Partial Purification and Characterization of the 15S Globulin of Soybeans, a Dimer of Glycinin

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During preparative gel filtration of crude glycinin (11S ultracentrifugal component of soybean proteins), the second fraction that eluted just before the major peak (glycinin) was found to be enriched in the 15S component. Gel electrophoresis under denaturing conditions indicated that this fraction was identical to glycinin, suggesting that the 15S component is an aggregate of glycinin. Upon rechromatography of pooled, freeze-dried, 15S-enriched (50% 15S prior to freeze-drying) fractions the purity of 15S was only 39%, apparently because of partial dissociation of the 15S fraction into 11S component as a result of dialysis and freeze-drying. When freeze-drying was avoided, two successive rechromatographies yielded 15S preparations of 63−66% purity. However, on storage in solution, the purified 15S fraction slowly dissociated into 11S component; after 20 days, the composition was 47% 15S and 31% 11S. Gel electrophoresis and amino acid analysis confirmed the identity of the 15S fraction with glycinin. Molecular weight estimations by gel filtration indicated that the 15S fraction is a dimer of glycinin.

Keywords: *Soybeans; 15S globulin; glycinin; dimer; purification; stability*

INTRODUCTION

The 7S (primarily β-conglycinin) and 11S (glycinin) fractions constitute the majority of soybean proteins, while the 15S fraction is a minor component and typically ranges from 3 to 7% of the water- or buffer-extractable proteins (Wolf and Briggs, 1956; Wolf et al., 1961, 1962). Little is known about the 15S fraction except that it often occurs in 11S preparations and that it undergoes some of the same reactions as the 11S fraction such as dissociation into subunits at low ionic strength and in the presence of detergents or urea (Wolf and Briggs, 1958). Early attempts to remove the 15S fraction from 11S preparations were usually unsuccessful (Wolf and Briggs, 1958, 1959; Wolf et al., 1962). Catsimpoolas (1969) suggested that the 15S fraction was a polymer or copolymer of glycinin, β-conglycinin, and γ-conglycinin. Later Catsimpoolas (1977) proposed that the 15S component is a polymer of β-conglycinin. Derbyshire et al. (1976) speculated that the 15S protein in legumes is an association product of the 11S protein linked by disulfide bonds. However, the soybean 15S fraction is stable to 2-mercaptoethanol at concentrations up to 0.25 M (Briggs and Wolf, 1957) and to 0.1 M dithiothreitol (Wolf, 1993), making it unlikely that it is a disulfide-linked polymer of glycinin.

There are no reports on purification and characterization of the 15S fraction of soybeans, but in the course of purifying glycinin by preparative gel filtration, we repeatedly observed that the fraction eluting just ahead of glycinin was enriched in the 15S fraction. Preliminary experiments indicated that it was indistinguishable from glycinin by gel electrophoresis (Wolf, 1993). We have therefore attempted to purify the 15S fraction more thoroughly and to characterize it as described here.

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MATERIALS AND METHODS

Purification of Glycinin. Crude glycinin was prepared from hexane-defatted flakes of Raiden cultivar, 1987 crop (stored at 4 °C), according to the procedure of Thanh and Shibasaki (1976) and subjected to concanavalin A affinity chromatography to remove β-conglycinin contaminants followed by gel filtration (Wolf, 1993). In a typical experiment, 4 g of freeze-dried crude glycinin was dispersed in 100 mL of buffer (pH 7.6, 0.5 ionic strength, 0.033 M K_2HPO_4, 0.0026 M KH_2PO_4, 0.4 M NaCl, 0.02% NaN_3, and 0.01 M 2-mercaptoethanol) and centrifuged. The supernatant (containing about 3.6 g of glycinin on the basis of absorbance at 280 nm) was applied to a concanavalin A−Sepharose 4B (Pharmacia Biotechnology Inc., Piscataway, NJ) column (12.5 × 2.6 cm i.d.) and eluted with buffer until the absorbance at 280 nm returned to the baseline. The unadsorbed protein fraction (primarily glycinin) was concentrated by pervaporation or ultrafiltration to about 25 mL (protein concentration in a typical experiment, 6.4%) and then applied to two Sephacryl S-300 high-resolution (Pharmacia) columns (93 × 2.6 cm i.d.) connected in tandem. The XK 26 columns were fitted with AK 26 adapters (Pharmacia) to ensure even flow and to minimize zone broadening as a result of connecting two columns together (Hagel et al., 1989). The columns were equilibrated and eluted at 4 °C with the buffer described above but also containing 1 mM disodium ethylenediaminetetraacetate (henceforth referred to as standard buffer). Fractions of 9.5−10.6 mL were collected at flow rates of 14−18 cm h^{-1}. Flow rates exceeding the manufacturer's recommendations (Pharmacia, 1993) were used because preliminary experiments showed that the 15S protein slowly dissociated into 11S protein. The high flow rates were a compromise between optimal flow rates and the need to minimize dissociation of the 15S protein during the time needed for preparation of multiple batches for pooling and subsequent rechromatography. Results with crude glycinin at flow rates of 3 cm h^{-1} were similar to those at 14−18 cm h^{-1} except for greater resolution of the peaks. Absorbance was measured at 280 nm with appropriate dilution when absorbance exceeded 1. Tubes were pooled as shown in the elution diagrams; fractions were concentrated by ultrafiltration or pervaporation, if necessary, and adjusted in concentration for ultracentrifugal analysis in standard buffer. After ultracentrifugal analysis, the samples were dialyzed against distilled water in the cold for 3−5 days and freeze-dried prior to analysis by gel electrophoresis.

