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Determination of Glycinin and β-Conglycinin in Soybean Proteins by Immunological Methods

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Glycinin and β-conglycinin contents in soybean seeds and in protein fractions were immunologically assayed. Whole extractable proteins (WBE) were composed of 32% glycinin, 23% β-conglycinin, and 45% remainder unassayed by anti-glycinin and anti-β-conglycinin. The acid-precipitable fraction (APP) at pH 4.8 was composed of 34% glycinin, 27% β-conglycinin, and 39% remainder. The protein composition of APP was similar to that of WBE. The crude 7S, precipitating at pH 4.8, was composed of 52% β-conglycinin, 3% glycinin, and 45% remainder. However, the remainder content in the pH 6.4 precipitated fraction (crude 11S) was reduced from 45% to 15%. Thus, the amount of contaminating protein in globulin fraction is proportional to the acidity. Treatment with 10% NaCl and ammonium sulfate fractionation improved the glycinin plus β-conglycinin content. Contaminating proteins were evidently concentrated in a 0–51% saturated ammonium sulfate precipitated fraction.

Isolation of the two major reserve soybean proteins, glycinin (11S globulin) and β-conglycinin (7S globulin), has been examined by many researchers (Wolf, 1972). A method by Thanh and Shibasaki (1976), however, is the only straightforward procedure for simultaneous preparation of the two globulins. Almost all researchers have, during the last 5 years, used this method for preparing both glycinin and β-conglycinin for studies of thermal denaturation (Damodaran and Kinsella, 1982), functional properties (Nakamura et al., 1984; Umeya et al., 1980), and other properties of these proteins (Damodaran and Kinsella, 1981; Honig et al., 1984) since it is a simple and large-scale separation method.

Purity and composition of the various fraction obtained during isolation were usually determined by ultracentrifugation. Thanh and Shibasaki (1976) reported that ultracentrifugal analysis, discontinuous gel electrophoresis, and immunodiffusion indicated very little cross-contamination between the prepared fractions; however, the color of the freeze-dried crude 7S fraction is tan relative to that of the crude 11S fraction. This suggests a contamination by other components. Compared to ultracentrifugal (Wolf, 1970) or densitometric analysis (Sato et al., 1986), an immunological method (Iwabuchi and Shibasaki, 1981; Murphy and Resurreccion, 1984) is well suited for measuring the absolute amounts of glycinin and β-conglycinin in various fractions.

The present study was undertaken to (a) obtain the glycinin and β-conglycinin content in soybean seeds, (b) obtain quantitative information concerning the purity of protein fractions, and (c) study the effect of a reductant on protein fractionation.

EXPERIMENTAL SECTION

Materials. Soybeans (*Glycine max* var. Raiden) were finely ground in a Waring Blendor and defatted with hexane at room temperature. Soybean meals for protein extraction were used without screening. Sepharose CL-6B, DEAE-Sephadex A-50, and Con A-Sepharose 4B were purchased from Pharmacia Co., and 2-mercaptoethanol (2-ME) and all other reagent-grade chemicals were obtained from Nakarai Chemicals.

Methods. *Preparation of Whole-Buffer Extract, Acid-Precipitated Protein, Glycinin, and β-Conglycinin. Step 1. Preparation of the Isoelectric Precipitated Fraction.* A sample (100 g) of defatted meal was extracted once with 2 L of 0.03 M Tris-HCl buffer containing 10 mM 2-ME, at pH 8.0 at room temperature. After centrifugation the supernatant was designated whole-buffer extract (WBE I; Figure 1a). The WBE I was acidified to pH 4.8 with 2 N HCl. After centrifugation, the SUP I was the whey protein fraction and was designated Whey I. The precipitated protein curd was washed twice with pH 4.8 water, dispersed in water, and titrated to pH 8.0 while stirring. By these operations the acid-precipitated curd was completely resolubilized. The clear supernatant was designated acid-precipitated protein (APP; Figure 1a).

