# Mungbean [*Vigna radiata* (L.) Wilczek] Globulins: Purification and Characterization

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Vicilin type (8S) and basic 7S globulins and legumin type (11S) globulins were isolated from mungbean [*Vigna radiata* (L.) Wilczek]. The native molecular weights of the different globulin types were 360000 for legumin, 200000 for vicilin, and 135000 for basic 7S. Some of the 8S globulin apparently complexed and coeluted with the 11S on gel filtration. On SDS-PAGE, 11S was composed of two bands of 40000 and 24000, 8S was composed of 60000, 48000, 32000, and 26000 bands, and basic 7S was composed of 28000 and 16000 bands. The percent composition of total globulins was estimated to be as follow: 8S, 89%; basic 7S, 3.4%; and 11S, 7.6%. The basic 7S and 11S but not the 8S globulins were found to have disulfide bonds. The presence of carbohydrates by conjugated peroxidase reaction was observed in all bands of 8S, the acidic polypeptide of basic 7S, and its complex but not in 11S. The 28000 basic 7S band and its 42000 complex and the first three major bands of 8S cross-reacted with antibodies to all types of soybean conglycinin subunits ($\alpha$, $\alpha'$, and $\beta$), whereas the fourth band cross-reacted only with the anti-$\beta$ subunit. None of the mungbean globulins cross-reacted with anti-soybean glycinin. Basic 7S was found to be easily extracted with 0.15 M NaCl, 11S was extracted with 0.35 M NaCl, and 8S was extracted over a wide range of NaCl concentrations. The N-terminal sequences of the different subunits/fragments of the globulins were determined and found to have strong homology with storage proteins of other legumes and crops.

**Keywords:** *Globulins; mungbean proteins; vicilin; legumin; storage proteins*

Mungbean [*Vigna radiata* (L.) Wilczek] is a popular legume in Asian countries. It contains 17–26% protein and is considered to be a major source of proteins in developing countries. Mungbean is consumed as a viand, boiled and cooked with vegetables and meat, as well as a dessert, cooked in syrup, or incorporated in bread or cake. It is also popularly used to make sprouts for egg rolls and other vegetable dishes.

Unlike soybean (*Glycine max* L.), faba bean (*Vicia faba*), or the jackbean and swordbean (*Canavalia gladiata* and *C. ensiformis*), there is limited work on the storage proteins of mungbean. Ericson and Chrispeels (*1*) reported the presence of two types of storage proteins in mungbean protein bodies, the 11S and 8S separated on sucrose gradient ultracentrifugation, corresponding to legumin and vicilin. They reported that legumin had three subunits of 56000, 44000, and 16500, whereas vicilin had four subunits of 63500, 50000, 29500, and 24000. Aside from determining the amino acid composition, Ericson and Chrispeels (*1*) detected the presence of carbohydrates, 0.2% glucosamine and 1% mannose for vicilin and 1% glucosamine for legumin.

Like other legume proteins, mungbean protein lacks the sulfur-containing amino acids methionine and cysteine. Factors affecting the nutritional quality and acceptability of mungbean are summarized in Mendoza et al. (*2*). Improvement of the nutritional quality of mungbean by increasing its methionine content will contribute to the improvement of the nutritional quality of the diet of people, especially the rural poor, in developing countries. Genetic engineering of legume storage proteins with the aim of improving nutritional quality and functional properties has been proposed (*3*–*7*). Soybean glycinins have been successfully engineered resulting in deletion of variable regions, insertion of tetramethionines in variable regions, deletion of disulfide bonds, and deletion and substitution of polyglutamic acid sequence with various amino acids, producing modified proglycinins with altered functionalities and nutritional values (*5*, *6*, *8*–*13*). More recently, Katsube et al. (*14*) reported the accumulation of soybean glycinins and the formation of hybrid soybean–rice proteins in rice. However, before attempts are made to alter the structure of the storage proteins of mungbean to improve its nutritional quality, it is important to first elucidate their nature and properties.

This study aimed (1) to isolate and purify the components of globulins from mungbean; (2) to characterize these components as to molecular weight, number and molecular weight of subunits, N-terminal amino acid sequences, presence of disulfide linkage and carbohydrate moiety, and solubility in salt solutions; and (3) to determine their immunological cross-reaction with soybean globulins.