Purification of 15S Fraction. Preparations enriched in 15S obtained in the purification of glycinin were pooled, concentrated, and resubmitted to gel filtration as described above for glycinin.

Calibration of Gel Filtration Columns. The Sephacryl S-300 columns used to prepare the 15S protein were calibrated with a MW-GF-1000 molecular weight marker kit (Sigma Chemical Co., St. Louis, MO) consisting of carbonic anhydrase (29 000), bovine serum albumin (66 000), alcohol dehydrogenase (150 000), β-amylase (200 000), apoferritin (443 000), thyroglobulin (669 000) and blue dextran (2 000 000). Ovalbumin (43 000) was also used. Except for thyroglobulin and carbonic anhydrase, the standards were run individually to avoid overlapping of eluting peaks. Flow rates were 3.6−4.9 cm h^{-1}. A calibration curve was prepared by plotting the log of molecular weight versus V_e/V_0, where V_e is the elution volume and V_0 is the void volume.

Ultracentrifugal Analysis. Samples were analyzed in standard buffer (described above) in a Beckman Model E analytical ultracentrifuge at 20−25 °C with a 30 mm double-sector cell at 48 000 rpm. Compositions were estimated by enlarging the ultracentrifuge patterns and measuring the area under each peak. Areas were corrected for radial dilution, and compositions are expressed as percentages of the total area.

Gel Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to the Fling and Gregerson (1986) modification of the Laemmli (1970) method using a Hoefer Scientific Instruments (San Francisco, CA) Model SE 600 unit. Gels of 1.5 mm were used in which AcrylAide (FMC BioProducts, Rockland, ME) was substituted for *N*,*N*′-methylenebis(acrylamide) as the cross-linking agent. Gel compositions were 13.5% T and 0.4% C. Proteins were stained with Coomassie Blue (Fling and Gregerson, 1986). A broad range of molecular weight standard proteins was used consisting of myosin, β-galactosidase, phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme, and aprotinin (Bio-Rad Laboratories, Hercules, CA).

Amino Acid Analysis. Analyses were performed on glycinin and 15S preparations by the University of Illinois Biotechnology Center, Genetic Engineering Facility (Urbana, IL), using an HP 1090 liquid chromatograph with AminoQuant (*o*-phthalaldehyde) derivatization and fluorescence detection according to the manufacturer's instructions (Hewlett-Packard, Palo Alto, CA). The samples were hydrolyzed with 6 M HCl at 110 °C for 24 h in sealed tubes after vacuum removal of air.

Statistical Analysis. Statistical analysis was done in procedures GLM and REG of SAS Software (SAS, 1989).

RESULTS

Purification. Numerous preparative purifications of glycinin by concanavalin A−Sepharose chromatography followed by gel filtration on Sephacryl S-300 yielded elution diagrams similar to Figure 1A. Tubes were pooled into six fractions for analysis by ultracentrifugation and SDS−PAGE. Fraction a eluted with the void volume and was turbid; on concentration and equilibration against standard buffer, the soluble portion contained primarily 15S and >15S fractions plus 11S component. Enrichment of the 15S fraction was almost 3-fold as compared to the starting preparation (Table 1). Further enrichment in 15S fraction occurred in fraction b. Depending on where the cut was made, content of 15S in fraction b ranged from 26 to 56%; the value of 43% in Table 1 is the mean for 10 runs. The main peak (fractions c and d) was predominantly glycinin; the leading edge of the peak generally was 90% or higher in 11S component with 15S as the major contaminant. The trailing edge of the peak (fraction d) often was free of 15S but contained 7S material and sometimes also 2S fraction. Fractions e and f were predominantly 2S and 7S proteins with almost 90% 2S