Another whole-buffer extract was prepared for preparation of crude 7S and 11S fractions. Following the procedure of Thanh and Shibasaki (1976), the 11S fraction was obtained by isoelectric precipitation at pH 6.4, resolubilized in a pH 7.6 phosphate buffer (2.6 mM KH_2PO_4, 32.5 mM K_2HPO_4, 0.4 M NaCl, 10 mM 2-ME, pH 7.6, ionic strength 0.5), and designated crude 11S fraction (Figure 1b). The SUP II was further separated into 7S and whey fractions by acidification to pH 4.8. The resulting precipitate was centrifuged, completely resolubilized in 0.03 M Tris-HCl buffer by back-titrating to pH 8.0, and designated crude 7S fraction (Figure 1b). The SUP III was designated Whey II. Usually pH 4.5 rather than pH 4.8 has been used to prepare globulins. Here and in Figure 1a we used pH 4.8 according to Thanh and Shibasaki (1976) and Wolf and Briggs (1959).

Step 2. Fractionation with 10% NaCl Extraction. Another resolubilization of the acid-precipitated curds of

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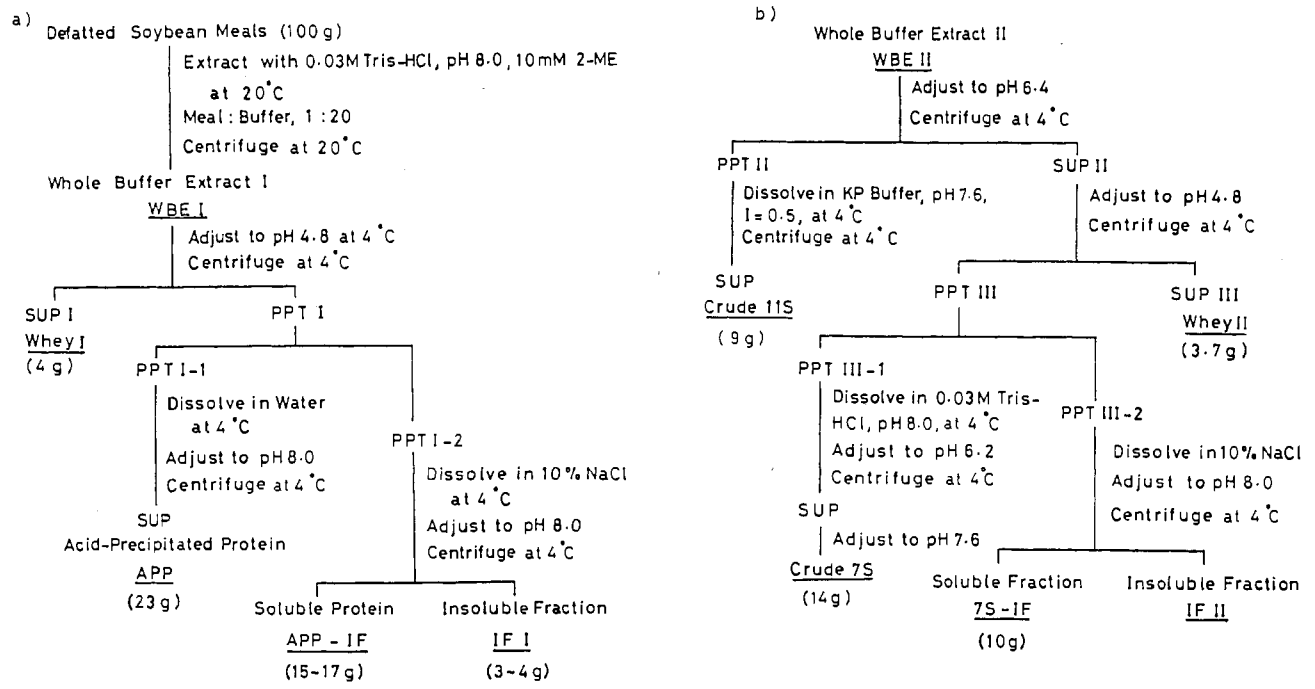

Figure 1. Schematic diagram for preparation of acid-precipitated protein (APP) and 7S and 11S fractions and removal of insoluble fraction (IF) by treatment with 10% NaCl. Whole-buffer extract in (b) was prepared as in (a). Each precipitated fraction was removed by centrifugation (15 000 rpm for 20 min) and washed with the appropriate solvent in order to remove the remaining supernatant.

