Chemical Form of Selenium in Soybean (*Glycine max* L.) Lectin†

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In hydroponically grown, intrinsically labeled soybeans, the major portion of ^{75}Se (89%) was associated with proteins. On a per unit weight basis, whey proteins incorporated 2.2 and 2.3 times more ^{75}Se than the major soybean storage proteins glycinin (11S) and β-conglycinin (7S). When the soluble proteins were fractionated according to the Thanh and Shibasaki procedure, 60% of the ^{75}Se radioactivity was associated with 11S (15.4%) and 7S (44.4%) proteins. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of soluble soy proteins indicated that ^{75}Se incorporation by 11S polypeptides was 5.12 times more than that by 7S polypeptides. One of the few biologically active proteins in soybeans is lectin, a whey protein. Lectin was capable of incorporating ^{75}Se and was purified to electrophoretic homogeneity using ammonium sulfate precipitation, DEAE DE-52 anion-exchange column chromatography, and Sephacryl S200 gel filtration column chromatography. The chemical form of selenium in soybean lectin was identified as selenomethionine.

INTRODUCTION

Selenium (Se) is an essential trace element with RDAs for males of 70 μg and for females of 55 μg (Food and Nutrition Board, 1989). Two selenium deficiency related diseases in humans have been documented: Keshan disease, a cardiomyopathy found in young children; and Kaschin–Beck disease (Dongxa, 1987), an osteoarthropathy causing ossification of affected joints (Keshan Disease Research Group, 1979a,b; Chen et al., 1980). Both of these diseases are responsive to selenium supplementation. The essentiality of selenium for animals has been long recognized (Schwarz and Foltz, 1957; Thompson and Scott, 1969), and the role of selenium in the functioning of glutathione peroxidase (GSH-Px) has been well established (Flohe et al., 1973; Rotruck et al., 1973). Subsequently, it has been shown that the chemical form of selenium in GSH-Px is selenocysteine (Kraus et al., 1983; Sunde and Evenson, 1987). New selenoproteins distinctly different from GSH-Px have been identified in animals and humans in recent years (Beilstein and Whanger, 1986; Yang et al., 1987; Takahashi et al., 1987; Sunde, 1984).

Soybeans are an excellent and relatively inexpensive source of protein. Soy proteins are used in infant formulas, food supplements, and a variety of foods as a functional ingredient. Soybeans contain about 35–40% protein on a dry weight basis. Glycinin (11S) and β-conglycinin (7S), the two major storage proteins, account for over 70% of the total seed proteins (Derbyshire et al., 1976), the remainder being 2S and whey proteins. In ^{75}Se intrinsically labeled, hydroponically grown soybeans, over 80% of the bean ^{75}Se is associated with proteins (Mason, 1984) and the bioavailability of selenium from soy protein isolate is 86–96% (Mason and Weaver, 1986).

Yasumoto et al. (1988) investigated the chemical form of selenium in soybeans and concluded "selenomethionine is at least one of the major chemical forms of selenium in soybean protein". They also prepared crude 11S, 7S, and whey fractions in an effort to study the selenium distribution in soy proteins. The major findings of these investigations were the following: (1) of the total soluble selenium (4.5 μg), 59.55% (2.68 μg) was recovered in all of the fractions (11S, 7S, polymerized 7S, and whey); (2) of the recovered selenium, 24.62 and 56.34% was associated with the 11S and 7S fractions, respectively; (3) on a per gram basis, 7S and 11S proteins contained 0.36 and 0.35 μg of selenium, respectively, while whey proteins had the highest amount (0.58 μg/g). These investigators did not purify soy proteins before determining the chemical form of selenium as selenomethionine and could account for 60% of recovered selenium from soy protein isolate as selenomethionine.

Because lectins are important biological tools in the study of cell surfaces and in medicine (Sharon and Lis, 1972; Sharon, 1977; Lis and Sharon, 1986; Strosberg et al., 1986) and it is not known whether lectins incorporate selenium, it was of interest to investigate whether intrinsically labeled, hydroponically grown soybeans incorporate selenium in soy lectin and in what chemical form.

MATERIALS AND METHODS

Materials. Soybeans (*Glycin max* L. cv. Century) were from Indiana Crop Improvement Association, W. Lafayette, IN. Electrophoresis grade acrylamide, bis[*N*,*N*′-methylenebis(acrylamide)], *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), tris(hydroxymethyl)aminomethane (Tris), ammonium persulfate, glycine, and AG 1X8 resin were from Bio-Rad Laboratories, Richmond, CA. Bromophenol blue, Coomassie Brilliant Blue R, glycerol, β-mercaptoethanol (β-ME), NaCl, soybean lectin, molecular weight (MW) marker kit [phosphorylase *b* (97 400), bovine serum albumin (66 000), ovalbumin (45 000), carbonic anhydrase (29 000), soybean trypsin inhibitor (20 100), and α-lactalbumin (14 200)], peptide MW kit [myoglobin backbone polypeptide (16 950), myoglobin fragment I and II (14 400), and myoglobin fragment II (6210)], TPCK–trypsin, and TLCK–chymotrypsin were from Sigma Chemical Co., St. Louis, MO. The standard proteins thyroglobulin, ferritin, bovine serum albumin, ovalbumin, chymotrypsinogen A, and ribonuclease A for calibration of the gel filtration column (Sephacryl S200) for Stokes radius were

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from Pharmacia, Inc., Piscataway, NJ. Hydrochloric acid (HCl) and acetic acid were from Mallinckrodt, Inc., Paris, KY. Bulk grade methanol was from Purdue University Chemistry Stores, W. Lafayette, IN. Fluoro-Hance was from Research Products International Corp., Mount Prospect, IL. Endoproteinase Lys-C and protease V8 were from Boehringer Mannheim Biochemicals, Indianapolis, IN. Reducible cross-linker dithiobis(succinimidyl propionate) (DSP) was from Pierce Chemical Co., Rockford, IL. TLC plates (20 × 20 cm, 250-μm thickness, Cellulose), were from Analtech Inc., Newark, DE. All other chemicals were of reagent grade.