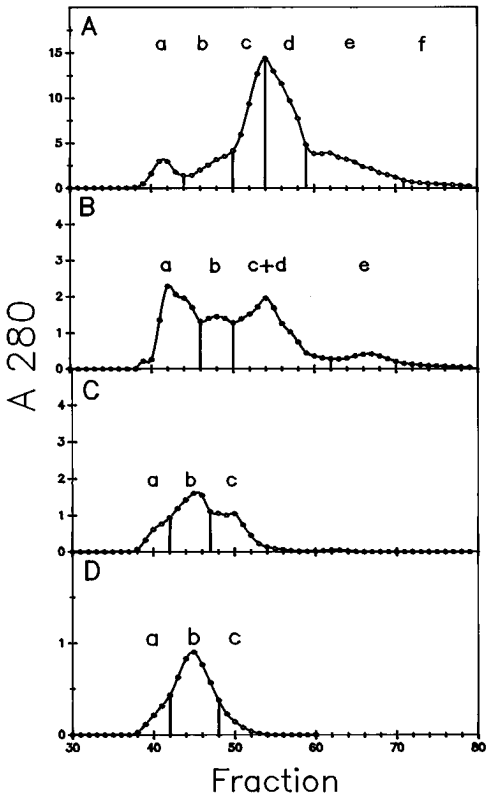

Figure 1. Gel filtration elution patterns for (A) crude glycinin after passage through a concanavalin A−Sepharose column; (B) pooled, freeze-dried 15S-rich fractions b from runs similar to (A); (C) pooled 15S-rich fractions b from runs similar to (A), concentrated by ultrafiltration and not freeze-dried; and (D) rechromatography of fraction b from (C). Variations in flow rates and fraction sizes account for horizontal displacement of similar fractions (e.g., fraction b) between different elution patterns.

in fraction f. Yields of the fractions for one experiment are also given in Table 1. Typically, the yield of glycinin (fraction c plus d) was about 1 g.

SDS−PAGE analysis of the fractions from the gel filtration depicted in Figure 1A is shown in Figure 2. Crude glycinin (lane 2), β-conglycinin contaminants removed from the crude glycinin by concanavalin A−Sepharose chromatography (lane 3), and the partially purified glycinin placed on the column (lane 4) are shown for comparison. Fractions a−d (lanes 5−8) show surprisingly few differences and are typical of patterns for glycinin. Fraction e (lane 9) contains what appears to be a major acidic polypeptide band of glycinin but only a minor basic polypeptide which is not in the normal ratio of acidic and basic polypeptide bands (lanes 7 and 8). In addition, fraction e contains bands at 44 (minor), 31, 18, and 17 kDa. Fraction f (lane 10) resembles fraction e but also has bands of 60 (minor), 23, and 15 kDa. The results obtained for fractions b−d strongly suggest that the 15S fraction is a polymer of glycinin.

Attempts were then made to purify the 15S fraction further by pooling freeze-dried preparations of fraction b from three separate runs and resubmitting them to gel filtration.

Although the pooled fractions contained 50% 15S component prior to freeze-drying (Table 1), the resulting elution diagram was more complex than expected (Figure 1B). Three major fractions and one minor fraction were obtained. Fraction a was complex, with about one-third of it consisting of very quickly sedi-

Table 1. Ultracentrifugal Composition of Samples Subjected to Gel Filtration and Resulting Fractions Obtained

protein sample	fraction	*n*	composition,[a] % 2S	7S	11S	15S	>15S	>>15S	>>>15S	yield,[b] mg
glycinin after concanavalin A chromatography	total	3	13.9[c]	14.4[b]	63.8[b]	7.9[c]	0.0[c]	0.0[b]		1651
	a	9	6.4[de]	6.0[c]	16.3[d]	21.7[b]	27.5[a]	18.9[a]		39
	b	10	2.2[e]	4.2[c]	34.1[c]	43.2[a]	12.7[b]	3.6[b]		124
	c	10	0.0[e]	3.3[c]	90.1[a]	6.6[c]	0.0[c]	0.0[b]		487
	d	10	3.6[de]	8.7[bc]	86.1[a]	1.6[c]	0.0[c]	0.0[b]		507
	e	10	47.3[b]	41.2[a]	11.5[d]	0.0[c]	0.0[c]	0.0[b]		243
	f	4	88.9[a]	10.4[bc]	0.7[e]	0.0[c]	0.0[c]	0.0[b]		6
pooled, freeze-dried 15S	total[c]	1	0.6	0.4	32.6	50.2	12.2	4.1		395
	a	1	7.9	4.0	12.3	17.6	14.7	11.2	32.4	58
	b	1	6.4	4.2	27.3	38.6	16.3	7.3		33
	c + d	1	9.2	10.0	68.9	11.7				55
	e	1	92	3.7	3.9					77
pooled, non-freeze-dried 15S	total	2	2.9[bc]	3.4[b]	37.0[b]	42.6[b]	9.6[b]	5.4[b]		644
	a	2	4.9[a]	3.6[b]	19.3[c]	25.8[c]	28.3[a]	18.1[a]		15
	b	2	0.8[c]	2.6[b]	25.5[c]	51.7[a]	11.4[b]	8.0[b]		90
	c	2	3.9[bc]	6.7[a]	72.6[a]	15.2[d]	1.7[c]	0.0[c]		72
fraction b from Figure 1C	total	2	0.8[a]	2.6[a]	25.5[b]	51.7[b]	11.4[a]	8.0[a]		69
	a	2	5.7[a]	13.5[a]	25.0[b]	20.4[c]	20.8[a]	14.7[a]		7
	b	2	0.0[a]	0.0[a]	19.6[b]	64.2[a]	14.2[a]	2.1[a]		36
	c	2	0.0[a]	2.4[a]	69.7[a]	26.8[c]	1.1[a]	0.0[a]		6

