation of the blot here is the reverse of Figure 2a. Arrows point to the 8 kDa proteins.

the other essential amino acids, lysine, threonine, and isoleucine, were absent or very low.

N-Terminal Sequence. Protein microsequencing of 2D-1, enriched in sufficient amounts by combining the 2D-1 spots from three 2D SDS−PAGE blots, revealed the following 20 amino acid N-terminal sequence:

EGKDEDEEEEGHMQKCATEM

DISCUSSION

In a previous work, we localized the MRP in the albumin fraction of soybean seed (Kho and de Lumen, 1991). In the present work, we first established the localization of the MRP in the LMW fraction and subsequently enriched the albumin with the LMW fraction before resolution by 2D SDS−PAGE. After a survey of several chromatography columns, Hi-Trap heparin, an affinity column based on a Sepharose matrix, was selected because it preferentially bound the high molecular weight proteins and facilitated the selective elution of the LMW proteins containing significant amounts of MRP as monitored through 1D SDS−PAGE and the iodoacetate assay. The 2D-1 MRP is distinct from the previously reported 10.8 kDa soybean MRP with 12% methionine (George and de Lumen, 1991), although it is possible that the two belong to a family of MRP. Although MRP have been reported in cereals (Yamagata et al., 1986) and oilseeds (Altenbach et al., 1987), this is only the second MRP reported in soybean. A cysteine-rich (7.8% cysteine) protein lacking methionine has been purified from lupine (*Lupinus angustifolius*) and the encoding cDNA isolated (Gayler et al., 1990).

Our initial attempt to clone the 10.8 kDa MRP was hampered by the small amount of protein recovered from the blots for definitive protein sequencing. The 10.8 kDa MRP had to be obtained directly from 2D SDS−PAGE of the total protein extract since the acidic methanol concentration step we were using to separate the LMW from the HMW proteins (Kho and de Lumen, 1988) caused a substantial loss of the MRP. In contrast, the use of heparin−Sepharose affinity chromatography clearly resulted in an enrichment of the LMW proteins. Consequently, sufficient amounts of MRP were recovered for amino acid analysis and protein microsequencing by using multiple blots. The middle N-terminal sequence of seven amino acids with the least codon degeneracy was used to design oligonucleotide primers for PCR amplification of 2D-1 cDNA fragments. The resulting PCR amplified product contained the partial N-terminal amino acid sequence and was used to probe a soybean seed midmaturation cDNA library. The cloning and characterization of the 2D-1 cDNA clone will be reported elsewhere.

Table 1. Amino Acid Composition[a] of 8 kDa Soybean MRP,[b] Defatted Soymeal,[c] Brazil Nut MRP,[d] and FAO Reference Protein[e]

amino acid	8kDa-1D MRP	2D-1 MRP	Brazil nut MRP	defatted soymeal	FAO ref protein
methionine	7.7	8.6	18.8	1.4	3.5 (met + cys)
cysteine	5.0	1.5	7.9	1.3	
lysine	12.7	1.4	0.0	6.0	5.5
tryptophan	nd[f]	nd	0.0	1.2	
threonine	2.3	3.1	0.0	3.7	4.0
isoleucine	4.0	4.0	1.0	4.4	4.0
leucine	7.7	7.7	5.0	6.7	7.0
phenylalanine	1.6	0.3	0.0	4.5	6.0 (phe + tyr)
tyrosine	nd	nd	0.0	4.6	5.0
asparagine	nd	nd	2.0	nd	
aspartic acid	10.3	11.2	1.0	10.4	
serine	4.8	5.6	6.9	4.6	
glutamine	nd	nd	11.9	nd	
glutamic acid	28.8	35.1	14.9	18.4	
proline	2.9	2.8	5.9	5.3	
glycine	2.1	3.1	5.9	3.4	
alanine	2.0	2.1	1.0	3.6	
histidine	1.7	0.0	2.0	2.2	2.2
arginine	6.0	3.4	14.9	7.6	

[a] Values are in g/100 g of protein as determined by amino acid analysis. [b] Amino acid analysis was carried out on 8 kDa protein band on Immobilon membrane after 1D SDS−PAGE (8 kDa-1D) and on 2D-1 after 2D SDS−PAGE of fraction enriched for LMW proteins by heparin−Sepharose chromatography. [c] Rackis (1961). [d] Determined from cloned cDNA encoding mature protein, % of total (Altenbach and Simpson, 1990). [e] FAO/WHO Food and Agriculture Organization/World Health Organization of the United Nations (1973). [f] nd, not detected.

The amino acid composition of 2D-1 showed high levels of both methionine (8.6%) and lysine (11.4%), the first limiting essential amino acids in legumes and cereals, respectively. This is significant since all of the MRP reported so far from rice, corn, sunflower, and Brazil nut contain very little or no lysine (Yamagata et al., 1986; Kirihara et al., 1988; Altenbach et al., 1987; Kortt et al., 1991). Also lacking or very low are threonine in Brazil nut and sunflower MRP and isoleucine in Brazil nut, corn, and rice MRP. Overexpression of genes coding for these cereal and oilseed MRP might cause an imbalance of other essential amino acids since there is evidence that transgenic MRP increases at the expense of other proteins (Nordlee et al., 1994).