Selenium Distribution in Soybean Proteins. Soybeans were hydroponically grown and intrinsically labeled with ^{75}Se according to the method of Mason and Weaver (1986). Mature seeds were harvested and stored at −20 °C until used.

The dry whole seeds were ground in a Wiley-type mill to obtain 40-mesh flour, and the flour was defatted with cold (4 °C) acetone. The defatted soy flour was air-dried in a fume hood and homogenized in a Waring blender for 1 min at the highest speed setting to obtain a homogeneous sample. The defatted soy flour was stored in an airtight container at −20 °C until further use.

To assess the radioactivity distribution, the defatted soy flour extracts were subjected to anion-exchange column chromatography, Thanh and Shibasaki (1976) protein fractionation, and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) as follows.

(*a*) *Anion-Exchange Column Chromatography.* Defatted soy flour (5 g) was extracted for 2 h at room temperature (25 °C) with 50 mM Tris-HCl, pH 8.5, containing 1 mM NaN_3 (flour to solvent ratio 1:10 w/v), with constant magnetic stirring, filtered through glass wool, and centrifuged (4 °C, 12600*g*, 20 min), and the supernatant was subjected to DEAE DE-52 anion-exchange column chromatography. The column (2.6 × 28.5 cm) was equilibrated with 50 mM Tris-HCl, pH 8.5, and eluted with 0–500 mM NaCl linear gradient developed in the equilibrium buffer (700 mL each). Fractions were collected every 20 min, and the flow rate was 40.8 mL/h. Fractions were analyzed for radioactivity [net counts per minute (cpm)/3 mL] and for protein composition by SDS–PAGE.

(*b*) *Thanh and Shibasaki Protein Fractionation.* Five grams of defatted soy flour was extracted with 100 mL of 20 mM Tris-HCl, pH 8.5, containing 0.1% β-ME and 1 mM NaN_3 at 25 °C for 2 h with constant magnetic stirring and filtered through glass wool. The filtrate was centrifuged (12600*g*, 4 °C, 20 min), and the supernatant was subjected to protein fractionation. The crude 11S, 7S, and whey fractions were analyzed for radioactivity and protein composition (SDS–PAGE).

(*c*) *Radioactivity Distribution by SDS–PAGE.* Defatted soy flour (100 mg) was extracted with 1 mL of 50 mM Tris-HCl, pH 8.5, containing 0.1% β-ME and 1 mM NaN_3 for 30 min with vortexing every 5 min and centrifuged in an Eppendorf centrifuge (15600*g*, 25 °C, 10 min). The supernatant was subjected to SDS–PAGE according to the Fling and Gregerson (1986) procedure. The 5 M urea in the sample buffer recommended by Fling and Gregerson was eliminated to avoid protein carbamylation. At the end of the gel run, the gel was stained briefly (30 min with gentle shaking) to visualize the protein bands. Appropriate gel slices containing protein bands and the blanks were cut, and the radioactivity was measured. A total of 12 samples was analyzed in this fashion using 3 mm thick, 8–25% linear acrylamide gradient gels.

Isolation and Purification of Soybean Lectin. The defatted soy flour was extracted with 50 mM Tris-HCl, pH 8.5, containing 0.1% β-ME and 0.02% NaN_3 (flour to solvent ratio of 1:10 w/v) with constant magnetic stirring for 2 h at room temperature (25 °C) and filtered through glass wool, and the filtrate was centrifuged (12600*g*, 4 °C, 20 min). The supernatant was subjected to ammonium sulfate precipitation, and the precipitate between 40 and 80% saturation was collected (centrifugation was at 4 °C, 12600*g*, 20 min). The pellet was dissolved in 50 mM Tris-HCl, pH 8.5, containing 1 mM NaN_3 and 0.1% β-ME, dialyzed against 50 mM Tris-HCl, pH 8.5, at 4 °C for 24 h (four changes of buffer), and centrifuged (12600*g*, 4 °C, 20 min). The supernatant was then loaded onto a DEAE DE-52 anion-exchange column previously equilibrated with 50 mM Tris-HCl, pH 8.5, and eluted with a linear NaCl gradient in the equilibrium buffer (0–250 mM NaCl, 300 mL each). Fractions were collected every 20 min. Fractions containing the lectin were pooled, concentrated in an Amicon stirred cell concentrator (MW cutoff of the membrane was 10 000), and chromatographed on a Sepharose CL-6B or Sephacryl S200 column for further purification. The course of lectin purification was monitored using SDS–PAGE. Details of columns and gels appear in appropriate figure legends.