[a] One to three ill-defined fractions sedimenting ahead of the 15S fraction were observed in some samples and are designated >15S, >>15S, and >>>15S. Means within a column followed by common letters are not significantly different as tested by protected ($p < 0.05$) least significant differences at $p < 0.05$. [b] Yield in total row is sample weight applied to the gel filtration column based on absorbance at 280 nm using $E^{1\%}_{1cm} = 8.4$ (Wolf, 1993); other values are weights of fractions recovered after dialysis and freeze-drying or estimates based on absorbance at 280 nm when samples were not freeze-dried. Data are from a single series of experiments. [c] Weighted averages for three pooled samples similar to fraction b, Figure 1A, prior to dialysis and freeze-drying.

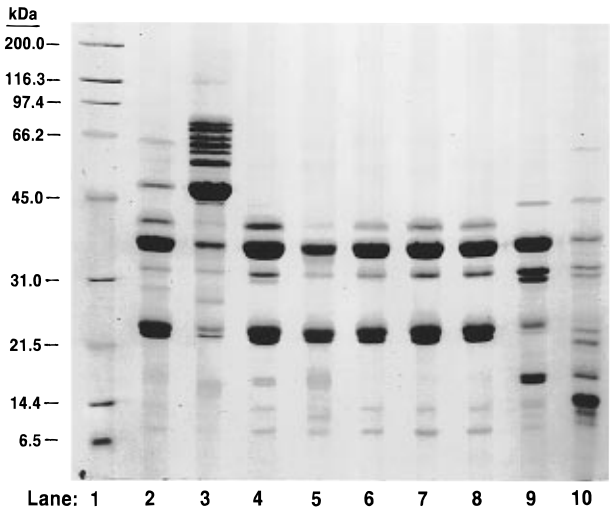

Figure 2. SDS−PAGE analysis of fractions obtained by gel filtration of partially purified glycinin as illustrated in Figure 1A: molecular weight standards (lane1); crude glycinin (lane 2); β-conglycinin contaminants removed from crude glycinin by concanavalin A chromatography (lane 3); crude glycinin after removal of β-conglycinin contaminants (lane 4); fraction a (lane 5); fraction b (lane 6); fraction c (lane 7); fraction d (lane 8); fraction e (lane 9); and fraction f (lane 10). Sample loads: lane 1, 23 μg; all others, 50 μg.

menting aggregates plus material ranging from 2S to >>>15S. Fraction b, which was expected to be enriched in 15S, contained only 39% 15S as compared to the initial content of 50% in the pooled sample prior to freeze-drying. Fractions c and d were combined and analyzed as a single sample and found to consist of about 70% 11S protein. Fraction e was essentially 2S material. Subsequent analysis of a portion of the freeze-dried protein originally placed on the column revealed the following composition: 2S, 9.7%; 7S, 6.1%; 11S, 48.6%; 15S, 20.9%; >15S, 9.2%; >>15S, 5.6%.

When compared with the typical composition shown in Table 1 (fraction b of glycinin after concanavalin A chromatography; Figure 1A), it is obvious that the pooled samples underwent considerable modification. The major change was a decrease in 15S from 50 to only 21% and an increase in 11S content from 33 to 49%. The 15S polymer of 11S apparently dissociated partially as a result of dialysis to remove the buffer salts followed by freeze-drying.

To avoid the effects of dialysis and freeze-drying, four 15S-rich fractions (fraction b from runs similar to Figure 1A) were concentrated by ultrafiltration, stored in the cold in standard buffer, and then pooled. The elution pattern for this pooled material is shown in Figure 1C. Again the pooled fractions eluted over a broader range (about 16 tubes) than expected considering that the original fractions were collected over a narrower range (4−6 tubes) in the initial gel filtration step. Fraction b was the major peak and contained 52% 15S compared to 43% for the pooled 15S-rich fraction that was placed on the column (Table 1). Fraction c was greatly enriched in 11S fraction.

