Soy Protein Isolate Components and Their Interactions

Silvana Petruccelli and María Cristina Añón*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata, Facultad de Ciencias Exactas, Calle 47 y 116, 1900 La Plata, Argentina

The electrophoretic behavior of a soy protein isolate was analyzed in both nonreducing and reducing SDS–PAGE. Aggregates formed by α and α′ subunits of β-conglycinin exhibited both ionic interactions and disulfide bonds. Higher molecular weight aggregates (180 000–190 000) consisted of trimers or dimers of α′ and α subunits, whereas those of intermediate molecular weight (115 000–120 000) were formed by α′,α subunits of β-conglycinin and A polypeptides of glycinin. The latter exhibited a higher sensitivity toward changes of ionic strength and thermal treatments. The proportion of α′ and α subunits of the isolate which was included in these aggregates is highly dependant on the freeze-drying conditions. These aggregates were readily reduced in the presence of Na_2SO_3, even at low concentrations and in the absence of denaturing agents, thus suggesting that the disulfide bonds involved were accessible. These aggregates were stable at different pH values, in the presence of both SDS and urea.

Keywords: *β-Conglycinin; aggregates; soybean isolates; 7S globulin*

INTRODUCTION

Soy protein isolates consist of 90% protein; their major components are glycinin, or 11S, and β-conglycinin, or 7S, which represent 34% and 27%, respectively, of the proteins occurring in the isolate (Iwabuchi and Yamauchi, 1987a,b). The rest of the proteins consist of whey proteins, such as γ-conglycinin (trimer of 170 kDa) (Hirano et al., 1987), the basic 7S globulin (Sathe et al., 1989; George and de Lumen, 1991), lipoxygenase, agglutinins, and β-amylases, which belong to the 7S fraction (Iwabuchi and Yamauchi, 1987b), and the soy trypsin inhibitors occurring in the 2S fraction.

11S globulin (glycinin) is a very heterogeneous oligomeric protein ranging from 340 to 375 kDa (Utsumi et al., 1981). It consists of six subunits: six acidic (A) polypeptide chains (37–45 kDa; pI = 4.2–4.8) and six basic (B) polypeptide chains (18–20 kDa; pI = 8.0–8.5). These polypeptides are joined by disulfide bonds, forming AB subunits (Badley et al., 1975). Six dimers are arranged in a trigonal antiprism structure (Plietz et al., 1983). Four different types of acidic (A_1–A_4) and basic polypeptides (B_1–B_4) have been found (Kitamura et al., 1976), although Lei et al. (1983) have reported that 13 acidic and 11 basic polypeptides are present.

7S globulin (β-conglycinin) is a trimeric glycoprotein of 140–170 kDa, which consists of three types of subunits: α′, α, and β of 58, 57, and 42 kDa, respectively. A subunit termed γ of 42 kDa copurifies with β subunits. Subunits α′ and α exhibit a high degree of homology, which is not restricted to N-terminal regions. Subunits β and γ also exhibit homology with the α and α′ subunits of β-conglycinin (Hirano et al., 1987). It has been reported that the β subunit consists of four components with isoelectric points between 5.8–6.2 (β_1–β_4), whereas α and α′ consist of single components having isoelectric points of 5.2 and 5.3, respectively. The trimeric structure of β-conglycinin is completely stabilized by ionic strengths higher than 0.5 M; it is formed by seven different types of oligomers: B_1 ($\alpha'\beta_2$); B_2 ($\alpha\beta_2$), B_3 ($\alpha\alpha'\beta$), B_4 ($\alpha_2\beta$), B_5 ($\alpha_2\alpha'$), B_6 (α_3) (Thanh and Shibasaki, 1976), and B_0 (β_3) (Yamauchi et al., 1981; Sykes and Gayler, 1981). The quaternary structure under those conditions of ionic strength is stabilized by hydrophobic interactions. Lowering of the ionic strength leads to dissociation of β-conglycinin into α and α′ subunits; however, β subunits remain associated when salts are removed (Thanh and Shibakasi, 1979; Sykes and Gayler, 1981; Iwabuchi et al., 1991b).

The aim of this study was to identify some of the components usually seen in an electrophoretic profile of a soy protein isolate, as well as the modifications introduced in that profile by changes of either the pH or the ionic strength, to identify which type of interactions take place between the components.

MATERIALS AND METHODS

Preparation of Isolates. Protein isolates were prepared from defatted soy flour (Sanbra S.A., Brazil), which was extracted with water at pH 8, made alkaline with 2 N NaOH [flour:water ratio 1:10 (w/v)] for 2 h at room temperature; pH was periodically adjusted. The suspension thus obtained was filtered through gauze, and the filtered material was centrifuged at 10000g for 30 min at 4 °C. The supernatant was adjusted to pH 4.5 with 2 N HCl. The precipitate was separated by centrifugation at 5000g for 15 min at 4 °C and suspended in water. Different treatments after this suspension were tested:

1. The isolates were adjusted to different pH values, 7, 8, 9, and 10, at two protein concentrations, i.e., 1 and 10% (w/v). Treatments were performed either at room temperature or at 100 °C for 90 min.

