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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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lowed up by measurements of $E^{1\%}_{1cm}$ values, SH groups, and nitrogen content in relation to the purification of glycinin and β-conglycinin fractions.

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.

The principal whey proteins of soybean were purchased from Sigma Chemicals: agglutinins (lectins), lipoxygenase (type I), trypsin inhibitor (type I-S). Sodium dodecyl sulfate ($NaDodSO_4$), urea, 2-mercaptoethanol (2-ME), acrylamide, bis(acrylamide), and other reagents were obtained from Nakarai Chemicals. γ-Conglycinin was prepared according to Sato et al. (1984). Commercial β-amylase from soybean (Nagase Biochemicals Ltd.) showed four bands with $NaDodSO_4$ gel electrophoresis; however, β-amylase could be estimated by a band at a position corresponding to its molecular weight (57 000).

Methods. *Preparation of Soybean Protein Fractions.* Defatted soybean meals were extracted once with 0.03 M Tris-HCl (pH 8.0) buffer in the presence of 10 mM 2-ME at a solvent-to-meal ratio of 20:1 at room temperature to yield whole-buffer extracts. Various protein fractions were prepared according to Iwabuchi and Yamauchi (1987). Outlines of the preparation methods are as follows:

Acid-Precipitated Fractions (APP). Whole-buffer extract was acidified to pH 4.8. APP were recovered by centrifuging and resolubilized in water by neutralization to pH 8.0.

Insoluble Fraction (IF). When the precipitates at pH 4.8 were resolubilized in 10% NaCl (pH 8.0) instead of water, a large amount of IF appeared. This fraction is thought to correspond to the "acid-sensitive fraction (ASF)" (Anderson, 1974; Yamauchi et al., 1980).

APP-IF. The 10% NaCl-soluble fraction of APP was obtained by removal of the IF from APP fraction by centrifugation.

Whey. The supernatant at pH 4.8 (whey) was brought to pH 8.0 and then precipitated with 90–100% ammonium sulfate.

Crude 11S. The crude 11S globulin was obtained by precipitation at pH 6.4 from the whole-buffer extract. The precipitated 11S was easily dissolved in 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, ionic strength 0.5).

Crude 7S. The supernatant at pH 6.4 after removal of the crude 11S fraction was further acidified to pH 4.8. The precipitated crude 7S fraction was readily solubilized in 0.03 M Tris-HCl buffer (pH 8.0).

7S-IF. In order to remove the salt-insoluble fraction (IF) present in the crude 7S fraction, the precipitates (7S) were extracted with 10% NaCl.

0–51% 7S. The crude 7S fraction was fractionated with ammonium sulfate. The precipitate at 0–51% saturation was collected. This fraction was relatively soluble in a pH 7.6 phosphate buffer in contrast with solubility of IF in this buffer.

51–100% 7S. After removal of the 0–51% 7S fraction, the remaining 7S globulin was precipitated at 90–100% saturation. This fraction corresponded to ammonium sulfate fractionated 7S.

51–66% 11S. The crude 11S fraction was fractionated with ammonium sulfate. The precipitate at 51–66% saturation was collected. This fraction corresponded to ammonium sulfate fractionated 11S.

0-51% APP and 51–100% APP. These fractions were prepared in a manner similar to the ammonium sulfate fractionation of the crude 7S fraction.

Glycinin and β-Conglycinin. Purified 11S and 7S globulins achieved by gel filtration on a Sepharose CL-6B (Pharmacia Chemicals) corresponded to glycinin and β-conglycinin, respectively.

The same preparation scheme, with all the steps described above, was repeated in the absence of 10 mM 2-ME. Finally, all preparations were dialyzed against distilled water. After readjustment to pH 7.0–7.2, the protein solutions were freeze-dried and stored at 5 °C in a desiccator.