Transformation of soybean with Brazil nut MRP cDNA driven by a β-phaseolin promoter leads to the accumulation of the Brazil nut MRP up to 8% of the total protein, which is equivalent to a 26% increase in methionine content (Nordlee et al., 1994; Townsend et al., 1992). It remains to be seen if this increase is nutritionally significant. Using the radioimmune allergosorbant test (RAST), the Brazil nut MRP in the transgenic soybean binds human IgE from sera of individuals allergic to Brazil nut, whereas no binding

is observed with comparable amounts of protein extracted from a genetically equivalent line of nontransformed soybean (Nordlee et al., 1996). In light of this initial evidence of Brazil nut MRP allergenicity, our identification of an endogenous MRP in soybean cotyledon has added significance. Although it remains to be proven, it is unlikely that soybean MRP is allergenic on the basis of the evidence that the sera of individuals allergic to Brazil nut MRP showed no reaction with endogenous proteins from nontransformed soybean (Nordlee et al., 1996).

The biological role of 2D-1 MRP is unknown. Comparison of the DNA sequence of the 2D-1 PCR amplified product with the GenBank database showed 53% homology with a DNA binding protein (GT-2) from *Arabidopsis thaliana* (Kuhn et al., 1993) and 60% homology with a cysteine-rich seed storage protein from lupine (*L. angustifolius*) (Gayler et al., 1990). A sulfur-rich amino acid protein isolated from the African cereal, *Digitaria exilis*, has 53% homology to a bovine transcription factor GHF-1 (de Lumen et al., 1993). Interestingly, the region of homology between 2D-1 and the GT-2 protein was in the acidic region near the carboxyl terminus. Acidic regions of many DNA-binding proteins have been shown to function as transcriptional activators (Ptashne, 1988). The in-frame translation product of the 2D-1 PCR fragment had only 41% amino acid sequence homology to the conglutin delta-2 large chain of lupine (Lilley and Inglis, 1986), although there was perfect alignment of the cysteine residues, a feature characteristic of the 2S albumin family of seed proteins (Shewry et al., 1995). Restriction fragment length polymorphism (RFLP) analysis of soybean genomic DNA probed with the 2D-1 PCR product revealed one to two copies in the soybean genome (results not shown), suggesting that 2D-1 was encoded by a unique gene with minimal sequence homology to other genes. This result is consistent with the sequence divergence found at the amino and carboxyl ends among members of the 2S albumin multigene family in *Arabidopsis thaliana* (Krebbers et al., 1988). The amino acid composition of 2S albumins diverges substantially among plant species but share a common structural identity based on the conserved position of the cysteine residues in the small and large subunits (Krebbers et al., 1988). To further elucidate the biological role of 2D-1 MRP, we plan to use an antibody raised against it for cellular immunolocalization and use the 2D-1 cDNA clone for genetic characterization and expression studies.

In summary, through a combination of selective enrichment of the albumin fraction by affinity chromatography, 2D SDS−PAGE, and radiolabeling of methionine-containing proteins with [1-^{14}C]iodoacetate, a new MRP from soybean seed was identified and isolated. The MRP has a MW of 8 kDa and contains 8.6% methionine and 11.4% lysine. This work lays the foundation for the cloning of the 2D-1 gene.

ABBREVIATIONS USED

MRP, methionine-rich proteins; 2D SDS−PAGE, two-dimensional sodium dodecyl sulfate−polyacrylamide gel electrophoresis; 1D SDS−PAGE, one-dimensional sodium dodecyl sulfate−polyacryalamide gel electrophoresis; LMW, low molecular weight; HMW, high molecular weight; IEF, isoelectrofocusing; PMSF, phenylmethanesulfonyl fluoride; MRP, methionine-rich protein; DTT, dithiothreitol; TEMED, *N*′,*N*′,*N*′,*N*′-tetramethylethylenediamine; PCR, polymerase chain reaction.

ACKNOWLEDGMENT

We acknowledge the advice and help of Jing-jie Liu, Bill Odegard, Enrique Colayco, Nancy Lin, and Margono Hadi in our department and of Jack Presley of the Protein Structure Laboratory at UC Davis for the amino acid analysis.

LITERATURE CITED

Altenbach, S. B.; Simpson, R. B. Manipulation of the methionine-rich protein genes in plant seeds. *Trends Biotechnol.* **1990**, *8*, 156−160.

Altenbach, S. B.; Pearson, K. W.; Leung, F. W.; Sun, 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.