2. Treatment was performed with 0.1% (w/v) Na_2SO_3, followed by removal of this agent by means of repeated isoelectric precipitations, until a negative reaction of sulfite in the supernatant with Ellman's reagent (Beveridge et al., 1974) was reached.

Isolates obtained were lyophilized.

Electrophoresis. *SDS–Electrophoresis (SDS–PAGE).* Dissociating electrophoresis was carried out in a continuous buffer system: 0.375 M Tris-HCl, pH 8.8, 1% (w/v) SDS for the separating gel. Sample buffer used was 0.125 M Tris-HCl, pH 6.8, 20% (v/v) glycerol, 1% (w/v) SDS, and 0.05% (w/v) bromophenol blue. In some instances, 5% (v/v) β-mercaptoethanol was added. The buffer used for the electrophoretic runs was 0.025 M Tris-HCl, 0.192 M Gly, and 0.1% (w/v) SDS, pH 8.3. The gel was prepared with a 5–15% acrylamide gradient. Runs

0021-8561/95/1443-1762$09.00/0

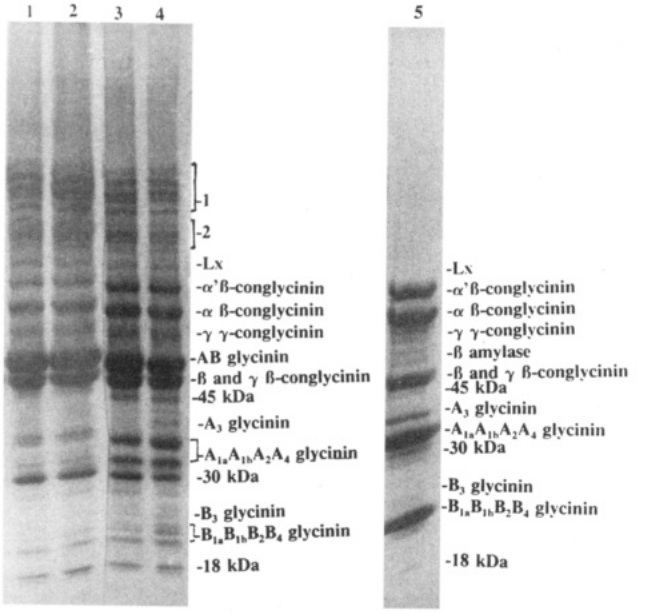

Figure 1. SDS–PAGE electrophoresis in a 5–15% polyacrylamide linear gradient. Lanes 1–4 correspond to a native, nonreduced isolate and lane 5 to an isolate reduced with β-mercaptoethanol. The protein concentration, prior to freeze-drying, was 10% (w/v) (lanes 1 and 2) and 1% (w/v) (lanes 3 and 4). Proteins identified: 1 and 2, aggregates of 100 000–200 000; Lx, lipoxygenase; α′ and α, β and γ subunits of β-conglycinin; AB subunit and A and B polypeptides of glycinin; β-amylase and proteins of molecular weights of 18 000, 30 000, and 45 000.

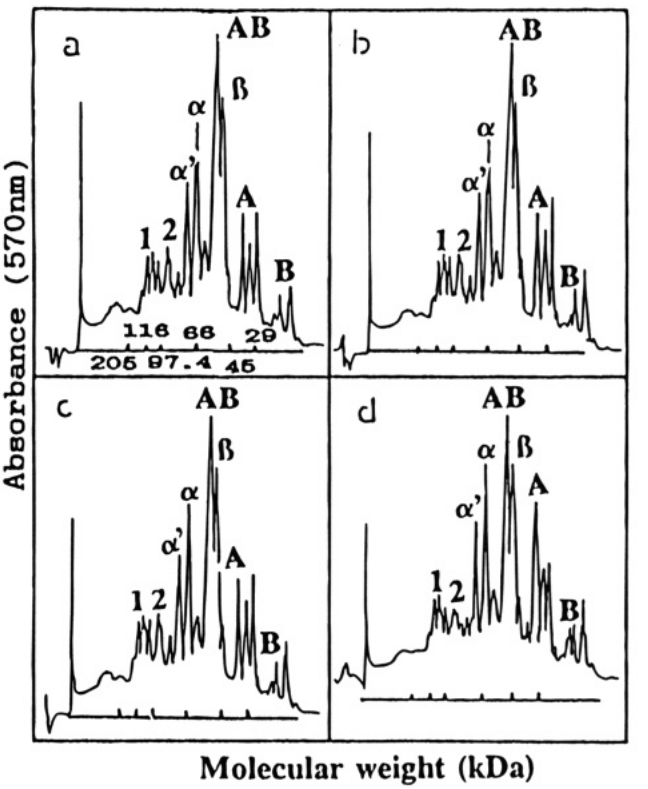

Figure 2. Densitograms corresponding to 5–15% linear gradient nonreducing SDS–PAGE electrophoresis of isolates treated at different pH values at a 1% protein concentration at room temperature: a, pH 7; b, pH 8; c, pH 9; d, pH 10.