## EXPERIMENTAL PROCEDURES

**Materials.** Seeds of mungbean variety Pagasa 7 were obtained from the Institute of Plant Breeding, dehulled, ground to 60 mesh using an intermediate Wiley mill, defatted

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in a Soxhlet fat extractor using *n*-hexane for 12 h, and dried in a forced draft oven at 40 °C overnight. Pagasa 7 is a green-seeded variety. The protein content of Pagasa 7 seeds is 25.80% dry weight basis.

**Extraction of Proteins.** Routine extraction of globulins from dried mungbean meal was done with 0.4 M NaCl in 35 mM potassium phosphate buffer, pH 7.6, 0.1 mM *p*-aminodino phenylmethanesulfonyl fluoride hydrochloride (APMSF, Wako Chemicals), $10^{-7}$ M pepstatin A, 10 mM $\beta$-mercaptoethanol, and 0.02% sodium azide at a ratio of 1 g to 18 mL by stirring for 1 h on ice. The homogenate was centrifuged at 13500*g* for 15 min and the supernatant (crude extract) collected and dialyzed against distilled water with 10 mM $\beta$-mercaptoethanol. The globulins started precipitating after 24 h, and precipitation was complete after another 18−24 h. The total globulins were separated from albumin by centrifugation at 13500*g* for 15 min and were washed three times with distilled water containing 10 mM $\beta$-mercaptoethanol.

**Ammonium Sulfate Fractionation.** A saturated solution of ammonium sulfate was slowly added to the crude extract to 60% saturation with stirring on an ice bath. The precipitate and supernatant were collected. The precipitate was taken up in 35 mM potassium phosphate buffer, pH 7.6, with 10 mM $\beta$-mercaptoethanol (buffer A) and dialyzed against distilled water with 10 mM $\beta$-mercaptoethanol. The supernatant was also dialyzed against distilled water with 10 mM $\beta$-mercaptoethanol. Globulins from the precipitate and supernatant of the 60% ammonium sulfate cut were subjected to column chromatography.

**Fractionation by Sephacryl S-200 and Mono Q Column Chromatography.** Total or ammonium sulfate-fractionated globulins (10−20 mg) were applied on a Hi-Prep 16/60 Sephacryl S-200 HR column (Pharmacia Biotech) using a Shimadzu AI liquid chromatography system, at a flow rate of 1 mL/min with buffer A containing either 0.5 or 1.0 M NaCl. Purification was also done on a Mono Q HR 10/10 column (Pharmacia Biotech) with a linear gradient of 0−0.5 M NaCl (500 mL each) in buffer A at a flow rate of 2 mL/min. Fractions were analyzed by SDS-PAGE. Fractions were routinely concentrated using a Centriconprep (Amicon).

The standard curve for the Sephacryl S-200 was prepared using the following protein standards and their respective molecular weights: ovalbumin, 45000; bovine serum albumin, 67000; proglycinin $A_{1a}B_{1b}$ homotrimer, 160000; and glycinin $A_3B_4$ monohexamer, 333000.

**Protein Measurement.** The protein content of samples was determined using the Bradford method (*15*) with bovine serum albumin as standard.

**Subunit Molecular Weight Determination by SDS—Polyacrylamide Gel Electrophoresis (SDS-PAGE).** SDS-PAGE was routinely done on a Bio-Rad mini gel electrophoresis apparatus using 11% gel according to the procedure of Laemmli (*16*). Subunit molecular weight was estimated by using the low molecular weight calibration kit (Pharmacia Biotech) consisting of the following proteins: phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20000), and $\alpha$-lactalbumin (14400).

Percent composition of the different proteins was determined by scanning the gels using the QuantiScan software (Biosoft).

**Detection of Carbohydrate Moiety.** The presence of the carbohydrate moiety of the mungbean globulins was detected by the peroxidase conjugated Con A. Samples were subjected to SDS-PAGE, transferred onto nitrocellulose membranes, and detected by peroxidase-conjugated Con A (Seikagaku Kogyo) as described by Katsube et al. (*12*, *13*).

**Detection of Disulfide Linkage.** Protein preparations were dialyzed against buffer A without mercaptoethanol for 24 h and run on SDS-PAGE in the absence of mercaptoethanol.