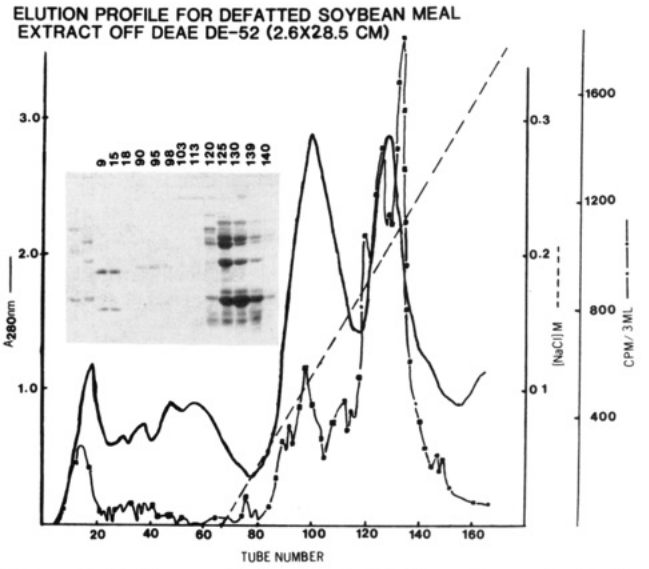

Figure 1. Elution profile for the defatted soy flour extract off DEAE DE-52. Extraction buffer was 50 mM Tris-HCl, pH 8.5, containing 1 mM NaN_3 (flour/solvent = 1:10 w/v). The DEAE DE-52 column (2.6 × 28.5 cm) was equilibrated with 50 mM Tris-HCl, pH 8.5, and eluted with the same buffer followed by a linear NaCl gradient (0–500 mM, 700 mL each) in the equilibrium buffer. Fractions were collected every 20 min. Column flow rate was 40.8 mL/h. Fractions were analyzed for ^{75}Se radioactivity (net cpm/3 mL), and protein was analyzed by SDS–PAGE (inset). For SDS–PAGE 50 μL of each fraction was used. (Inset) The first two lanes from the left, respectively, were molecular weight (MW) markers (from top to bottom: bovine serum albumin, 66 000; ovalbumin, 45 000; and soybean trypsin inhibitor, 20 100) and sample onto the column. The numbers on the top of each lane indicate the fraction number.

Analytical Methods. Protein concentrations of appropriate samples were determined according to the method of Lowry et al. (1951) after sample dialysis against 1 mM $NaHCO_3$ (MW cutoff of the tubing was 1000). SDS–PAGE was performed according to the method of Fling and Gregerson (1986), typically using 8–25% linear gradient gels, 1.5 mm thick. Native gel electrophoresis (pH 7.5) was done in 7.5% acrylamide separating slab gel (1.5 mm thick, acrylamide to bis ratio 30:0.8) with a 2.5% acrylamide stacking gel (acrylamide to bis ratio 10:2.5). The running buffer was Tris–EDTA–diethylbarbituric acid (4 g:1.168 g:22.08 g per 4 L). The gels were run at 30-mA constant current until the tracking dye migrated to the gel edge. The sample was dissolved in 33% glycerol containing 1 mM NaN_3 and 0.025% bromophenol blue as tracking dye. Gel staining (for all types of gels) was in 50% methanol containing 10% acetic acid and 0.25% Coomassie Brilliant Blue R for 16 h. The gels were destained with 50% methanol containing 10% acetic acid for 2 h followed by 5% methanol containing 7.5% acetic acid.

Chemical cross-linking of appropriate proteins was done at 25 °C for 2 h using DSP as the cross-linker. At the end of the cross-linking, sample buffer (30% glycerol, 10% SDS, 0.02% bromophenol blue, with or without 2% β-ME) was added to the reaction mixture, and the sample was heated in a boiling water bath for exactly 2 min. Samples were analyzed immediately using a 5% acrylamide slab gel, 1.5 mm thick (25 mM sodium phosphate, 0.1% SDS, acrylamide to bis ratio 30:0.8), using 25 mM sodium phosphate, pH 7.5, containing 0.1% SDS as the running buffer at constant voltage (25 V/gel) until the tracking dye migrated to the gel edge.

Table I. Radioactivity (^{75}Se) Distribution in Soybean Proteins Prepared According to the Thanh and Shibasaki (1976) Procedure[a]

sample	total protein, mg	total radioactivity, net cpm ($\times 10^{-3}$)	net cpm/mg of protein
20 mM Tris-HCl, pH 8.5, containing 0.1% β-ME and1 mM NaN_3 extract	686.15	113.901	166
crude 11S	126.00 (18.36)[b]	17.575 (15.43)	139
crude 7S	380.48 (55.45)	50.622 (44.44)	133
soy whey	101.58 (14.80)	31.050 (27.26)	306

[a] Data are average of two separate preparations starting with 5 g of defatted soy flour. The average total protein recovery was 88.62% based on total protein content of Tris-HCl, pH 8.5, extract as 100%. [b] Figures in parentheses are percent of the Tris-HCl, pH 8.5, extract.

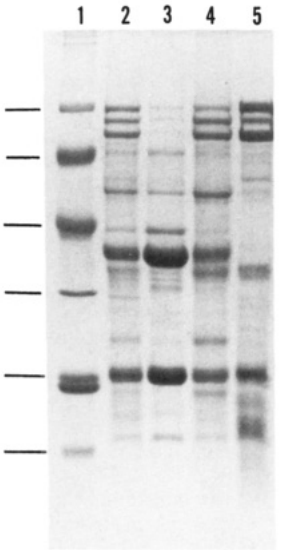

Figure 2. SDS–PAGE analysis of soy proteins fractionated according to the Thanh and Shibasaki procedure. (Lane 1) MW markers. (from top to bottom: phosphorylase *b*, 94 400; bovine serum albumin, 66 000; ovalbumin, 45 000; carbonic anhydrase, 29 000; soybean trypsin inhibitor, 20 100; and α-lactalbumin, 14 200; (lanes 2, 3, 4, and 5, respectively) defatted soy flour extract (50 mM Tris-HCl, pH 8.5, containing 0.1% β-ME and 1 mM NaN_3), crude 11S, crude 7S, and whey. Approximately 100 μg of protein was loaded onto the gels for soy proteins.