When fraction b isolated from the pooled 15S-rich material (Figure 1C) was concentrated to about 5 mL by ultrafiltration and resubmitted to gel filtration, a more symmetrical peak was obtained (Figure 1D). Nonetheless, the peak again was about 15 tubes wide compared to 5 tubes when it eluted originally. Moreover, the purity of fraction b rose from 52 to only 64% as a result of repeating the gel filtration and the sample still contained 20% 11S as a contaminant. Figure 3 (day zero) shows the sedimentation pattern for the 15S-enriched fraction.

Figure 4 shows SDS−PAGE analysis of 15S-rich fraction after one (lanes 4 and 5), two (lane 6), and three gel filtrations (lane 7). Partially purified glycinin after concanavalin A chromatography (lane 2) and purified glycinin (lane 3) are shown for comparison. There is no obvious effect of repeated gel filtration, and the 15S fractions are indistinguishable from glycinin. These results provide additional evidence that the 15S fraction is an aggregate of glycinin which is disrupted by SDS and 2-mercaptoethanol with the result that identical SDS−PAGE patterns are obtained for glycinin and 15S-

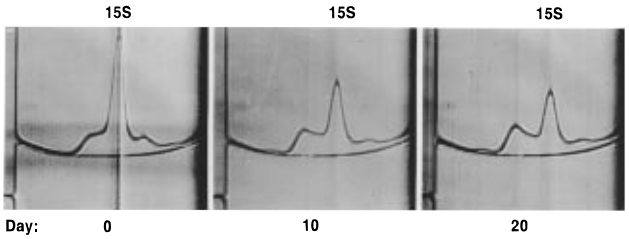

Figure 3. Ultracentrifugal patterns of 15S fraction obtained by three successive gel filtrations (fraction b, Figure 1D) at three storage times in about 0.5% solution in standard buffer. Sedimentation is from left to right.

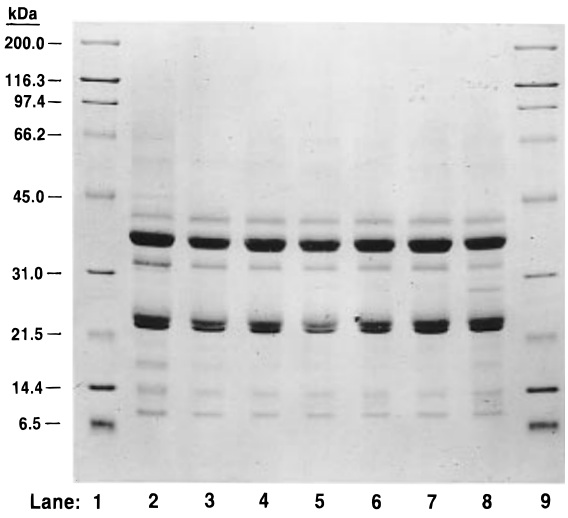

Figure 4. SDS−PAGE analysis of partially purified glycinin, purified glycinin, and 15S-rich fraction: molecular weight standards (lanes 1 and 9); partially purified glycinin after passage through concanavalin A column (lane 2); purified glycinin comparable to fraction d in Figure 1A (lane 3); 15S-rich material comparable to fraction b in Figure 1A (lane 4); pooled 15S-rich fractions used for second chromatography in Figure 1C (lane 5); 15S-rich material after second chromatography, fraction b, Figure 1C (lane 6); fraction b from third chromatography, Figure 1D (lane 7); and fraction b from third chromatography, Figure 1D, after storage in solution for 20 days (lane 8). Sample loads: lanes 1 and 9, 23 μg; all others, 50 μg.

rich fractions. The role of 2-mercaptoethanol under these conditions is to cleave the disulfide bond linking the acidic and basic polypeptides within the subunits of glycinin. In the absence of SDS, 2-mercaptoethanol at concentrations up to 0.25 M had no effect on the ultracentrifugal behavior of the 15S protein (Briggs and Wolf, 1957).