**Solubility in Different Molarities of NaCl.** Total globulins (∼5.9 mg; precipitated after extraction from mungbean meal and extensive dialysis; please Extraction of Proteins) were sequentially treated with 0.5 mL of various concentrations of NaCl (0−0.4 M) by gently pipetting in and out ∼20

times, followed by centrifugation at 14000 rpm for 10 min. Dissolved protein was measured according to the Bradford method and analyzed using SDS-PAGE.

**N-Terminal Amino Acid Sequencing.** Purified or partially purified proteins were run on SDS-PAGE in the presence of mercaptoethanol and blotted onto PDVF membrane, followed by staining with 0.1% Ponceau S in 0.1% acetic acid. Destaining was done with 0.1% acetic acid. N-Terminal amino acid sequences of the proteins from the PVDF membrane were determined using a protein sequencer Procise 492 (Applied Biosystems Inc.), either gas-phase or liquid-phase system.

Removal of the blocked N-terminal amino acid was done on the 11S band 1 by the following method: The PVDF membrane was soaked in 100 mM acetic acid with 0.5% (w/v) PVP-30 for 230 min at 37 °C. After incubation, the membrane was washed 10 times with distilled water and once with methanol and then immersed in deblocking solution (50 mM sodium phosphate buffer, pH 7.0, 10 mM DTT plus 2 milliunits of Pfu pyroglutamate amino peptidase) (Takara Shuzo) for 5 h at 50 °C. The membrane was then washed twice with distilled water and blotted dry before protein sequencing.

**Sequence Analysis.** The N-terminal amino acid sequences of the different globulins of mungbean were compared with known sequences by using the FASTA database version 3.0t74 December 1996 (*17*) and the BLAST database (*18*).

**Immunological Cross-Reaction with Antibodies to Soybean Glycinin and $\beta$-Conglycinin.** Proteins were run on SDS-PAGE and blotted onto a nitrocellulose membrane followed by reaction with antibodies to soybean glycinin and $\beta$-conglycinin ($\alpha$, $\alpha'$, and $\beta$ subunits) using a standard procedure. Immunological cross-reaction was detected using alkaline phosphatase−NBT−BCIP reaction.

## RESULTS AND DISCUSSION

**Isolation and Purification of Globulins from Mungbean.** *Purification of 8S and 11S.* Application of total globulins on Sephacryl S-200 resulted in the separation of two major peaks corresponding to 360000 and 200000, of almost equal proportions (1:1.16) (Figure 1). The second peak (II) corresponds to the 8S vicilin and consists of four bands with molecular weights of 60000, 48000, 32000, and 26000 on SDS-PAGE (Figure 1). The same bands are seen in peak I. The first peak (I) therefore consists of the 11S legumin and the 8S vicilin, which apparently complexed to coelute with the 11S. The 11S consists of bands of 40000 and 24000 (with arrows). A 60000 band was also evident in peak I but was not observed in peak II. As shown later, this could be the complex of the 40000 and 24000 bands.

A preliminary experiment showed that with 60% ammonium sulfate fractionation of total globulins, only the 8S globulin was present in the supernatant, whereas the 11S globulin and some 8S globulin were present in the precipitate. Thus, when the 60% ammonium sulfate supernatant fraction was subjected to Mono Q ion exchange column, the 8S globulin was also readily purified, giving the same pattern as in Figure 1 (lane b) (data not shown).