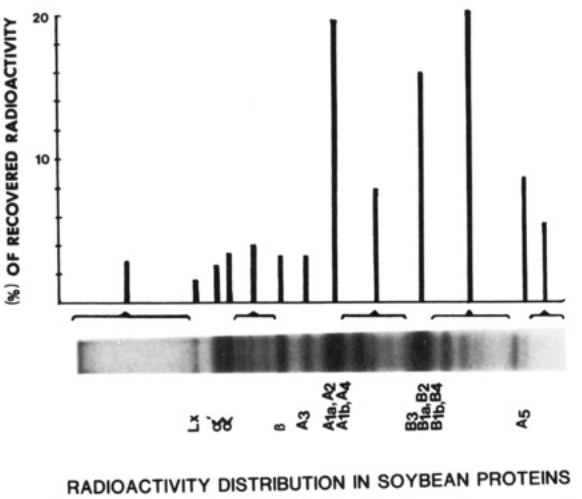

Figure 3. Radioactivity (^{75}Se) distribution in soybean proteins. Vertical bars indicate average percent of total radioactivity recovered ($n = 12$). Proteins were separated using 8–25% linear acrylamide gradient gels 3 mm thick. Lx, lipoxygenase; α', α, and β, 7S subunits; A_3, A_{1a}, A_{1b}, A_2, A_4, and A_5, acidic subunits of the 11S; B_{1a}, B_{1b}, B_2, and B_4, basic subunits of 11S; peptides under acidic 11S subunits, soy lectin peptides.

One-dimensional peptide mapping was done by digesting heat-denatured (boiling water bath at 100 °C for 10 min) proteins under final digestion conditions of 50 mM Tris-HCl, pH 8.1, 1 mg of substrate protein/mL, 30 min at 37 °C (controlled-temperature water bath), and protein to enzyme ration of 50:1 w/w. TPCK–trypsin, TLCK–chymotrypsin, V8 proteinase, and endoproteinase Lys-C were the enzymes used. At the end of the digestion, an equal volume of SDS–PAGE sample buffer was added, and the samples were heated for 5 min at 100 °C (boiling water bath) to inactivate the enzymes. Appropriate controls were run simultaneously. Samples were immediately analyzed using 8–25% linear acrylamide gradient SDS–PAGE. The commercial lectin used in this and subsequent experiments was first purified by gel filtration column chromatography (Sephacryl S200, 2.6 × 80.0 cm column, equilibrium and elution buffer being 20 mM sodium phosphate, pH 7.5, containing 0.02% NaN_3 and 100 mM NaCl) to remove minor contaminants present. The purest fractions were pooled, dialyzed against distilled water at 4 °C, lyophilized, and stored at −20 °C until used.

For autoradiography, a 12% acrylamide SDS–PAGE gel was used. After electrophoresis, the gels were treated with Fluoro-Hance solution for 30 min and dried under vacuum on a filter paper. The dried gel was exposed to Kodak X-Omatic film for 3 weeks at −80 °C.

To determine the chemical form of selenium in the lectin, purified lectin was reduced and carboxymethylated according to the procedure of Crestfield et al. (1963), dialyzed against distilled water (4 °C, 24 h, three changes), and lyophilized. The reduced and carboxymethylated protein was then hydrolyzed in vacuo in 6 N HCl (110 °C, 24 h) and dried under vacuum. The dry hydrolysate was reconstituted in distilled water and filtered through a 0.45-μm filter (Whatman nylon filter), and the filtrate was loaded onto a Dowex AG 1X8 ion-exchange resin column (0.7 × 14 cm). The column was eluted sequentially with 12 mL each of distilled water, 1.5 M HCl, and 4.0 M HCl. Three milliliter fractions were collected and counted for radioactivity in a γ counter (TM Analytic 1191, Elk Grove Village, IL) to identify radioactive fractions. Fractions containing radioactivity were pooled and lyophilized. The lyophilized fractions were reconstituted in distilled water and subjected to one-dimensional analytical thin-layer chromatography (TLC). The developing solvent was acetone/water/pyridine/acetic acid (75:20:5:1 v/v/v/v). Selenomethionine and carboxymethylated selenocysteine were chromatographed simultaneously. Amino acids were visualized using ninhydrin spray and subsequent heating of plates (80 °C, 20 min) to develop the color.

Carbohydrate analysis was done according to the method of Dubois et al. (1956); Stokes radius was measured using a 2.6 × 80.0 cm Sephacryl S200 column calibrated with standard proteins according to the method of Siegel and Monty (1966). Lectin activity was assayed using purified lectins and trypsinized human blood (blood group O) erythrocytes according to the method of Liener and Hill (1953).

RESULTS AND DISCUSSION

Selenium Distribution in Soybean Proteins. Since selenium in food is associated with proteins (Franke, 1934) and the chemical forms of selenium appear to be dependent on the food source (Underwood, 1977), the differences in selenium bioavailability from different food and feed sources have been partly attributed to the different chemical forms of selenium in the diet (Yoshida et al., 1981; Beilstein and Whanger, 1986; Schwarz and Foltz, 1958; Cantor et al., 1975; Laws et al., 1986; Mason and Weaver, 1986). We therefore investigated the selenium

Table II. Summary of Soybean Lectin Purification[a]

purification step	total protein, mg	total radioactivity, net cpm ($\times 10^{-5}$)	net cpm/mg of protein
50 mM Tris-HCl, pH 8.5, containing 0.1% β-ME and 0.02% NaN_3 extract	3892.63	323.347	5969
ammonium sulfate (40–80% saturation) precipitate	1887.13	79.404	4208
soy lectin off DEAE DE-52 column	11.15	1.086	9739
soy lectin off Sephacryl S200 column	7.81	0.712	9113

[a] Data are for a typical preparation starting with 20 g of defatted soy flour containing 2.45 μg of ^{75}Se/g of flour (approximately 5×10^6 cpm/g). The protein content of samples was determined according to the Lowry et al. (1951) procedure after the samples were dialyzed against 1 mM $NaHCO_3$.

distribution in soybean protein fractions to discern which protein fractions incorporated selenium.