Stability of 15S in Solution. Results described earlier with pooled, freeze-dried, 15S-rich fractions (Figure 1B) suggested that the 15S fraction is partially disaggregated as a result of dialysis to remove buffer salts followed by freeze-drying. These results together with the inability to obtain pure 15S fraction upon repeated gel filtration suggested that disaggregation of 15S fraction may also occur in solution. Accordingly, four preparations of 15S-rich fraction ranging from 45 to 66% 15S were stored in about 0.5% solution at 4 °C except for about 1 h necessary for each of the ultracentrifugal analyses. The solutions were analyzed periodically at room temperature. All samples showed a slow decrease in 15S fraction with an accompanying increase in 11S fraction. Figure 3 shows sedimentation patterns at 0, 10, and 20 days for the 66% 15S preparation (fraction b, Figure 1D). Compositional data for the entire time series are shown in Figure 5. Curves fitted

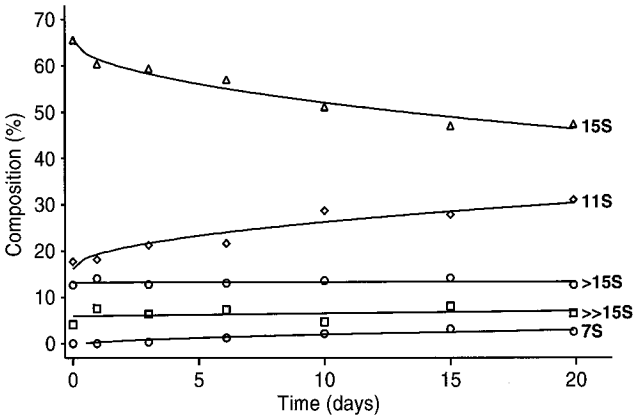

Figure 5. Ultracentrifugal composition of 15S fraction obtained by three successive gel filtrations (fraction b, Figure 1D) as a function of storage time in about 0.5% solution in standard buffer at 4 °C except for about 1 h at room temperature required for each ultracentrifugal analysis.

by least-squares procedures are described by the equations

$$\% \text{ 7S} = -0.46 + 0.75t^{1/2} \qquad (R^2 = 0.87)$$

$$\% \text{ 11S} = 16.10 + 3.20t^{1/2} \qquad (R^2 = 0.91)$$

$$\% \text{ 15S} = 65.78 - 4.35t^{1/2} \qquad (R^2 = 0.90)$$

where t is the time in days. The >15S and >>15S fractions did not change significantly with time of storage. Clearly the 15S component is being converted into 11S component. The 15S fraction decreased from 66 to 47% while the 11S fraction increased from 18 to 31% over a period of 20 days. The other fractions showed no changes except for the appearance of about 3% 7S component.

SDS−PAGE analysis of the sample after 20 days of storage (Figure 4, lane 8) resulted in a gel pattern typical of glycinin and the original 15S-rich fraction except for the appearance of a minor band of about 28 kDa. Apparently only very minor changes occurred in 3 weeks of storage (in the presence of sodium azide) except for interconversion of 15S to 11S (Figure 5).

Early studies on the stability of 11S contaminated with 4−5% of 15S fraction indicated that the 15S component dissociated into smaller units when the ionic strength was lowered from 0.5 to 0.01 at pH 7.6 (Wolf and Briggs, 1958). When the ionic strength was returned to 0.5, the 15S fraction did not reappear. A similar experiment was performed with the 15S-rich fraction (fraction b, Figure 1D). A portion of a stock solution in standard buffer was diluted with Tris buffer, pH 7.6, μ = 0.01, and then dialyzed against the Tris buffer. The solution became turbid shortly after dialysis began but cleared up again on continued dialysis. After equilibration against Tris buffer, the sample was analyzed in the ultracentrifuge and brought back to 0.5 ionic strength buffer by dialysis against standard buffer. This resulted in formation of turbidity and precipitation of a small amount of protein. The sample was then reanalyzed in the ultracentrifuge (Table 2). Lowering the ionic strength from 0.5 to 0.01 decreased the 15S from 66 to 21% and increased the 11S from 18 to 41%, accompanied by appearance of 14% 2S and 24% 7S. Restoring the ionic strength to 0.5 had no effect on the 15S fraction but some of the 2S and 7S fractions reassociated to the 11S form. Breakdown of the 15S

Table 2. Effect of Lowering and Raising the Ionic Strength on the Ultracentrifugal Composition of the 15S Component

	ultracentrifugal composition, %					
buffer	2S	7S	11S	15S	>15S	>>15S
standard, $\mu = 0.5$			17.7	65.6	12.6	4.1
Tris, $\mu = 0.01$	14.2	24.1	40.6	21.1		
standard, $\mu = 0.5$	9	5.1	52.4	19.9	13.5	