To further purify the 11S globulin, the 60% ammonium sulfate precipitate fraction was taken up in buffer A and dialyzed against distilled water with $\beta$-mercaptoethanol and the resultant precipitate (dissolved in buffer A) was subjected to chromatography on Mono Q. However, the 8S globulin still coeluted with the 11S globulin using buffer A with a linear gradient of 0−0.5 M NaCl, and the results did not show further purification (data not shown). A lot of fine bands were also observed in the fractions coming from the 60% ammonium sulfate precipitate, and this could be due to degradation of the proteins during the process of fractionation and dialysis. Application of the fractions
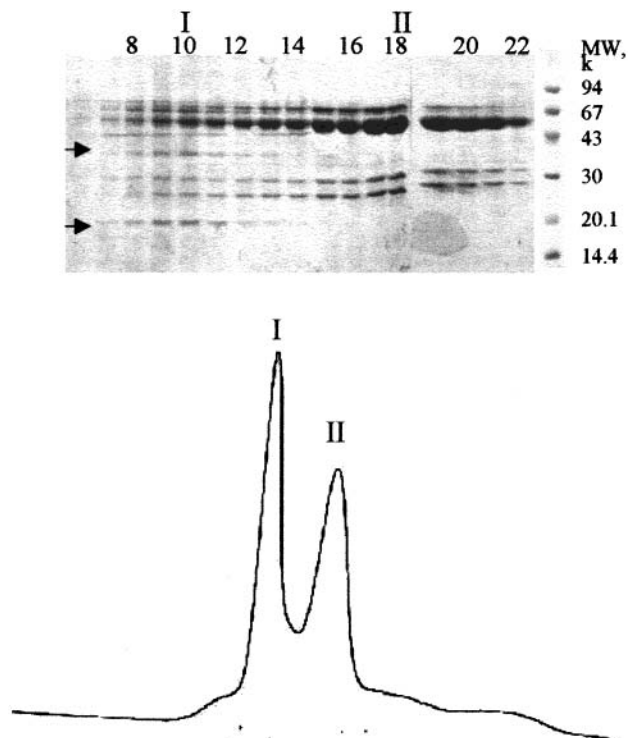
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**Figure 1.** Gel filtration of mungbean globulins on Sephacryl S-200. Inset shows the SDS-PAGE pattern of the fractions. Peaks I (lane 10) and II (lane 18) denote elution of 11S (with arrows) and 8S globulins, respectively. Take note that the 8S coeluted with 11S at peak I.
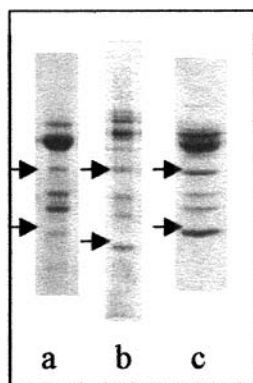


**Figure 2.** Enrichment of 11S globulins: (lane a) total globulins; (lane b) after Sephacryl S-200; (lane c) after Mono Q ion exchange chromatography. The positions of the 11S globulin components are indicated by arrows.

of peak I from Sephacryl S-200 gel filtration on the Mono Q ion exchange column resulted in the enrichment of 11S, although the 8S still persistently coeluted with the 11S. Enrichment of the 11S legumin was estimated to be from 7.6 to 14.8% after Sephacryl S-200 and to 25.6% after Mono Q (Figure 2). Use of 1 M NaCl in buffer A did not prevent the complexation of the 8S vicilin and its coelution with the 11S legumin.

The number and subunit molecular weights obtained for 8S and 11S mungbean globulins are similar to those earlier reported by Ericson and Chrispeels (*1*), which were 56000, 44000, and 16500 for 11S and 63500, 50000, 34500, and 24000 for 8S.

*Purification of Basic 7S.* The basic 7S was obtained from the chromatography of 16-day-old 60% ammonium sulfate globulin precipitate on Sephacryl S-200 with a molecular weight of ~135000 (not shown) and exhibited two bands of 28000 and 16000 on SDS-PAGE (Figure
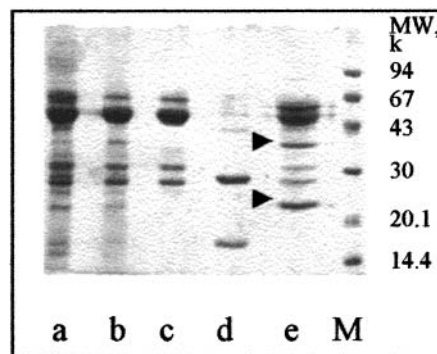


**Figure 3.** SDS-PAGE patterns of mungbean proteins: (lane a) total crude extract; (lane b) globulins; (lane c) 8S (vicilin); (lane d) basic 7S; (lane e) 11S-enriched fraction (marked with arrowheads); (lane M) markers.