Initial experiments using DEAE DE-52 anion-exchange column chromatography fractionation (Figure 1) indicated that the major portion of radioactivity was associated with the major proteins in soybeans (11S and 7S). Two other protein fractions (tubes 9–18 and 90–100) contain appreciable amounts of radioactivity but do not account for major portions of the total soluble seed proteins. Fraction 90–100 contained the soybean lectin, and fraction 9–18 contained a sulfur-rich salt-soluble globulin.

The association of ^{75}Se with the major soybean proteins was further confirmed by fractionating the soluble proteins according to the Thanh and Shibasaki (1976) method. This method separates the major proteins on the basis of their differential solubility in dilute buffer solutions at different pHs. The 11S fraction is quantitatively precipitated at pH 6.4–6.6, and the 7S is precipitated at pH 4.5. The proteins remaining soluble at pH 4.5 after the removal of 11S and 7S are termed whey proteins. Results of these fractionations are summarized in Table I. These data indicated that crude 11S fraction contained less total radioactivity than the crude 7S fraction. On a protein weight basis, crude 11S and 7S had comparable radioactivity and the whey registered the highest amount of radioactivity.

These data are in agreement with those of Yasumoto et al. (1988). On the basis of selenium analysis of crude fractions, these investigators concluded "the absolute amount of selenium in the subfraction was most abundant in the 7S globulin fraction". The data of Yasumoto et al. (1988) indicate that the amounts of Se (micrograms per gram of protein) in their 11S and 7S glubulin fractions (Table I in their paper) were similar (0.35 and 0.36 μg, respectively) and whey protein contained the highest amount (0.58 μg/g of protein). Of the total selenium (whole buffer extract contained 4.5 μg of selenium) they recovered 59.6% selenium in various fractions; 11S contained 0.66 μg and 7S contained 1.51 μg of selenium, representing 15.1 and 33.6%, respectively, of the selenium present in the whole buffer extract.

Our data indicate that the amounts of radioactivity recovered (total recovery was 87.13%) in crude 11S and 7S fractions were, respectively, 15.4 and 44.4% of the total radioactivity in the 50 mM Tris-HCl, pH 8.5, extract. However, because protein fractions prepared according to the Thanh and Shibasaki procedure do not yield biochemically pure proteins (see Figure 2), determining selenium radioactivity distribution by the glycinin and β-conglycinin polypeptides by such fractionation procedures may not yield accurate results. We therefore analyzed the whole soybean extracts by preparative high-resolution gel electrophoresis followed by the radioactivity measurement of appropriate gel slices (Figure 3). Typically, we recovered 65.6% (mean ± SEM = 65.50 ± 4.69%, $n = 12$) of the radioactivity loaded onto the gel (the mean ± SEM for the loaded radioactivity was 229 628 ± 18 068 net cpm). Of the recovered radioactivity, the 7S globulin (β-conglycinin) polypeptides α', α, and β accounted for 2.58, 3.43, and 3.21%, respectively, for a combined total of 9.22%. The 11S globulin polypeptides A_3, acidic subunits, basic subunits, and A_5, respectively, contained 3.19, 19.61, 15.93, and 8.49% for a combined total of 47.22% of the total recovered radioactivity, suggesting 11S protein incorporated 5.12 times more ^{75}Se than the 7S globulin (β-conglycinin). The ^{75}Se radioactivity in 11S by these analyses may also be somewhat overestimated because the soybean trypsin inhibitor, which has high amounts of sulfur amino acids, may comigrate with the basic subunits of 11S because of their similar molecular weights. However, this should not introduce a large error in the estimate because the acidic subunits of 11S account for twice the amount of radioactivity compared to that of the basic subunits, and the quantity of the soybean trypsin inhibitor is small when compared with the 11S. Rackis et al. (1985) have shown that, on average, 57.8 mg of trypsin inhibitor/g of protein is present in raw soy flour (that is less than 6% of the total protein). On the basis of these data 11S globulin (glycinin) incorporates more selenium than the 7S globulin (β-conglycinin). This is also consistent with