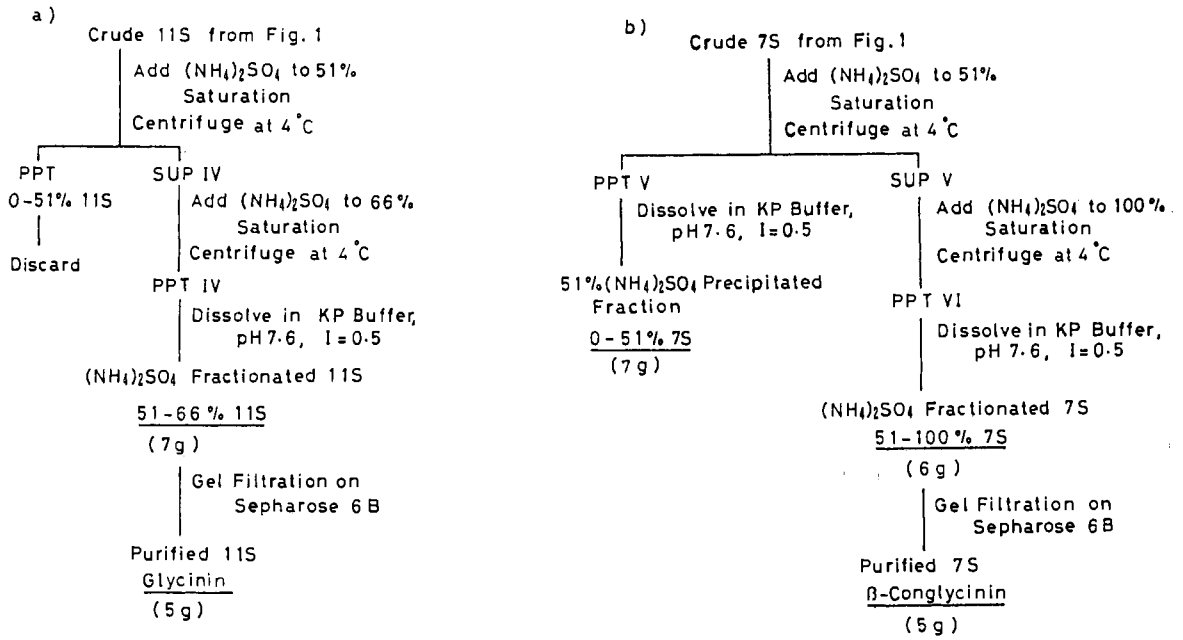

Figure 2. Schematic diagram of the purification of 7S and 11S fractions by ammonium sulfate fractionation. Proteins for ammonium sulfate fractionation have concentrations in a range of 0.5–1.0% in pH 7.6 phosphate (KP) buffer containing 10 mM 2-ME.

PPT I and PPT III was examined with 10% NaCl instead of water or 0.03 M Tris-HCl buffer. By treatment with 10% NaCl containing 10 mM 2-ME, the curd was divided into salt-soluble protein and -insoluble fractions; PPT I-2 was fractionated into a soluble fraction (APP-IF) and an insoluble fraction (IF-I). PPT III-2 was also fractionated into 7S-IF and IF-II (Figure 1b).

Step 3. Fractionation with Ammonium Sulfate. The crude 11S fraction was further fractionated with ammonium sulfate according to Wolf et al. (1962). To the crude 11S fraction was added solid ammonium sulfate to 51% saturation. The small amount of precipitate at 51% saturation was centrifuged and discarded. To the supernatant was added still more solid ammonium sulfate to give 66% saturation. The resulting precipitate was centrifuged and designated ammonium sulfate fractionated 11S (51–66% 11S; Figure 2a).

The crude 7S fraction was fractionated with ammonium sulfate according to a modified method by Roberts and Briggs (1965). Adjusting the crude 7S fraction to 51% saturation precipitated more than half of the protein. This was collected and designated 51% ammonium sulfate precipitated fraction (0–51% 7S; Figure 2b). The remaining 7S globulin was precipitated at 90–100% saturation, centrifuged, and designated ammonium sulfate fractionated 7S (51–100% 7S; Figure 2b).

The acid-precipitated fraction (PPT I in Figure 1a) was also fractionated with ammonium sulfate at 51% saturation followed by 100% saturation. The resulting precipitates were referred to as 51% ammonium sulfate precip-

itated fraction of APP (0–51% APP) and ammonium sulfate fractionated APP (51–100% APP).

Step 4. Gel Filtration on a Sepharose CL-6B Column. Each fraction of the ammonium sulfate fractionated APP, 7S, and 11S was finally purified on a Sepharose CL-6B column (4.2 × 130 cm). Elution was performed with the phosphate buffer (pH 7.6, ionic strength 0.5). Purified 7S and 11S globulins achieved by these operations were designated β-conglycinin and glycinin, respectively (Figure 2).