**Table 1. Summary of Molecular Weight, Number of Polypeptides/Subunits, and Percent of Globulins from Mungbean**

| globulin | mol wt × $10^{-3}$ | | % of protein |
|---|---|---|---|
| | native | subunit/peptide | |
| basic 7S | 135 | 28, 16 | 3.4 |
| 8S | 200 | 64, 48, 32, 26 | 89.0 |
| 11S | 360 | 40, 24 | 7.6 |

3d). This is the first report of direct evidence for the presence of the basic 7S globulin in mungbean, which was earlier suggested by analysis using antibody raised against soybean basic 7S (*19*) and the release of basic 7S type of proteins from legume seeds by immersion in hot water (*20*). A repeat of this purification step using freshly prepared 60% ammonium sulfate globulin precipitate also separated the basic 7S; however, many contaminating fine bands of various sizes were evident. Apparently, storing the said fraction for some period of time even at 5 °C resulted in the denaturation and insolubilization of other proteins but not the basic 7S globulin. It was noted that the 60% ammonium sulfate globulin precipitate was quite difficult to dissolve, especially the older preparation. The basic 7S globulin was not detected in two runs on Sephacryl S-200 using total globulins. This could only be explained by the low level of this component in the total globulins (3.4% as shown in Table 1).

*Summary of the Number of Subunits/Polypeptides, Molecular Weight, and Percent Composition.* The native and subunit molecular weights and percent of 8S, basic 7S, and 11S globulins of mungbean are summarized in Table 1. The 8S globulin was estimated to constitute 89% of the total globulins with 11S and basic 7S being 7.6 and 3.4%, respectively. From the sucrose gradient centrifugation data of Ericson and Chrispeels (*1*), the 8S and 11S globulins were estimated to be 85 and 15%, respectively, of total globulins. The 7S and 11S globulins of soybean are the major components of soybean, each comprising 30−50% (*21*). Two other 7S globulins, γ-conglycinin and basic 7S globulin, account for less than a few percent of soybean proteins (*22*). β-Conglycinin has a molecular weight of 150000−200000 and three major subunits, namely, α′ (72000), α (68000), and β (52000) (*23, 24*). On the other hand, the 11S globulin of soybean, glycinin, is a hexamer of 300000−380000 molecular weight. Each subunit consists of an acidic polypeptide (~35000) and a basic polypeptide (~20000), which are linked together by a disulfide bond (*24*). Coconut, a monocot, has an 11S legumin type as its major globulin (*25*).

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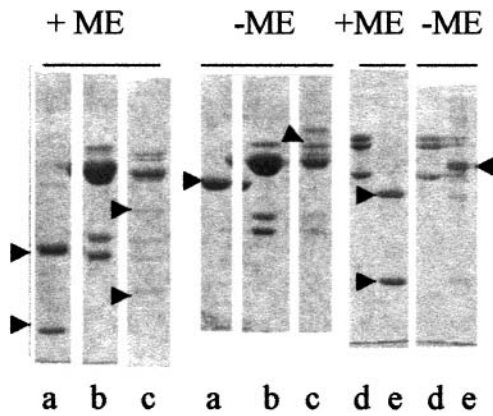*J. Agric. Food Chem.,* Vol. 49, No. 3, 2001 **1555**



**Figure 4.** Mungbean globulins in the presence and absence of mercaptoethanol. Samples are basic 7S (lanes a), 8S (lanes b), and 11S (lanes c) fraction. As control, β-conglycinin (laens d) and glycinin (laens e) are also shown. Arrowheads denote bands of basic and acidic components and their complexes.



**Figure 5.** Detection of carbohydrates in mungbean globulins: (A) carbohydrate staining; (B) protein staining; (lanes a) basic 7S; (lanes b) 8S; (lanes c) 11S; (lanes d) soybean β-conglycinin; (lanes e) soybean glycinin.



**Figure 6.** Serial extraction of mungbean globulins with increasing concentration of sodium chloride.

The SDS-PAGE profiles of the different mungbean globulins and total crude extract are shown in Figure 3. On the basis of the SDS-PAGE profiles of the purified and/or enriched components, the different globulin types—the major 8S bands, the 11S bands, and the basic 7S bands—could now be detected and differentiated in the total globulin lane (Figure 3b).