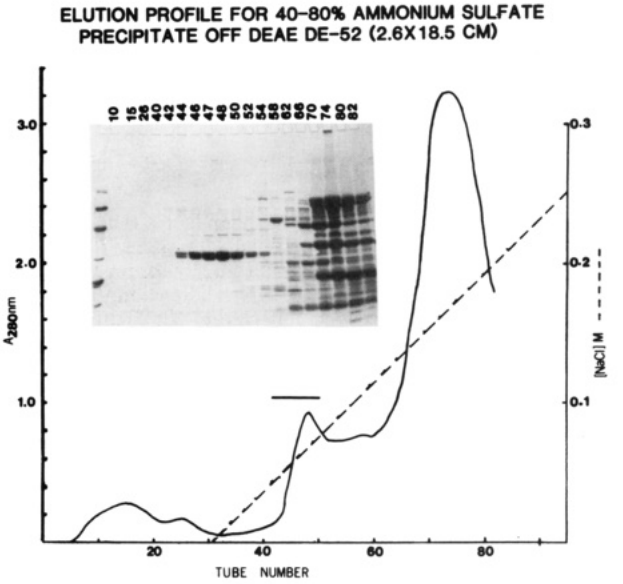

Figure 4. Elution profile for the 40–80% ammonium sulfate fraction off a DEAE DE-52 column (2.6 × 18.5 cm). Equilibrium buffer was 50 mM Tris-HCl, pH 8.5. The column was eluted with equilibrium buffer followed by 0–250 mM NaCl linear gradient in equibrium buffer (300 mL each). Fractions were collected every 20 min, and the column flow rate was 34.2 mL/h. (Inset) SDS–PAGE analysis of fractions (fraction numbers indicated on top). The end lane (left-hand side) contained MW markers (same as in Figure 2). Fifty microliters of each fraction was used for analysis. The lectin eluted at a NaCl concentration of 40–100 mM. The horizontal bar indicates fractions pooled (tubes 42–51). The pooled fractions were concentrated (Amicon concentrator fitted with a YM 10 membrane, under nitrogen gas pressure) to 10 mL and directly loaded onto a Sepharose CL-6B column.

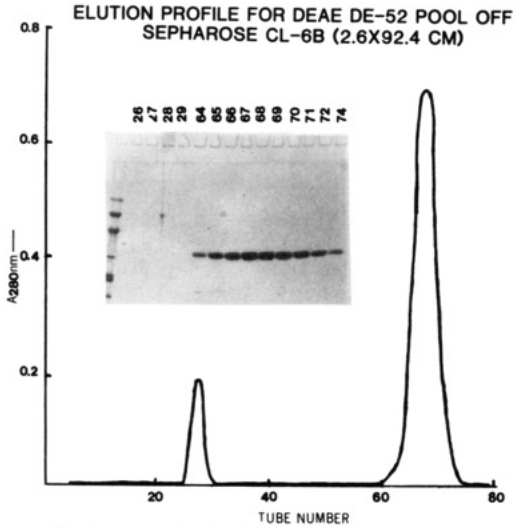

Figure 5. Elution profile for the soybean lectin (DEAE DE-52 pool) off a Sepharose CL-6B column (2.6 × 92.4 cm). The column was equilibrated and eluted with 150 mM sodium phosphate buffer, pH 7.5, containing 0.02% NaN_3. The column flow rate was 20.9 mL/h. Fractions were collected every 20 min. (Inset) SDS–PAGE analysis of fractions (50 μL from each fraction was loaded onto the gels). Fraction numbers are indicated on top. MW markers (end lane on left) used were the same as in Figure 2. The pooled fractions (tubes 65–74) were dialyzed against distilled water (4 °C, 24 h, three changes, dialysis tubing MW cutoff of 30 000), concentrated (Amicon concentrator fitted with a YM 10 membrane) under nitrogen gas pressure, lyophilized, and stored at −20 °C in airtight containers until further use.

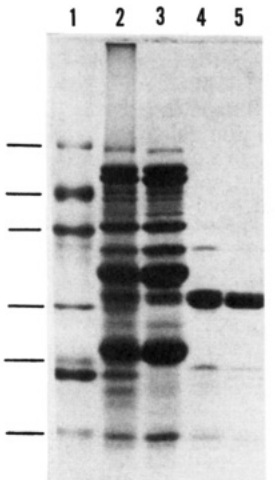

Figure 6. SDS–PAGE analysis of soybean lectin purification. (Lane 1) MW markers (same as in Figure 2); (lane 2) 50 mM Tris-HCl, pH 8.5, extract; (lane 3) 40–80% ammonium sulfate saturation pellet; (lane 4) pooled fractions from the DEAE DE-52 column; (lane 5) pooled fractions from the Sepharose CL-6B column. Protein load in lanes 2 and 3 was 100 μg each and in lanes 4 and 5 was 50 μg each.

the amino acid composition of these proteins. Published data indicate 11S globulin contains 1.1 residues of methionine and 1.5 residues of cysteine per 100 residues compared to 0.3 residues each of methionine and cysteine per 100 residues in 7S globulin.

Lectin Purification and Characterization. Because soy whey incorporated the highest amount of selenium per gram in our studies and soybean lectin is one of the major whey proteins, we were interested in the ability of soy lectin to incorporate selenium. Lectin was also chosen for this investigation because it does not contain cysteine and has 6 mol of methionine/mol of lectin (Lis et al., 1966). Recent studies using molecular cloning and sequencing

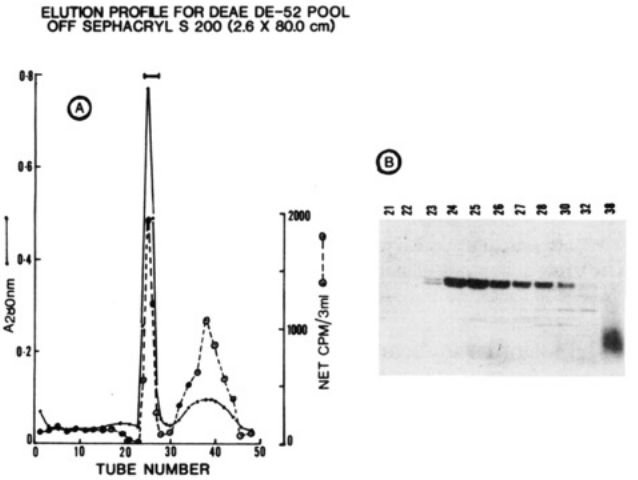

Figure 7. Elution profile for the soybean lectin off a Sephacryl S200 (2.6 × 80.0 cm) column (A) and the SDS–PAGE analysis of the fractions (B). The fractions pooled from the DEAE DE-52 column were loaded onto this column after concentrating (Amicon concentrator fitted with a YM10 membrane, under nitrogen gas pressure). The equilibrium and elution buffer was 20 mM sodium phosphate, pH 7.5, containing 100 mM NaCl and 0.02% NaN_3. Fractions were collected every 20 min. Column flow rate was 18 mL/h. Seventy-five microliters from each fraction were used for SDS–PAGE analysis (fraction number indicated on top). Horizontal bar (panel A) indicates fractions pooled.