Polyacrylamide Gel Electrophoresis (PAGE). Slab gel electrophoresis in 0.1 M H_3PO_4/Tris buffer (pH 6.8) containing 8 M urea and 0.1% $NaDodSO_4$ was performed according to Swank and Munkres (1971) using 8% polyacrylamide gel (0.1 × 16 × 16 cm) and 1:30 cross-linkage. Protein samples (0.1%) in an 8 M urea solution containing 1% $NaDodSO_4$, 1% 2-ME, 10mM H_3PO_4/Tris (pH 6.8), and 0.005% bromophenol blue were treated at 60 °C for 10 min. A 10-μL aliquot of sample was applied on top of the gels. Electrophoresis was carried out at a constant voltage of 120 V for 5–6 h. Gels were stained with 0.3% Coomassie Blue G-250 in methanol/acetic acid/water (20:10:5, v/v) for 1 h and destained in methanol/acetic acid/water (20:7:73, v/v). Densitometric tracings were determined by using a Shimazu dual-wavelength TLC scanner, Model CS 900.

Determination of Sulfhydryl (SH) Groups. The content of SH groups in various fractions was determined with Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Ellman, 1959). Protein was dissolved in distilled water containing 1 mM Na_2EDTA. To 1 mL of the solution was added 4 mL of 0.1 M phosphate buffer, pH 8.0, containing 1 mM Na_2EDTA and 0.1 mL of DTNB solution (40 mg of DTNB in 10 mL of 0.1 M phosphate buffer, pH 8.0). The color was developed for 15 min and read at 412 nm. To calculate the SH content, a molar absorptivity of 13 600 M^{-1} cm^{-1} was used. The results were expressed in terms of moles of SH/mole of protein.

Determination of $E^{1\%}_{1cm}$ Values of Various Protein Fractions. The specific extinction coefficient ($E^{1\%}_{1cm}$) was determined at 280 nm in a pH 7.6 phosphate buffer and in 0.02 N HCl. For the estimation of the protein fractions when the protein/buffer solutions were turbid, the protein samples were dissolved in buffer/6 M urea solution, and the absorbance at 280 nm was measured against the buffer/6 M solution as a reference.

Nitrogen and Moisture Content. The nitrogen content of various protein fractions was determined by micro-Kjeldahl analysis. For the determination of moisture, 100 mg was dried to a constant weight in vacuo over P_2O_5 at 70 °C. The moisture contents were found to be 8–10%.

RESULTS

Identification of Constituents of Soybean Proteins by PAGE. $NaDodSO_4$/PAGE was used to check the purity of preparations with fractionation steps, the effect of 10 mM 2-ME on the isolation of glycinin and β-conglycinin, and identification of the contaminating constituents in the acid-precipitated protein fraction. The electrophoretic bands have been assigned to the known soybean proteins with purified glycinin, β-conglycinin, and partially purified γ-conglycinin, of which the subunit structures are electrophoretically well characterized. Commercial lipoxygenase, agglutinin, β-amylase, and trypsin inhibitor were used as standards. In this paper, the nomenclature systems of Kitamura et al. (1976) and that of Thanh and Shibasaki (1977) were used for the glycinin polypeptide chains (acidic chains A_1–A_4 and basic chains B_1-B_4) and