The same purification procedure was repeated in the absence of 10 mM 2-ME.

Finally, the preparations were dialyzed against water. After readjustment to pH 7.0–7.2, the protein solutions were freeze-dried and stored at 5 °C in a desiccator. These materials were used as protein samples.

Assay of Glycinin and β-Conglycinin Contents by an Immunological Method. Antibody Production. Glycinin for antibody production and single radial immunodiffusion standard was purified by the method reported by Kitamura et al. (1976) using a Con A-Sepharose 4B column and Sepharose CL-6B column to remove the β-conglycinin. β-Conglycinin for antibody production and standards of analysis were purified according to Thanh and Shibasaki (1977) on a DEAE-Sephadex A-50 column, Con A-Sepharose 4B column, and Sepharose CL-6B column.

Antisera to the glycinin and the β-conglycinin were prepared by immunization procedures using young adult rabbits as described previously (Iwabuchi and Shibasaki, 1981). Antisera of glycinin and β-conglycinin showed no cross-reaction with β-conglycinin and glycinin antigens, respectively.

Single Radial Immunodiffusion. A single radial immunodiffusion was done according to the modified method of Mancini et al. (1965). A thoroughly cleansed 10 × 7.5 cm glass slide was placed on a level surface, and 10 mL of 1.2% agarose in the phosphate buffer (pH 7.6, ionic strength 0.5) containing 0.1–0.2 mL of antiserum was applied to each slide. A series of wells (3-mm diameter) were formed, and exactly 4 μL of antigen solution was applied to each well by a microsyringe. Solutions of standard glycinin and β-conglycinin antigens dissolved in the phosphate buffer were used for calibration. Protein samples (0.1%) were applied to the wells. On every slide, calibrations and protein samples for assay were applied at the same time to reduce experimental error. The standard curves had correlation coefficients of 0.9997 for glycinin and 0.9988 for β-conglycinin.

Protein Determination. After solubilization of lyophilized proteins in the phosphate buffer, sample solutions were analyzed for protein concentration with a biuret reagent. Purified glycinin and β-conglycinin were used to prepare the standard curves. In order to obtain the yield of proteins distributed into various fractions, protein concentrations were determined by micro-Kjeldahl using a nitrogen content of 16%.

RESULTS

Glycinin and β-Conglycinin Contents of Various Fractions. The absolute glycinin and β-conglycinin contents in various protein fractions are shown in Figure 3. A stable value for glycinin and β-conglycinin content was obtained by a number of determinations on 3–16 preparations.

Isoelectric Precipitation. The glycinin and β-conglycinin contents in whole-buffer extract (WBE) were determined to be 32% and 23%, respectively. Of the protein 45% remained unassayed.