Table 3. Amino Acid Composition (Mole Percent) of Glycinin and 15S-Rich Fractions

amino acid	glycinin[a]	15S-rich fraction[b]	pooled 15S-rich fraction[c]	purified 15S[d]	glycinin from lit.[e]
Asp	13.72	12.76	13.90	12.41	12.65
Glu	20.03	19.49	21.03	19.21	15.49
Ser	5.79	5.71	5.80	5.66	5.3
His	1.69	1.49	1.62	1.47	1.8
Gly	8.39	8.59	8.06	8.77	7.73
Thr	4.16	4.10	4.12	4.02	3.71
Ala	5.68	5.76	5.77	5.75	5.55
Arg	6.07	6.02	6.03	6.04	5.51
Tyr	2.57	2.55	2.46	2.52	2.77
Cys[f]	0.62	0.66	0.54	0.64	0.70
Val	5.27	5.12	5.01	4.99	5.72
Met	1.13	1.37	1.05	1.31	1.56
Phe	4.45	4.41	4.52	4.35	4.3
Ile	4.61	4.73	4.94	4.61	4.61
Leu	7.71	7.74	7.76	7.76	7.04
Lys	4.10	4.40	4.11	4.42	4.23
Pro	4.33	5.43	3.56	6.37	6.17

[a] Preparation similar to fraction d, Figure 1A. [b] Preparation similar to fraction b, Figure 1B. [c] Pooled fractions b from four runs similar to Figure 1B. [d] Fraction b, Figure 1D. [e] Badley et al. (1975). [f] Based on cystine values; samples were not oxidized with performic acid.

component apparently is irreversible when ionic strength is changed as described here.

Amino Acid Analysis. Additional evidence for identity of the 15S fraction with glycinin was obtained by amino acid analysis (Table 3). The estimates of amino acid composition of the three 15S fractions were all within 0.5% of the estimates for glycinin with the exception of the values for aspartic acid, glutamic acid, and proline. The differences in amino acid composition between the 15S-rich fraction and glycinin were similar ($r = 0.98$) to the differences in composition between purified 15S and glycinin. Published values for glycinin (Badley et al., 1975) are also shown in Table 3. Except for glutamic acid and proline, agreement is good with our data for glycinin considering that Badley and co-workers prepared their glycinin from a commercial soybean meal and by a different method than used here.

Molecular Size of 15S Protein Relative to 11S Protein. The size of the 15S protein relative to the 11S protein was estimated by gel filtration by calibrating the Sephacryl columns with protein standards. The calibration curve shown in Figure 6 was utilized to estimate the molecular sizes of the 11S and 15S proteins at various stages of purification of the 15S protein (Table 4). The first two samples of Table 4 (crude glycinin) were chromatographed under preparative conditions of large sample volume (23−26 mL) and high flow rates (14−18 cm h^{-1}). The pooled 15S-enriched samples were in smaller volumes (4−17 mL) but still at high flow rates (14−15 cm h^{-1}). The last sample, crude glycinin, was chromatographed under more ideal conditions of small sample volume (9 mL) and low flow rate (3 cm h^{-1}). The molecular sizes of the 11S and 15S fractions varied from run to run, possibly because nonideal conditions were used for the first five samples.

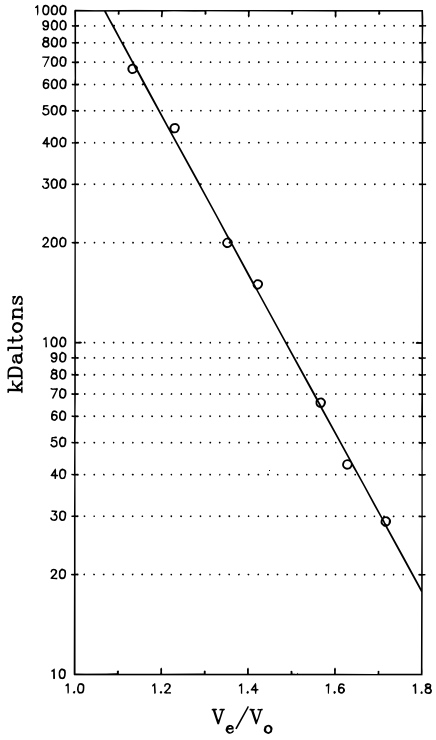

Figure 6. Calibration curve for Sephacryl S-300 columns connected in tandem. The log of molecular weight of the protein standards is plotted against V_e/V_0, where V_e is the elution volume and V_0 is the void volume.

The center of the 15S peak was difficult to estimate when large samples were chromatographed (Figure 1A), resulting in small changes in V_e/V_0 but large changes in molecular size; in the high molecular weight range of the calibration curve (Figure 6) the molecular weights are very sensitive to small variations in V_e/V_0. Under more ideal conditions, crude glycinin (last sample in Table 4) gave a value of 320 kDa for 11S, in agreement with the value reported by Badley et al. (1975). Under these conditions the 15S peak was well-defined (data not shown) and the molecular size of 660 kDa was very close to twice that of the 11S protein. Despite variability of molecular sizes for the various runs, the ratios of 15S:11S were nearly constant at 2.14 ± 0.16, indicating that the 15S protein is a dimer of glycinin.