**Detection of Disulfide Linkage.** In the presence of β-mercaptoethanol, both basic 7S and 11S globulins exhibited two bands, 28000 and 16000 and 40000 and 24000, respectively (Figure 4a,c). In the absence of β-mercaptoethanol, these two bands combined to form 42000 and ~60000 for basic 7S and 11S, respectively. Such bands but very lightly stained were also observed in the purified or enriched fractions of basic 7S and 11S (Figures 1−3). No change was observed with the 8S bands (Figure 4b). For comparison, β-conglycinin and glycinin of soybean were subjected to similar treatment, which resulted in the combination of the 35000 and 20000 bands of glycinin to form a 55000 band (Figure 4e) as observed previously for soybean glycinin (*26*) and no change in β-conglycinin (Figure 4d). The results indicate the presence of a disulfide bridge in basic 7S and 11S and none in 8S of mungbean. Basic 7S of soybean also has a disulfide bridge linking two polypeptides, 26000 and 16000 (*27*).

**Detection of the Presence of Carbohydrates.** The presence of carbohydrate moieties linked to mungbean globulins was determined using Con A conjugated to peroxidase. Some of the bands of the 8S and basic 7S but none of the 11S globulins stained positively for carbohydrates (Figure 5a−c). The 28000 band of the basic 7S and most of the 8S bands were found positive for carbohydrates. The 42000 band in the basic 7S lane, which stained positive for carbohydrate, could be a contaminant or the complex resulting from the combination of the 28000 and 16000 bands. The 26000 band of 8S stained very lightly. The bands attributed to 11S were not stained; those that stained could be contaminating 8S globulin. As a control, soybean β-conglycinin and glycinin run simultaneously with mungbean globulins showed positive reaction for the former and negative for the latter (Figure 5d,e). For comparison, SDS gels stained for protein by Coomassie blue are shown in Figure 5B.

Ericson and Chrispeels (*1*) measured carbohydrate in the 8S vicilin to be 0.2% glucosamine and 1% mannose and in the 11S legumin to be 1% glucosamine. They determined the carbohydrate in the fractions purified
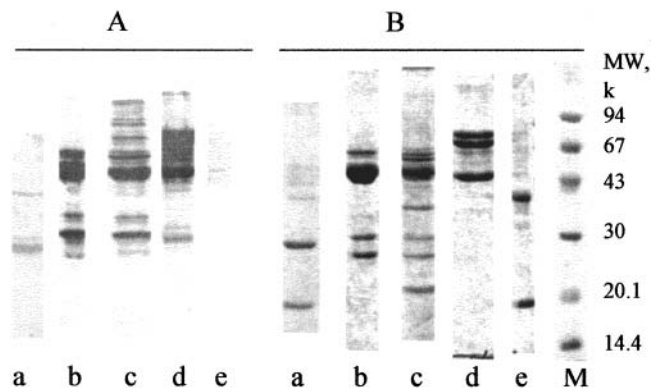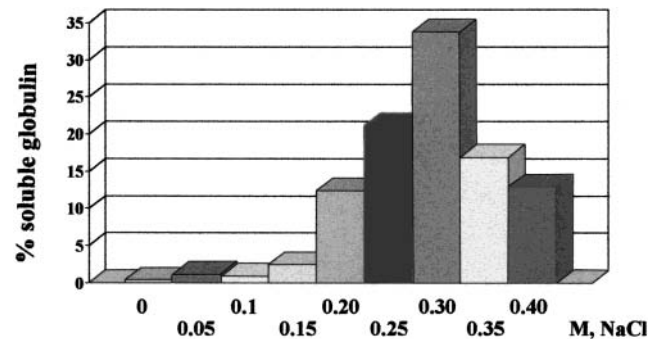
from ion exchange chromatography, and the high level found in the 11S could be due to the 8S contaminating this fraction. As shown in the present study, it is very difficult to separate the 8S from 11S globulins by various chromatography and salt precipitation techniques.

**Solubility of Mungbean Globulins in Various Concentrations of Sodium Chloride.** When total globulins were serially extracted with increasing concentrations of sodium chloride, small amounts (<1%) were extracted with 0−0.1 M and this increased to 12.4% with 0.2 M and 33.8% with 0.3 M. The remaining 17 and 13% were extracted with 0.35 and 0.4 M NaCl, respectively (Figures 6 and 7). It is to be noted that basic 7S could be extracted with 0.15 M NaCl and 11S by 0.35 M NaCl, whereas 8S could be extracted by a wide range of salt concentrations. It should also be noted that the ~60000 band of 11S (representing the combined acidic and basic subunits) was quite enriched in the 0.35 M fraction. The results present the possibility of purifying the various globulins on the basis of their solubility in NaCl.