Adjusting WBE to pH 4.8 precipitated all of the storage

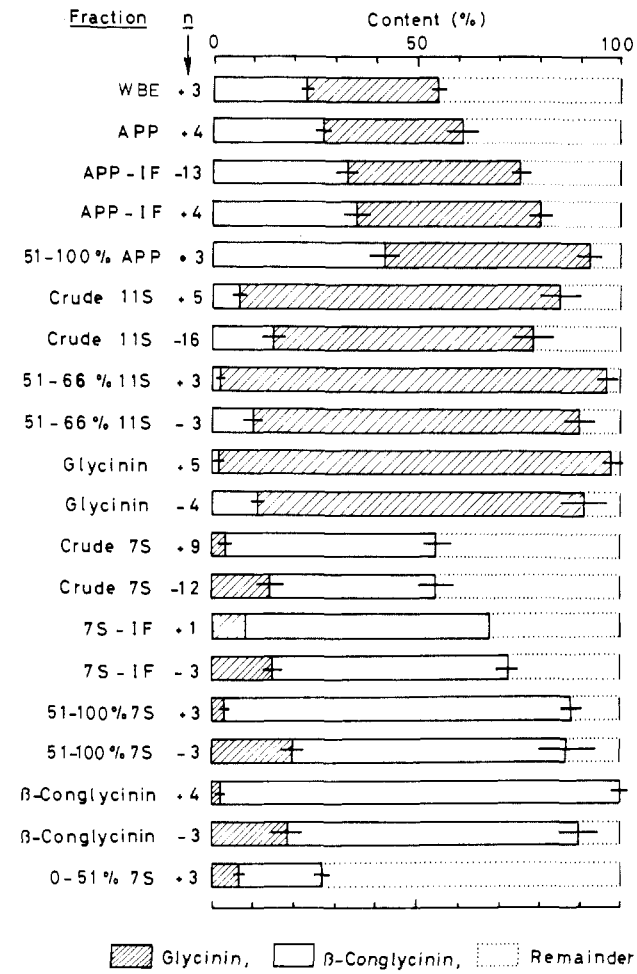

Figure 3. Glycinin and β-conglycinin contents of soybean protein fractions. Contents of the protein component are expressed in percent of total protein. Remainder was determined by total protein minus glycinin and β-conglycinin content. See the Experimental Section or Figures 1 and 2 for abbreviations. Number of determinations is shown by n; $+n$ corresponds to the preparations in the presence of 10 mM 2-ME; $-n$ corresponds to those in the absence of 2-ME. The horizontal line indicates standard deviation.

globulins present in the extract; however, the actual contents of glycinin and β-conglycinin in the APP fraction were 34% and 27%, respectively. Considerable amounts of protein (39%), other than glycinin and β-conglycinin, remained in the APP fraction. Total protein concentration of samples was determined by a biuret reagent, and there was no distinct difference in the protein concentrations between the crude fraction containing a large amount of remainder and the purified globulin fraction of 90–100% purity. These results indicate that the bulk of remainder should be proteins, although slight amounts of nucleic acids and phosphorus may be present.

The crude 11S fraction was comprised of 79% glycinin, 6% β-conglycinin, and 15% remainder (Figure 3). The crude 7S fraction was comprised of 52% β-conglycinin, 3% glycinin, and 45% remainder (Figure 3). Thus, the fractionation procedure by Thanh and Shibasaki (1976) was effective for simultaneously separating the glycinin and β-conglycinin components. The results of Figure 3 show, however, that the absolute content of β-conglycinin in the crude 7S fraction was unexpectedly low, indicating that the purity of the crude 7S was much lower than what Thanh and Shibasaki (1976), and Damodaran and Kinsella (1981) had documented. On the other hand, the glycinin content in the crude 11S was high.

Table I. Yields of Various Fractions Obtained from the Procedure for Isolation of Glycinin and β-Conglycinin (Protein Fractions Prepared with a Buffer Containing 10 mM 2-ME Used as Samples)

fraction[a]	wt of protein,[b] g/100 g meal	fraction of total protein, %	immunochem compn,[c] g/100 g meal			
			glycinin	β-conglycinin	others[d]	whey
WBE II	30.0	100	9.6	6.9	13.5	
SUP II (sup of pH 6.4)	19.4	64				
PPT II (ppt at pH 6.4)	9.3	31	6.9	0.9	1.4	
SUP III (sup of pH 4.8)	3.8	13				3.8
PPT III (ppt at pH 4.8)	13.2	44	0.5	6.7	5.9	
$(NH_4)_2SO_4$ fractionation of PPT III						
0–51% ppt	6.9	23	0.4	1.4	5.1	
51–100% ppt	5.0	17	0.2	4.3	0.5	
glycinin[e]	7.4–7.5	24.7–25.0				
β-conglycinin[e]	6.7–7.7	22.3–25.5				
glycinin + β-conglycinin	14.1–15.2	47.0–50.6				
whey & others[e]	10.7–11.1	35.6–37.0				
β-conglycinin/glycinin ratio	0.9–1.0					

[a] See Figures 1 and 2 for preparation. [b] Determined by micro-Kjeldahl analysis. [c] Estimated by calculation with the results of Figure 3. [d] Determined as remaining proteins except glycinin and β-conglycinin present in acid-precipitated fraction. [e] Obtained by adding all the individual values distributed in each fraction.

All precipitates prepared by isoelectric precipitation were washed twice in order to remove the occluded proteins arising from the supernatant components; however, the results of Figure 3 show that acid-precipitated fractions at pH 4.8 (APP and crude 7S) still contained large amounts of contaminating proteins other than glycinin and β-conglycinin. It is known that when isoelectric precipitation is employed at the initial step of fractionation for the crude extract, a contamination of unwanted proteins possessing a similar isoelectric point will take place in the acid precipitate. The acidification of WBE to pH 4.8 will result in the precipitation of many kinds of minor globulins and the partial precipitation of whey proteins such as lipoxygenase, agglutinin, and trypsin inhibitor, whose isoelectric points range between 5.8 and 4.5, together with the glycinin and β-conglycinin. Actually, there was only a small improvement in the protein composition after acid precipitation as shown by the fact that the content of glycinin plus β-conglycinin in the APP, 61%, increased by 11% over that in the WBE, 55%, after the removal of whey fraction.