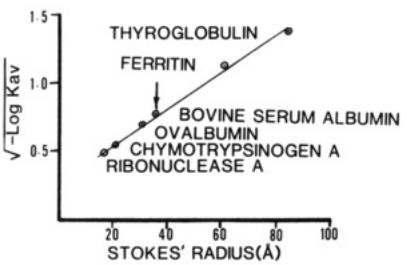

Figure 8. Calibration curve for measuring Stokes radius of soybean lectin using a 2.6 × 80.0 cm Sephacryl S200 column. The standard curve was prepared using proteins of known Stokes radii (average of two runs for each protein). The column equilibrium and elution buffer was 20 mM sodium phosphate, pH 7.5, containing 100 mM NaCl and 0.02% NaN_3. Column flow rate was 18 mL/h. Arrow indicates elution position of purified soybean lectin.

confirm the lack of cysteine in soybean lectin and report 4 mol of methionine/molecule (Vodkin et al., 1983). Consequently, the chemical form of selenium in soybean lectin could be determined without ambiguity.

A summary of lectin purification appears in Table II. The yield was 39 mg/100 g of defatted flour. Chromatography profiles and the SDS–PAGE analysis at every stage during purification are shown in Figures 4–6. On the basis of SDS–PAGE (Figure 6), the lectin purity was ≥95%. An agglutination assay (data not shown) indicated that the protein was biologically active. Homogeneity of the lectin was further confirmed by (a) elution of the lectin off gel filtration Sephacryl S200 column chromatography as a single peak in the same position as that of the commercial soybean lectin (Figure 7) with a Stokes radius of 35–37 Å (Figure 8), (b) migration of the lectin as a single band in native polyacrylamide gel at pH 7.5 (Figure 9), (c) the lectin having the same oligomeric nature as the commercial soybean lectin (Figure 10), and (d) one-dimensional polypeptide mapping with four different proteinases compared with those of the commercial soybean lectin (Figure 11). The gel filtration, chemical cross-linking, and SDS–PAGE data are consistent with

Figure 9. Nondenaturing nondissociating polyacrylamide gel electrophoresis of purified soybean lectin. This is a 7.5% acrylamide gel (1.5 mm thick, acrylamide/bis 30:0.8) with a 2.5% acrylamide stacking gel (acrylamide/bis 10:2.5). The running buffer was Tris–EDTA–diethylbarbituric acid, pH 7.5. The direction of migration was from top (anode) to bottom (cathode). The lectin migrated as a single band.

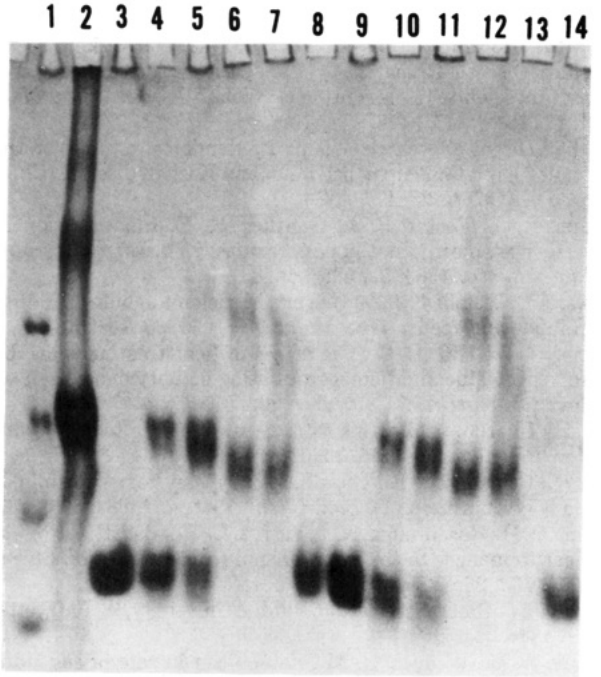

Figure 10. SDS–PAGE analysis of chemical cross-linking of the purified soybean lectin and the commercial soybean lectin (Sephacryl S200 purified). This is a 5% acrylamide gel 25 mM in sodium phosphate, pH 7.5, and 0.1% in SDS. Proteins were cross-linked using a reducible cross-linker dithiobis(succinimidyl propionate) at protein to cross-linker molar ratios of 1:0 (lanes 3 and 9), 1:2.5 (lanes 4 and 10), 1:5 (lanes 5 and 11), 1:20 (lanes 6 and 12), 1:40 (lanes 7 and 13), and 1:40 treated with β-ME (lanes 8 and 14). (Lane 1) MW markers (from top to bottom: phosphorylase *b*, bovine serum albumin, ovalbumin, and carbonic anhydrase); (lane 2) cross-linked bovine serum albumin. The oligomeric natures of both proteins were identical.

the literature data that the soybean lectin is a tetrameric protein containing four polypeptides with an estimated molecular weight of 110 000 (Lis et al., 1966). The carbohydrate content of our preparation (mean ± SEM = 4.27 ± 0.28%, n = 4) was also comparable to 4.5% mannose reported by Lis and co-workers. These data suggest that the lectin isolated from ^{75}Se-labeled soybeans was identical to the lectin that is obtained from nonradioactive beans. Incorporation of ^{75}Se therefore did not alter the lectin with respect to the polypeptide composition, molecular size and molecular weight, primary structure, carbohydrate content, oligomeric nature, and biological activity.