**Immunological Cross-Reactions with Antibodies to Soybean β-Conglycinin and Glycinin.** Storage proteins are known to show extensive homologies with each other. To test the possible genetic relationship between mungbean globulins and soybean globulins, the purified globulins from mungbean were subjected to an enzyme-linked immunological test with antibodies to soybean β-conglycinin α, α′, and β subunits. The 28000 band of basic 7S cross-reacted with anti-α and α′ subunits and to a lesser degree with the anti-β subunit (Figure 8). The 42000 complex of basic 7S also cross-reacted with the different antibodies. The first three major bands of 8S cross-reacted with antibodies to all

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**Table 2. N-Terminal Amino Acid Sequence of Mungbean Globulins and Sequence Analysis for Homology with Other Storage Proteins**

| globulin | kDa | N-terminal amino acid sequence | | | | sequence analysis[a] |
|---|---|---|---|---|---|---|
| 8S band 1 | 60 | EDKEE | Q | | | similar to sequences of the extension regions of α and α′ subunits of β-conglycinin (*28*) |
| 8S band 2 | 48 | IDAAE | VSVSR | GKNNP | FYFNN | 45.4% identity in 11 aa overlap with phaseolin precursor; 66.7% identity in 9 aa overlap with phaseolin β-type and α-type; 54% identity in 13 aa overlap with soybean β-conglycinin |
| 8Sband 3 | 32 | SKTLS | SQNEP | FNLRL | N | 78.6% identity in 14 aa overlap with canavalin precursor; 71.4% identity in 14 aa overlap with soybean β-conglycinin, β-chain precursor; 64.3% identity in 14 aa overlap with pea and *Vicia faba* vicilin precursor; 66.7% identity in 12 aa overlap with soybean β-conglycinin α-chain precursor |
| 8S band 4 | 26 | IDGAE | VSVSR | GKNNP | | very similar to 8S band 2 |
| Basic 7S band 1 | 28 | NYVMN | PAYVL | MKPTQ | KDAAL | 80−90% similar to the N-terminal sequence of 27000 MW protein of mungbean released in hot water (*20*) |
| Basic 7S band 2 | 16 | STTVG | HSGGT | MIST | | ∼80% similar to the N-terminal sequence of 16000 MW protein of mungbean released in hot water (*20*); 80.0% identity in 15 aa overlap with soybean basic 7S globulin precursor |
| 11S band 1 | 40 | SSSST | NNRF | | | no homology found with plant storage proteins |
| 11S band 2 | 24 | GLEET | IXSSK | | | identity in 10 aa overlap with 35 various types of storage proteins in legumes and cereals ranging from 50 to 90% |

[a] Using FASTA (*17*), unless otherwise stated.
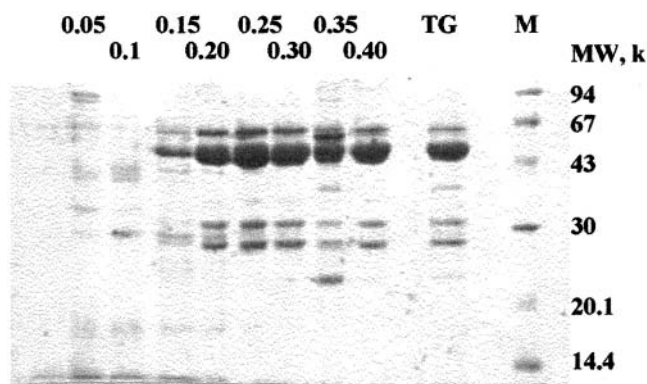


**Figure 7.** SDS-PAGE electrophoretic patterns of mungbean globulins serially extracted with increasing concentrations of sodium chloride.
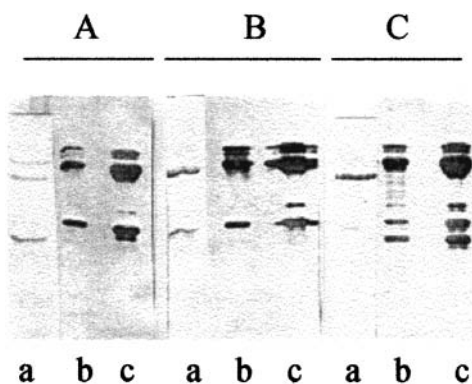