Extraction with 10% NaCl. When the isoelectric precipitates (PPT I and PPT III in Figure 1) were treated with a 10% NaCl solution instead of water or 0.03 M Tris-HCl buffer, the 7S-IF fraction and the APP-IF fractions increased in absolute contents of glycinin plus β-conglycinin from 55–61% to 68–80% as the result of the removal of the contaminating proteins (Figure 3). Although experimental conditions are not the same, the insoluble fraction formed after acidification and solubilization by salt solutions may closely correspond in composition and properties to the acid-sensitive fraction (ASF) reported previously (Lillford and Wright, 1981; Anderson and Warner, 1974).

Ammonium Sulfate Fractionation. Ammonium sulfate fractionation significantly improved the purity of every globulin fraction to more than 90% (Figure 3). By adjusting the solution of the crude 7S fraction to 51% saturation, most of the contaminating proteins were removed as the 0–51% 7S fraction, which consisted of 6% glycinin, 21% β-conglycinin, and 73% remainder. The protein components of the remainder fraction are still unidentified. Electrophoretical studies on this fraction will be described elsewhere (Iwabuchi and Yamauchi, 1987).

The slight cross-contaminants of glycinin and β-conglycinin and remainder were almost completely removed by gel filtration on a Sepharose CL-6B column under the reducing conditions (Figure 3).

Effect of Reductant Removal on Fractionation. The removal of 10 mM 2-ME from the buffers for preparation resulted in a lowering of the efficiency of protein separation into glycinin and β-conglycinin as reported previously (Nash et al., 1974). The immunlogical assay of the crude 7S fraction prepared without 2-ME detected 14% of glycinin impurity (Figure 3). Thus, the glycinin impurity was increased fivefold over that in the crude 7S fraction prepared with 2-ME. The amount of β-conglycinin impurity in the crude 11S fraction was increased from 6% to 15% in the absence of 2-ME. Ammonium sulfate fractionation of crude protein fractions prepared without 2-ME removed contaminating proteins and yielded a globulin content of 90%; however, even after ammonium sulfate fraction and gel filtration, the cross-contamination by glycinin and β-conglycinin, especially glycinin impurity in the β-conglycinin fraction, was not eliminated (Figure 3). These results show that the presence of 2-ME has an important effect on the isolation of glycinin and β-conglycinin in the initial isoelectric precipitation step.

Yield of Various Fractions and Distribution of Soybean Proteins within the Fractions. Yields of various fractions of whole-buffer-extractable proteins according to Figures 1 and 2 and contents of glycinin and β-conglycinin distributed in the fractions on the basis of micro-Kjeldahl analysis are listed in Tables I and II. Each fraction was dialyzed against distilled water to remove Tris buffer and then subjected to analysis. The 0.03 M Tris-HCl buffer at pH 8.0 extracted 30 g of total protein from the defatted meals (100 g); this corresponds to 100% yield (Table I). This value is relatively low since the extraction had not been repeated; however, this is consistent with the value of Lillfod and Wright (1981). At pH 6.4, 31% of whole-buffer extract precipitated (PPT II composed of the 11S fraction) and 64% of the whole protein remained in the supernatant. After adjustment to pH 4.8, 44% of the whole proteins precipitated (PPT III composed of the 7S fraction) and 13% of the whole buffer extract, corresponding to the whey proteins, remained (Table I).