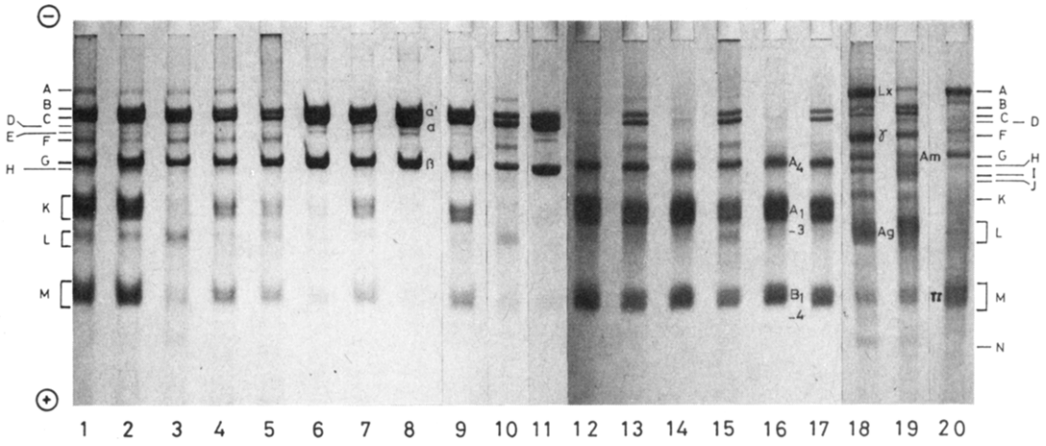

Figure 1. Various protein fractions with increasing purity on $NaDodSO_4$/8% polyacrylamide gels. The protein fractions were prepared as described in the Experimental Section. Key: lane 1, APP (15 μg of protein) prepared with 10 mM 2-ME; lane 2, APP-IF (15 μg) prepared with 10 mM 2-ME; lane 3, crude 7S (10 μg) prepared with 10 mM 2-ME; lane 4, same as lane 3 but prepared without 2-ME; lane 5, 7S-IF (10 μg) prepared without 2-ME; lane 6, 51–100% 7S (10 μg) prepared with 10 mM 2-ME; lane 7, same as lane 6 but without 2-ME; lane 8, β-conglycinin (10 μg) prepared with 10 mM 2-ME; lane 9, same as lane 8 but prepared without 2-ME; lane 10, same as lane 3 but only 7 μg of protein applied to the gel; lane 11, same as lane 8 but only 7 μg applied; lane 12, crude 11S (10 μg) prepared with 10mM 2-ME; lane 13, same as lane 12 but prepared without 2-ME; lane 14, 51–66% 11S (10 μg) prepared with 10 mM 2-ME; lane 15, same as lane 14 but prepared without 2-ME; lane 16, glycinin (10 μg) prepared with 10mM 2-ME; lane 17, same as lane 16 but prepared without 2-ME; lane 18, insoluble fraction (IF-1) obtained from APP; lane 19, 0–51% 7S (10 μg) prepared with 10 mM 2-ME; lane 20, whey (10 μg) prepared with 10 mM 2-ME. α, α′, and β are subunits of β-conglycinin, and γ is a subunit of γ-conglycinin. A_1–A_4 and B_1–B_4 are polypeptide chains of glycinin. Lx, Am, Ag, and TI are polypeptides of lipoxygenase, β-amylase, agglutinin, and trypsin inhibitor, respectively. N may be a Bowman–Birk type trypsin inhibitor.

the β-conglycinin subunits (α, α′, β), respectively.

The $NaDodSO_4$/PAGE profiles of various fractions are shown in Figure 1. The profile in lane 1 shows that the APP fraction is mainly composed of glycinin (bands K and M) and β-conglycinin subunits (bands B, C, and G) but includes bands A, F, and L, which correspond to lipoxygenase, the γ-conglycinin subunit, and the agglutinin subunit, respectively, and other minor components. There was no distinct difference in the intensity of protein bands between APP and WBE (profile of WBE not shown). The treatment of APP with 10% NaCl resulted in a decrease in the amounts of bands A and F (lane 2).

The profile of the crude 7S fraction prepared with 2-ME (lane 3) shows α, α′, and β subunits of β-conglycinin plus weak A, F, and L bands corresponding to whey components and minor amounts of acidic and basic polypeptide chains of glycinin. Judging from the intensity of the bands in lane 3, the proportion represented by agglutinin (band L) was higher than that contributed by glycinin (bands K and M). The crude 7S fraction prepared in the absence of 2-ME (lane 4) distinctly contained glycinin polypeptides. On treatment of crude 7S with 10% NaCl, lipoxygenase (band A) was decreased in the 7S-IF fraction (lane 5). By ammonium sulfate fractionation, bands A, F, and L were removed from the crude 7S fraction (lane 6). In the absence of 2-ME, the contaminating glycinin and band L were not removed from the 7S fraction by ammonium sulfate precipitation (lane 7) or gel filtration (lane 9).

The preceding paper demonstrated that the treatments with 10% NaCl and ammonium sulfate caused a rise of glycinin plus β-conglycinin contents in the crude 7S fraction from 55% up to 73% and from 55% up to 87%, respectively. It is unclear yet whether the mere removal of bands A, F, and L can have such a large effect on the increase of glycinin plus β-conglycinin contents. However, as a result of the removal of the contaminating proteins, the intensity of β-conglycinin subunit bands varied in proportion to the β-conglycinin content (see lanes 3–9). This trend was demonstrated clearly by the following experiments.