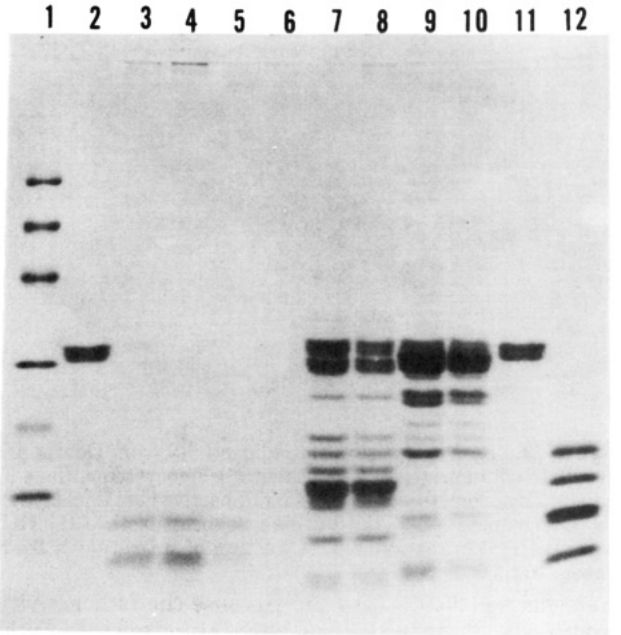

Figure 11. One-dimensional peptide mapping of laboratory-prepared soybean lectin (I) and commercial soybean lectin purified by Sephacryl S200 column chromatography (II). This is a 8.25% linear acrylamide gel. (Lane 1) MW markers (same as in Figure 2); (lane 12) polypeptide MW markers (from top to bottom: myoglobin backbone polypeptide, 16 950; myoglobin fragments I and II, 14 400; myoglobin fragment I 8160; myoglobin fragment II, 6210; (lanes 2 and 11, respectively) I and II, no enzyme controls; (lanes 3, 5, 7 and 9, respectively) I digested with TPCK–trypsin, TLCK–chymotrypsin, V-8 proteinase, and endoproteinase Lys-C; (lanes 4, 6, 8, and 10, respectively) II digested with TPCK–trypsin, TLCK–chymotrypsin, V-8 proteinase, and endoproteinase Lys-C. Final digestion conditions were 1 mg of protein/mL, 50 mM Tris-HCl, pH 8.1, 37 °C, and protein to enzyme ratio of 50:1 w/w. Proteins I and II were heat denatured (100 °C, 10 min) prior to subjecting them to proteolysis. Proteins I and II have identical polypeptide maps and therefore have identical primary structure (amino acid sequence).

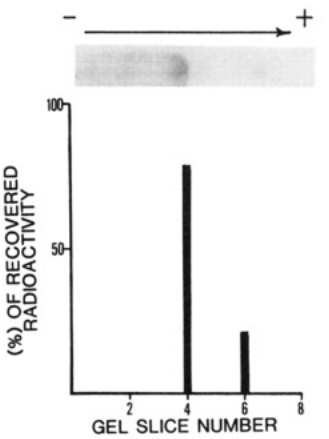

Figure 12. Autoradiograph of purified soybean lectin obtained from a 12% acrylamide gel (SDS–PAGE) after the dried and Fluoro-Hance-treated gel was exposed to Kodak X-Omatic film for 3 weeks at −80 °C. Protein load was 300 μg. Directon of migration is indicated by an arrow, gel top (−), gel bottom (+). About 80% of the recovered radioactivity was associated with the lectin polypeptides when the gel slices were measured for the radioactivity (lower portion, vertical bars). The gel was sliced from left to right.

Selenium Incorporation and Chemical Form. Selenium was incorporated by the soy lectin covalently. An autoradiograph of the purified lectin from SDS–PAGE and the radioactivity measurements of gel slices are shown in Figure 12. The average recovery of the radioactivity

Figure 13. TLC analysis of the radioactivity from Dowex AG 1X8 resin column. (Lane 1) Authentic selenocysteine; (lane 2) authentic selenomethionine; (lane 3) radioactive pool from Dowex AG 1X8 column when the purified soybean lectin 6 N HCl hydrolysate was fractionated. Direction of migration is from bottom to top.

from gels was 65.9% (n = 2). Because the radioactivity in purified soybean lectin could be recovered quantitatively both from gel filtration (Sephacryl S200) and from SDS–PAGE, the selenium incorporation by lectin must be covalent. This is because ionic interactions, hydrogen bonds, and weak interactions between the protein and mineral would be completely disrupted by the conditions used in these experiments (salt concentration of 100 mM, 2% β-ME, and the presence of 1% SDS). This is further confirmed by the association of selenium with amino acid when the protein was hydrolyzed in 6 N HCl and fractionated by ion-exchange column chromatography. Thin-layer chromatographic analysis (Figure 13) of the radioactive fractions from the Dowex AG 1X8 resin column indicated that the chemical form of selenium in soybean lectin was selenomethionine on the basis of the identical mobility of sample when compared with the authentic selenomethionine standard.

Conclusions. On the basis of the data presented, the following conclusions can be derived. (1) The majority of selenium in hydroponically grown, intrinsically labeled soybeans is covalently incorporated by proteins. (2) On a per unit weight basis, the soy whey incorporated 2.2 and 2.3 times more selenium compared to the two major storage proteins glycinin and β-conglycinin, respectively. (3) Soybean lectin incorporated selenium, and the chemical form of selenium in the lectin was selenomethiomine. (4) Selenium incorporation in the lectin did not cause significant alteration in the biochemical properties of the lectin when compared to those of the lectin from nonradioactive soybeans.

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