De Clerq, A.; Vabdewiele, M.; Van Damme, J.; Guerche, P.; Van Montagu, M.; Vandekerchove, J.; Krebbers, E. Stable accumulation of modified 2S albumin seed storage proteins with higher methionine contents in transgenic plants. *Plant Physiol.* **1990**, *94*, 970−979.

de Lumen, B. O. Molecular approaches to improving the nutritional and functional properties of plant seed as food sources: developments and comments. *J. Agric. Food Chem.* **1990**, *38*, 1779−1788.

de Lumen, B. O.; Kho, C. J. Identification of methionine containing proteins and quantification of their methionine contents. *J. Agric. Food Chem.* **1987**, *35*, 688−691.

de Lumen, B. O.; Thompson, S.; Odegard, W. J. Sulfur amino acid-rich proteins in Acha (*Digitaria exilis*), a promising underutilized African cereal. *J. Agric. Food Chem.* **1993**, *41*, 1045−1047.

Gayler, K. R.; Kolivas, S.; Macfarlane, A. J.; Lilley, G. G.; Baldi, M.; Blagrove, R. J.; Johnson, E. D. Biosynthesis cDNA and amino acid sequences of a precursor of conglutin *δ*, a sulphur-rich protein from *Lupinus angustifolius*. *Plant Mol. Biol.* **1990**, *15*, 879−893.

George, A. A.; de Lumen, B. O. A novel methionine-rich protein in soybean: Identification, amino acid composition and N-terminus sequence. *J. Agric. Food Chem.* **1991**, *39*, 224−227.

Gupta, Y. P. *Chemistry and Biochemistry of Legumes;* Arora, S. K., Ed.; Oxford and IBH Publishing: New York, 1983.

Hoffman, L. M.; Donaldson, D. D.; Herman, E. M. A modified storage protein is synthesized, processed and degraded in the seeds of transgenic plants. *Plant Mol. Biol.* **1988**, *11*, 717−729.

Kho, C. J.; de Lumen, B. O. Identification and isolation of methionine and cysteine rich protein fraction in soybean seed. *Plant Foods Hum. Nutr.* **1988**, *38*, 287−296.

Kirihara, J. A.; Hunsperger, J. P.; Mahoney, W. C.; Messing, J. W. Differential expression of a gene for a methionine-rich storage protein in maize. *Mol. Gen. Genet.* **1988**, *211*, 477−484.

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

Krebbers, E.; Herdies, L.; De Clerq, A.; Seurinck, J.; Leemans, J.; Van Damme, J.; Segura, M.; Gheysen, G.; Van Montagu, M.; Vandekerckhove, J. Determination of the processing sites of an *Arabidopsis* 2S albumin and characterization of the complete gene family. *Plant Physiol.* **1988**, *87*, 859−866.

Kuhn, R. M.; Caspar, T.; Dehesh, K.; Quail, P. H. DNA binding factor GT-2 from *Arabidopsis*. *Plant Mol. Biol.* **1993**, *23*, 337−348.

Lilley, G. G.; Inglis, A. S. Amino acid sequence of conglutin delta, a sulfur-rich seed protein of *Lupinus angustifolius* L. *FEBS Lett.* **1986**, *195*, 235−241.

Nordlee, J. A.; Taylor, S. L.; Townsend, J. A.; Thomas, L. A.; Townsend, R. Investigations of the allergenicity of Brazil nut 2S seed storage protein in transgenic soybean. Presented at the OECD Workshop of New Foods, Oriel College, Oxford, U.K., 1994.

Nordlee, J. A.; Taylor, S. L.; Townsend, J. A.; Thomas, L. A.; Bush, R. K. Identification of a Brazil-nut allergen in transgenic soybeans. *New Engl. J. Med*. **1996**, *334*, 688−692.

O'Farrell, P. H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem*. **1975**, *250*, 4007−4021.

Ptashne, M. How eukaryotic transcriptional activators work. *Nature* **1988**, *335*, 683−689.

Rackis, J. Amino acids in soybean hulls and oil meal fractions. *J. Agric. Food Chem*. **1961**, *9*, 409−412.

Schagger, H.; Von Jagow, 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.; Napier, J. A.; Tatham, A. S. Seed storage proteins: structure and biosynthesis. *Plant Cell*. **1995**, *7*, 945−956.

Townsend, J. A.; Thomas, L. A.; Kulisek, E. S.; Daywalt, M. J.; Winter, K. R. K.; Altenbach, S. B. Improving the quality of seed proteins in soybean. In *Proceedings of the 4th Biennial Conference of Molecular and Cellular Biology of Soybean,* July 27−29, 1992; Iowa State University: Ames, IA, 1992; p 4.

Yamagata, H.; Tamura, K.; Tanaka, K.; Kasai, Z. Cell-free synthesis of rice prolamin. *Plant Cell Physiol*. **1986**, *27*, 1419−1422.

Received for review January 30, 1996. Revised manuscript received July 5, 1996. Accepted July 9, 1996. This work is partially funded by USDA NRICGP 94-02167 and American Soybean Association Grants to B.O.deL. M.J.R. is funded by a Fulbright Hays Fellowship and the ESEP of the Department of Science and Technology of the Philippine Government. [⊗]

JF960063U

[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1996.