The high loading level of protein (10–15 μg) necessary to visualize the less abundant whey protein components in the APP and 7S fractions gave poor separation of the most abundant proteins of β-conglycinin, the α′ and α subunits. A loading level of 7 μg was best for resolution of these subunits of β-conglycinin (lanes 10 and 11). There was a distinct difference in the intensity of bands B, C, and G between the crude 7S (lane 10) and purified β-conglycinin (lane 11) fractions in spite of the same loading level. These results can be explained on the basis of the previous results that the actual content of β-conglycinin in the crude 7S fraction is only 52% of that in the purified β-conglycinin fraction (Iwabuchi and Yamauchi, 1987).

When the crude 11S fraction was prepared in the presence of 2-ME, α and α′ subunit bands appeared in very low amounts (lane 12). These bands and a part of band G were easily removed by ammonium sulfate fractionation (lane 14). Band G in lane 12 is thought to contain a β subunit in addition to the A_4 polypeptide of glycinin. The densitometric scanning showed that band G in lane 12 was higher than that of the purified glycinin in lanes 14 and 16 (data not shown). When the 11S fractions were prepared in the absence of 2-ME, distinct bands of the α and α′ subunits appeared in the crude 11S fraction (lane 13) and could not be removed by the subsequent purification steps (lanes 15 and 17). Under the nonreducing conditions, γ-conglycinin (band F) and agglutinin (band L) tend to be retained in the 11S fraction and cannot be removed by ammonium sulfate fractionation (lane 15).

Distinct evidence indicating contamination by multiple whey protein components in the acid-precipitated globulins at pH 4.8 was obtained by subjecting 0–51% 7S and IF-1 fractions to electrophoresis. It must be remembered that all constituents of these fractions originally existed in the crude 7S or APP fraction. The IF-1 and 0–51% 7S fractions consisted of more than 10 and 13 bands, respectively (lanes 18 and 19). This shows that, by the removal of major globulins such as β-conglycinin and glycinin from the crude 7S or the APP fraction, many minor components originally present in the globulin fractions were concentrated. Some of these bands corresponded to α and α′ subunits and a γ-conglycinin subunit. The remaining

Table I. Chemical Compositions of Soybean Protein Fractions

fraction[a]	N content, %	$E^{1\%}_{1cm}$[b]	A_{280}/A_{260}[g]	SH content mol/g ($\times 10^6$)[c] +ME[d]	SH content mol/g ($\times 10^6$)[c] -ME[e]	SH content mol/mol[f] +ME[d]	SH content mol/mol[f] -ME[e]
APP	16.2	10.60	1.07				
APP-IF	16.4	9.63	1.08	2.7	0.7		
purified APP	17.3	6.80	1.32				
crude 11S	17.1	9.07	1.20	4.0	0.2	1.4	0.07
51–66% 11S	16.9	7.62	1.71				
glycinin	17.4	7.40	1.81	3.2		1.1	
crude 7S	16.7	10.40	1.02	5.1	1.0	0.8	0.15
7S-IF	16.7	9.02	1.01				
51–100% 7S	16.2	5.76	1.43				
β-conglycinin	16.5	4.22	1.62	0.2		0.03	
0–51% 7S	15.6	12.10	1.00				
whey	16.4	9.00	1.64				

[a] See the experimental section for preparation. [b] Values were estimated on protein fractions prepared with a buffer containing 10 mM 2-ME. [c] Values were expressed as moles of SH/gram of protein. [d] Protein fractions were prepared with a buffer containing 10 mM 2-ME. [e] Protein fractions were prepared with a buffer containing no 10 mM 2-ME. [f] Values were expressed as moles of SH/mole of protein. M_r 350 000 for glycinin and Mr_r 150 000 for β-conglycinin were used. [g] Absorbance ratios at 280 and 260 nm.