CONCLUSIONS

Attempts to purify the 15S component beyond 66% homogeneity were unsuccessful because of the inherent instability of this protein. It appears that dissociation of 15S is more rapid during gel filtration than in solutions allowed to stand after gel filtration (Figure 5). Possibly the dissociation reaction rate is increased by removal of the 11S fraction as the protein migrates through the gel filtration column. Nonetheless, results of SDS−PAGE (Figures 2 and 4) and amino acid analysis leave little doubt that the 15S is an aggregate of glycinin (11S).

Marcone et al. (1994) recently isolated a fraction from crude glycinin by Sephacryl S-300 gel filtration, estimated its size to be about 506 kDa, and concluded that it was an aggregate of glycinin. On the basis of a molecular mass of 320 kDa for glycinin (Badley et al., 1975), this aggregate is apparently a dimer and may correspond to the 15S component. Contrary to the results shown in Figure 1A, Marcone and co-workers obtained almost baseline resolution of this putative 15S

Table 4. Gel Filtration Conditions, Molecular Sizes, and Estimates of 15S:11S Ratio

sample	sample vol, mL	sample wt, mg	flow rate,[a] cm h^{-1}	molecular size,[b] kDa 11S	15S	ratio of 11S:15S
crude glycinin (Figure 1A)[c]	25.8	1651	17.9	340	790	2.32
crude glycinin[c]	23.2	1191	14.1	435	900	2.07
pooled 15S, freeze-dried (Figure 1B)[c]	9.5	371	14.1	275	630	2.29
pooled 15S, non-freeze-dried (Figure 1C)[c]	17.0	644	15.0	460	900	1.96
pooled 15S, rechromatographed (Figure 1D)[c]	4.2	69	14.0		780	
crude glycinin[d]	9.2	232	3.3	320	660	2.06
mean ± SD				366 ± 79	777 ± 115	2.14 ± 0.16

[a] Cross-sectional area of column = 5.31 cm^2. [b] Estimated from calibration curve (Figure 6). [c] Preparative conditions, especially flow rate (14.0−17.9 cm h^{-1}). [d] Analytical conditions of low sample volume and low flow rate.

fraction from the 11S fraction and it represented a much greater proportion of the total protein than the 11S fraction. In the chromatography system used here, the 15S-rich fraction was always much smaller than the 11S fraction, which is the major peak. For example, Table 1 shows that the freeze-dried yield of 15S (fraction b, Figure 1A) was only about 12% of the yield of 11S fraction (fractions c and d, Figure 1A). The purification procedure used here, involving concanavalin A chromatography followed by gel filtration on Sephacryl S-300 columns, has been used routinely to prepare gram quantities of glycinin for other studies.

Interconversion of 15S into 11S (Figure 5) provides additional evidence that the 15S fraction is an aggregate of 11S, and this instability presumably accounts for the inability to achieve a higher degree of homogeneity in this study. Although the 15S protein was slowly converted into 11S protein, the reverse reaction was not observed. The experiment in Table 2 indicates that when 15S is dissociated by lowering ionic strength, it is not re-formed when the ionic strength is raised. Early studies on 11S also showed that it is stable in solution; no generation of 15S was noted upon dialysis of 11S in buffer for up to 70 days (Wolf et al., 1962). These same studies showed, however, that conversion of 11S to 15S fraction occurs when 11S preparations are frozen and thawed or precipitated by dialysis against water and freeze-dried. High concentrations of 11S such as occur on freezing or precipitation may be required to facilitate formation of the 15S aggregate.

Danielsson (1949) first reported the presence of 15−18S fractions in some legume proteins and subsequently suggested that they are association products of the 11−13S major globulins (Danielsson, 1956). Later, Mori and Utsumi (1979) isolated the 15S fraction of broad beans (*Vicia faba* L.) by sucrose density gradient centrifugation and showed it to be 1.88 times the size of the 11S fraction, i.e., a dimer. On recentrifugation of their isolated 15S fraction they observed an appreciable amount of 11S (roughly one-third of the total protein), but apparently they did not investigate the possible dissociation of 15S into 11S protein. The results of gel filtration (Table 4) indicate that in soybeans the 15S fraction is also a dimer of the 11S protein (glycinin). Similar studies with almond meal proteins have shown that the almond 15S protein likewise is an unstable dimer of amandin, the 11S globulin of almonds (unpublished results). It therefore appears that dimerization of the 11S protein of seeds may be a common phenomenon.

It is not clear whether the soybean 15S exists as a native protein in the seed or is an artifact formed during isolation of the proteins. It is, however, invariably found in simple extracts obtained by treatment consisting only of stirring defatted meal in water or salt solution followed by dialysis against buffer (Naismith, 1955; Wolf and Briggs, 1956), suggesting that it preexists in the meal.

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