Additional fraction of PPT III with ammonium sulfate revealed that more than half of the crude 7S fraction precipitated at 51% saturation. By the results of immunological composition shown in Figure 3, components distributed in the various fractions have been calculated and are shown in Table I. The weights of each protein, distributed in various fractions, were added. The contents

Table II. Yields of Various Fractions Obtained from the Procedure for Isolation of Acid-Precipitated Protein

fraction[a]	wt of protein,[b] g/100 g meal	fraction of total protein, %	immunochem compn, g/100 g meal		
			APP[c]	others[d]	whey
WBE I	29.7	100	16.3	13.4	
SUP I (sup of pH 4.8)	4.2	14			4.2
PPT I (ppt at pH 4.8)	24.0	81	14.4	9.6	
$(NH_4)_2SO_4$ fractionation of PPT I					
0–51% ppt	11.4	38	3.4	8.0	
51–100% ppt	11.0	37	9.9	1.1	
glycinin + β-conglycinin[e]	13.3–14.4	44.7–48.5			
whey & others[e]	13.3–13.8	44.7–46.4			

[a] See Figure 1 for preparation. [b] See Table I. [c] Estimated by calculation with the results of Figure 3 and expressed as the sum of glycinin and β-conglycinin contents. [d] See Table I. [e] See Table I.

of glycinin, β-conglycinin, and others contained in 100 g of defatted soybean meal (Raiden) were 7.5 g (25%), 7.7 g (25.5%), and 11.1 g (37%), respectively (Table I). As shown by Arabic numerals in Figures 1 and 2, 5 g glycinin and 5 g β-conglycinin were obtained from 100 g of meal. The recoveries of the purified glycinin and β-conglycinin were approximately 65% of the total glycinin or β-conglycinin present initially. These are the highest yields that have ever been reported. The great difference in the recovery may be due to the use of different buffers: the dilute Tris-HCl buffer and the phosphate buffer containing NaCl. The Tris-HCl buffer developed by Thanh and Shibasaki (1976) was well suited for the preparation of soybean protein fractions, since all fractions precipitated by acidification were resolubilized in this buffer without formation of an insoluble fraction (see yields in Figures 1 and 2).

When WBE I was titrated to pH 4.8, 81% of the protein precipitated as the APP fraction and 14% of the protein remained in the supernatant (Table II). Ammonium sulfate fractionation of PPT I revealed that this fraction contained 9.1 g of other components in addition to 13.3 g of glycinin and β-conglycinin. The contents of glycinin plus β-conglycinin and whey proteins plus other remaining proteins contained in 100 g of defatted meals were evaluated at 13.3 g (44.7%) and 13.3 g (44.7%), respectively. Yields of the protein fractions in Tables I and II agreed approximately with those in Figures 1 and 2.

On the other hand, the protein composition of the whole-buffer extract was immunologically calculated to be 9.6 g (32%) of glycinin, 6.9 g (23%) of β-conglycinin, and 13.5 g (45%) of whey protein plus other remaining proteins on the assumption that 30 g of the total protein was recovered from 100 g of defatted meals (see Figure 3).

DISCUSSION

Generally, it is recognized that glycinin and β-conglycinin account for approximately 70% of the total protein in seeds (Hill ane Breidenbach, 1974). Our data on the protein composition were compared with those reported in the literature. There was no difference in the glycinin concentration between the reported values (Thanh and Shibasaki, 1976; Wolf and Sly, 1965; Hughes and Murphy, 1983) and our data. The 7S fraction contains lipoxygenase, agglutinin, β-amylase, and γ-conglycinin in addition to β-conglycinin. It is, therefore, reasonable to consider that out of a high 7S protein content of 37–52% reported in the literature, 23% of the protein is β-conglycinin. Our data for β-conglycinin content are compatible with those reported recently by Murphy and Resurreccion (1984) using an immunological assay. In contrast, their estimates for glycinin are higher than our data. The glycinin content of 50% by Murphy and Resurreccion (1984) appears incorrect for the following reasons: They employed severe conditions of sodium dodecyl sulfate gel electrophoresis for purifying glycinin, which may induce an alteration of molecular conformation. We assume that the immunological reactivity probably changes depending on the glycinin conformations and whether it is native or denatured, since the antigenicity of glycinin is more conformation dependent than β-conglycinin (Iwabuchi and Yamauchi, 1984).

Soybean globulins, comprising about 90% of the total protein, are commonly prepared by acidifying an aqueous extract of meal to pH 4.5–5.0 (Wolf and Sly, 1965). The composition of acid-precipitated globulins is not clear although ultracentrifugal data have been reported (Koshiyama, 1983; Wolf and Smith, 1961). Recently, Sato et al. (1986) measured densitometrically the proportion of glycinin and β-conglycinin in APP. However, an immunological assay is preferable to ultracentrifugal or densitometric analysis since gel electrophoresis alone is not sufficient for the quantitation of proteins in a mixture because of the proximity and overlapping of bands. In this paper, we clarified that the acid-precipitated fractions at pH 4.8 (APP and crude 7S) are composed of 55–61% glycinin plus β-conglycinin and a large amount of remaining globulins (39–46%). The composition of these remaining proteins must be clarified in the future.