Table II. Chemical Compositions and Properties of Principal Enzymes in Soybean Seed

enzyme	$S_{20,w}$	M_r	isoelec pt	$E^{1\%}_{1cm}$	N content, %	SH content, mol/mol	ref
Kunitz trypsin inhibitor	2.3S	21 500	4.5	7.2	16.7	0	Koshiyama et al. (1981); Wolf (1972)
lipoxygenase	5.6S	100 000	5.4	17.8		5	Theorell et al. (1949); Wolf (1972)
agglutinin	6.0S	120 000[a]	5.8	12.8	13.2	0	Lotan et al. (1974)
β-amylase	4.67S	57 000	5.85	17.3	16.3	5	Morita et al. (1976)

[a] Composed of four identical subunits of M_r 30 000.

major bands and many minor bands are considered to belong to the whey proteins as shown by mobilities that agreed with the corresponding standards. Although there are some differences in the profiles, similarities between IF-1 and 0–51% 7S fractions are clear. Thus, the fractionation steps of the 51% ammonium sulfate precipitation and 10% NaCl treatments were effective in removing contaminating whey proteins found in the globulin fraction. The amounts of glycinin polypeptides present in bands K and M may be slight, if any, since the 0–51% 7S fraction contained only 5% glycinin (Iwabuchi and Yamauchi, 1987).

The whey fraction contained three major bands (lipoxygenase, β-amylase, trypsin inhibitor), many minor bands present between bands A and M, and a low molecular weight protein of band N (possibly, a Bowman–Birk trypsin inhibitor; lane 20). Agglutinin (band L) was detected stronger in lanes 18 and 19 than in lane 20.

Chemical Properties of the Various Protein Fractions. The extinction coefficients ($E^{1\%}_{1cm}$), the number of SH groups, and the nitrogen content in the different protein fractions are shown in Table I. Values were corrected for moisture content.

The $E^{1\%}_{1cm}$ value of each fraction became progressively smaller as the purity of the 7S and 11S fractions was raised with successive fractionation by isoelectric precipitation, 10% NaCl treatment, ammonium sulfate fractionation, and gel filtration. Finally, the $E^{1\%}_{1cm}$ values of purified β-conglycinin and glycinin reached minima of 4.22 and 7.40, respectively. The $E^{1\%}_{1cm}$ value of β-conglycinin agreed with that of Thanh and Shibasaki (1978); however, the $E^{1\%}_{1cm}$ value of glycinin was slightly lower than that of Koshiyama (1972). The greatest $E^{1\%}_{1cm}$ value was observed for the 0–51% 7S fraction. These results indicate that components present in the 0–51% 7S fraction and contaminating proteins in the crude 7S and APP fractions, except for glycinin and β-conglycinin, contribute to the high $E^{1\%}_{1cm}$ values. Table II lists the chemical composition and physical properties of the principal enzymes taken from the literature and shows that the $E^{1\%}_{1cm}$ values of whey proteins are high. Contamination by these whey proteins, therefore, would result in an increase in the $E^{1\%}_{1cm}$ values of the globulin fractions.

Absorbance ratios of A_{280}/A_{260}, where A_{280} is the absorbance at 280 nm and A_{260} is that at 260 nm, are also shown in Table I. These data indicate that the protein purities of APP, APP-IF, 7S, 7S-IF, and 0–51% 7S fractions are low.

The number of SH groups in glycinin and β-conglycinin fractions at various stages of fractionation is shown in Table I. Considerable differences in SH contents were noticed between the two preparations. All fractions prepared with 2-ME contained a larger number of SH groups than those without 2-ME. When the 11S fraction was prepared in the presence of 2-ME, 1.1 mol of SH groups/mol of glycinin was found in the freeze-dried glycinin after purification. There was no great difference in the number of SH groups in the 11S fraction before and after purification. Under the nonreducing conditions, only 0.07 mol of SH groups was revealed in the native 11S protein, indicating that almost all SH groups present on the surface of glycinin readily form SS bonds and exhibit inactivity in the native state. The SH content of glycinin, according to literature data, varies widely. Simard and Boulet (1978) reported that 11S globulin contains 1.1 mol of SH groups on the surface. This agreed well with us; however, Hoshi and Yamauchi (1983) reported that glycinin contains 10.3 mol of SH groups, located on the surface. This discrepancy may arise from differences in the protein preparation, i.e., with or without Na_2EDTA and with or without freeze-drying, which will greatly affect the number of SH groups. In this paper, protein fractions were prepared without Na_2EDTA.