**Figure 8.** Immunological cross-reaction of mungbean globulins with antibodies to soybean globulins. Samples are basic 7S (lanes a), 8S (lanes b), and 11S (lanes c) versus anti-α (A), anti-α′ (B), and anti-β subunits (C). Not shown are the negative reactions of the different mungbean globulins against anti-glycinin.

types of conglycinin. Interestingly, only the fourth band of 8S crosss-reacted with the anti-β subunit. The bands of the 11S did not cross-react with any of the β-conglycinin antibodies. With soybean anti-glycinin, none of the mungbean globulins cross-reacted, although the positive control, soybean glycinin, reacted positively (data not shown).

**N-Terminal Amino Acid Sequences of Mungbean Globulins and Comparison with Other Storage**

**Proteins.** N-Terminal amino acid sequence analysis was done on the purified globulins from mungbean, and the results are shown in Table 2. The N-terminal sequence of the 8S band 1 was found to be very acidic with four of the first six amino acids consisting of aspartate and glutamate. β-Conglycinin α and α′ subunits have extension regions that are rich in acidic amino acids and have bigger molecular masses than that of β subunit (*28*), suggesting that the 8S band 1 belongs to the subunit type corresponding to the α and α′ subunits.

Of the first 20 amino acids determined for 8S band 2, which is the major band, the sequence KNNPYFNN was found to have 54−67% homology with phaseolin and β-conglycinin. The third 8S band had very strong homology (67−79%) in a 14 amino acid overlap with various storage proteins such as canavalin precursor, β-conglycinin, and faba bean vicilin precursor. The N-terminal amino acid sequence of the 8S band 4 was similar to that of the 8S band 2, except for the third amino acid, G instead of A. It is possible that the 26 kDa 8S band 4 is a fragment of 8S band 2. A similar phenomenon is known to occur with pea vicillin (*29, 30*).

The basic 7S band 1 was found to have 80−90% similar N-terminal amino acid sequence with the M1 and M2 mungbean proteins released from mungbean seeds when immersed in hot water and varying similarities with proteins from other similarly treated legume seeds such as adzuki bean, cowpea, winged bean, and soybean (*20*). The basic 7S band 2 had 80% homology with soybean basic 7S globulin precursor in a 15 amino acid overlap. Its N-terminal amino acid sequence was ∼90% similar to that of M3 protein from hot-water-treated seeds of mungbean (*20*). Such proteins released from legume seeds immersed in hot water were found to strongly cross-react with antibodies to soybean basic 7S, either the 27000 or 16000 subunit.

No results were obtained with 11S band 1 after two attempts despite the much higher amount of sample applied in the second attempt. It is known that the N-terminal Gln could easily form pyroglutamic acid under acidic conditions. However, even when the PVDF membrane was not stained in acidic Ponceau S, the same results were obtained. This suggests that the 11S band 1 could have a blocked N-terminal amino acid. The N terminals of the 11S type proteins of oat (*31, 32*), cotton (*33*), rice (*34*), and rape (*35*) are known to be

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blocked. By treating the 11S band 1 on the PVDF membrane with pyroglutamate amino peptidase, its N-terminal amino acid sequence was determined to be SSSSTNNRF. However, sequence analysis did not show homology of the mungbean 11S band 1 with plant storage proteins.

The N-terminal amino acid of the 11S band 2, the basic polypeptide, was identified to be glycine, which is typical of the basic polypeptides of 11S groups (*6*). The 11S band 2 had 50–90% homology with storage proteins in legumes as well as cereals in a 10 amino acid overlap. High (90%) homology was found with pea legumin K fragment and J precursor and *Vicia faba* legumin type B, but only 50% homology was found with soybean glycinin G1, G2, and G3 and 60% with G4. On the other hand, rice glutelin had 50–60% homology with mungbean basic polypeptide of the 11S globulin.

The results show the great homology of the 8S bands 2, 3, and 4 with soybean $\beta$-conglycinins, basic 7S with soybean basic 7S, and 11S with soybean glycinin and other globulins from other legumes and cereals.

## ACKNOWLEDGMENT

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