Contents of glycinin plus β-conglycinin in protein samples described in the literatures may vary between 50 to 100% according to preparations. These protein samples may show different functional properties. Many researchers have reported that a minimum protein concentration of 8–10% is required for gelation of soybean protein (Circle et al., 1964). If purified proteins, instead of crude preparations, are used as samples, the minimum protein concentration will decrease to less than 8%. Actually, Nakamura et al. (1984) reported that only 2.5% of purified glycinin is required to form gels. The finding that both APP and crude 7S fractions contain considerable amounts of contaminating proteins indicates that when these crude fractions are studied, e.g., interactions between soybean proteins and ligands (Damodaran and Kinsella, 1981) or distributions of phytate into protein fractions (Honig et al., 1984), contaminating proteins will have some effect on results.

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Electrophoretic Analysis of Whey Proteins Present in Soybean Globulin Fractions

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Whole extractable soybean proteins were separated into globulin and whey fractions by acidification to pH 4.8; however, this globulin fraction (acid-precipitated fraction) included large amounts of contaminating proteins in addition to the two major globulins glycinin and β-conglycinin and the minor globulin γ-conglycinin. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that the acid-precipitated fraction included bands corresponding to lipoxygenase, agglutinin, trypsin inhibitor, and β-amylase. On treatment with ammonium sulfate, these bands, belonging to the whey proteins, were concentrated in the 0–51% ammonium sulfate precipitated fraction. Contaminating proteins in the acid-precipitated fraction were thus cleanly isolated from the globulin fractions of glycinin and β-conglycinin. Similar results were obtained by measurements of extinction coefficients and SH groups, which are related to the purity of protein fractions.

Glycinin and β-conglycinin are the two major storage proteins in soybean and have been reported to account for about 70% of the protein in the seed (Hill and Breidenbach, 1974). Glycinin has a sedimentation coefficient of 12.3S and a molecular weight of about 350 000 and is composed of acidic polypeptide chains (M_r 45 000–37 000) and basic polypeptide chains (M_r 20 000), which are paired by a disulfide bond (Kitamura et al., 1976; Staswick et al., 1984b). β-Conglycinin has a sedimentation coefficient of 7S and a molecular weight of about 150 000 and exists in at least seven forms (B_0–B_6) as a result of various combinations of the three subunits α, α′, and β (Thanh and Shibasaki, 1977; Yamauchi et al., 1981). Recently, Staswick et al. (1984a) have revealed the complete primary structure of a glycinin polypeptide.

In contrast to the remarkable progress in the field of the protein chemistry of glycinin and β-conglycinin, quantitative aspects of soybean proteins containing globulins and whey proteins are still poorly understood. Generally, the main soybean proteins are classified as globulins; about 90% of the total protein consists of four major fractions with S_{20w} of 2, 7, 11, and 15S. These are prepared simply by acidifying an aqueous extract of soybean meal. A smaller portion, about 10% of the total protein composed of only 2S and 7S proteins, remains soluble in the whey (Wolf, 1972).

Recently, Koshiyama et al. (1981) reported that some of the 2S globulins precipitated between pH 5.8 and 4.5 were identical with Kunitz trypsin inhibitor. Kunitz (1947) has reported that his trypsin inhibitor is a globulin. It is partially soluble in whey and also partially precipitates with the acid-precipitated globulin fraction; Rackis et al. (1985) have reported that acid-precipitated proteins contain trypsin inhibitor. Therefore, other globulins may also be partitioned between the whey and the acid precipitates.

Recently, we immunologically quantitated the contents of glycinin and β-conglycinin in whole extractable protein and its various fractions (Iwabuchi and Yamauchi, 1987). In contrast to the earlier reports, we demonstrated that the acid-precipitated fractions at pH 4.8 contained large amounts of contaminating globulins and were composed of 61% glycinin plus β-conglycinin and 39% remainder; this composition is similar to the whole-buffer extract of soybean. However, the composition of this remaining fraction was unknown. In this paper, we have electrophoretically explored the presence of whey proteins in the acid-precipitated globulin fractions. Removal of the contaminating proteins from the globulin fractions was fol-

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