When the SH content was expressed as mole/gram of protein, approximately the same number of SH groups, 5.1×10^{-6} mol/g, as in glycinin was measured in the crude 7S fraction prepared in the presence of 10 mM 2-ME; however, a dramatic decrease was observed in the purified

β-conglycinin. This result indicates that the contaminating proteins in the crude 7S fraction caused a high SH content. This idea is also supported by the data in Table II. Amino acid analysis data show that β-conglycinin is devoid of cysteine (Thanh and Shibasaki, 1977) or contains only two SS groups (Koshiyama, 1968). Our finding of an insignificant amount of SH (0.03 mol/mol) in β-conglycinin is consistent with the amino acid analysis data. The idea that almost all SH groups quantitated in the crude 7S fraction may be derived from the contaminating protein fractions predicts that even slight contamination by whey proteins should result in the presence of SH groups in β-conglycinin. Consequently, if SH groups are detected in β-conglycinin preparations, the possibility of contamination by whey proteins must be considered.

Nitrogen contents of protein fractions are shown in Table I. As a result of the successive fractionation steps, the nitrogen contents of glycinin and β-conglycinin increased to 17.4 and 16.5, respectively. The nitrogen contents of whey protein and 0–51% 7S fractions were relatively low.

DISCUSSION

Soybean globulin is a general term for soybean storage proteins or acid-precipitated proteins, although the acid-precipitated fractions are contaminated by small amounts of nonprotein impurities (Wolf and Smith, 1961). It has been believed that soybean globulin is completely separated from whey proteins by acidification (Catsimpoolas and Ekenstam, 1969) and that the occluded amount of whey in the acid-precipitated fraction is negligible since there is no appreciable change in the protein composition even after repeated precipitation and washing (Wolf and Briggs, 1959). On the other hand, the possible presence of the whey proteins in the globulin fraction has been suggested (Koshiyama and Fukushima, 1976; Wolf and Smith, 1961; Kunitz, 1947). Our previous data revealed that the glycinin plus β-conglycinin accounts for 55% of total protein. Then, the remaining 45% fraction was calculated to be composed 13% whey and 32% other globulins precipitating by acidification. These remaining globulins were concentrated in the 0–51% ammonium sulfate precipitated fraction or the IF-1 fraction. Electrophoretic analysis of these fractions indicates that lipoxygenase, β-amylase, agglutinin, and trypsin inhibitor are partitioned between the whey and the acid-precipitated globulin fraction. The proportion represented by these whey proteins in the remaining globulins is unknown. It is difficult, however, to consider that the whey proteins make up all of the remaining fraction. Therefore, the bulk of remaining globulins may be still unidentified. An immunological assay of the whey protein components distributed in various fractions is needed.

Precipitation of soybean proteins at pH 4.5–5.0 results in the formation of a protein complex (ASF) that does not resolubilize in solutions containing NaCl. The protein components of ASF have been reported to be 2S and 7S globulins (Nash et al., 1971) and to contain agglutinin and trypsin inhibitor (Lillford and Wright, 1981). This idea is supported by the $NaDodSO_4$/PAGE information on the proteins composing the IF-1 fraction (Figure 1, lane 18). On the basis of our results, we attempted to explain why the ASF is formed. The conditions for preparation of crude 11S fraction do not cause ASF but those of crude 7S and APP fractions bring about formation of ASF. These results indicate that ASF development is closely related to the proportion of the glycinin plus β-conglycinin and the remaining globulins comprising the protein fractions. If the proportion of the glycinin plus β-conglycinin in the acid-precipitated globulins is high, e.g. 80% of total protein, exposure to the low pH will not form the ASF. As the crude 7S and APP fractions are composed of only 55–60% glycinin plus β-conglycinin and approximately 40% remaining globulins, the bulk of the remaining globulins may have different solubilities than the major globulins of glycinin and β-conglycinin and cause ASF development.

Registry No. Lipoxygenase, 9029-60-1; trypsin inhibitor, 9088-41-9; β-amylase, 9000-91-3.

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