were performed at 20 mA/0.75 mm plate thickness. A Cole Palmer electrophoresis unit was used.

Gels were fixed and dyed with R-250 Coomassie (0.1%) in water/methanol/acetic acid (5:5:2) for 12 h. They were later destained with 25% (v/v) methanol and 10% (v/v) acetic acid. Densitograms corresponding to each gel were performed in a Shimadzu double-wavelength spectrodensitometer TLC Scanning CS-910. Wavelengths used were 570 and 395 nm for the sample and reference, respectively.

Two-Dimensional Electrophoresis. Two-dimensional electrophoresis was performed on samples that were run in a SDS–PAGE gel in a 5–15% linear polyacrylamide in the first dimension, the second dimension being performed in a SDS–PAGE gel in a 5–15% linear polyacrylamide gradient in the presence of β-mercaptoethanol. After the first dimension run, gels were cut and the different lanes were frozen in 10% (v/v) glycerol at −20 °C. Before the second dimension run, the lanes were treated with 10 volumes of 62.5 mM Tris-HCl buffer, pH 6.8, 1% (w/v) SDS, and 20% (w/v) sucrose, with 0.2 M β-mercaptoethanol, for 30 min at 55 °C, with two changes of solution (Utsumi et al., 1984). Gels prepared for the first dimension were 0.75 mm thick; those corresponding to the second dimension were 1.5 mm thick, so as to avoid difficulties in placing that portion of the gel corresponding to the first dimension. Once the lane had been placed on the gel corresponding to the second dimension, a solution containing 6.25 mM Tris-HCl, pH 6.8, 1% (w/v) SDS, and 0.01% (w/v) bromophenol was added; the run was then performed at a constant current of 40 mA/plate.

Viscosity. Aqueous solutions containing 10% (w/v) of the isolates were prepared. In some cases, Na_2SO_3, Na_2SO_4, or NaCl was added. These were homogenized, and the apparent viscosity at 128 rpm at 20 °C was measured with a Haake Rotavisco (HAAKE Mess Technik GmbH u Co., Karlsruhe, Germany).

RESULTS AND DISCUSSION

Figure 1 shows a typical electrophoretic run in a SDS–PAGE gel of a native isolate, both with and without β-mercaptoethanol. The different components present in an isolate can be identified as follows: Lx, lipoxygenase (Iwabuchi and Yamauchi, 1987b); α′, α, β, and γ subunits of β-conglycinin (Thanh and Shibasaki, 1977; Davies et al., 1985); AB subunits and A and B polypeptides of glycinin (Nielsen, 1985); γ subunit of γ-conglycinin (Hirano et al., 1987). The 45 kDa fraction may belong to the basic 7S globulin, as well as the 30 kDa fractions (H_I and H_{II} basic 7S globulin) and the 18 kDa fractions (L_I and L_{II} basic 7S globulin) (Sathe et al., 1989). However, the 30 kDa fraction could be an agglutinin and the 18 kDa band that of the Kunitz trypsin inhibitor (Iwabuchi and Yamauchi, 1987b; Pearson, 1982). Fractions labeled 1 and 2 are 100–200 kDa aggregates, consisting of more than eight protein components, as can be seen in Figure 1. These aggregates disappear in the presence of β-mercaptoethanol; at the same time an increase of α′ and α subunits occurs. It is characteristic of these aggregates to form at least four well-defined bands (two corresponding to 1 and the other two to 2).

An important difference in the amount of aggregates of the α′ and α subunits of β-conglycinin is observed at the protein concentration at which neutralization and further freeze-drying are performed (Figures 1, 2, and 3). At 10% (w/v) protein concentration, these treatments promote the formation of a higher amount of aggregates (the intensity of the bands labeled 1 on lanes 1 and 2 is higher than that corresponding to α and α′ 7S subunits; the opposite behavior is detected on lanes 3 and 4), because of the closer proximity of β-conglycinin molecules, thus favoring their interaction (Figure 1). These aggregates are stable, since diluting the isolates to 1%, 0.1%, and 0.01% (w/v) concentration resulted in no change of their level. Changes do occur when reducing or thermal treatments are used.

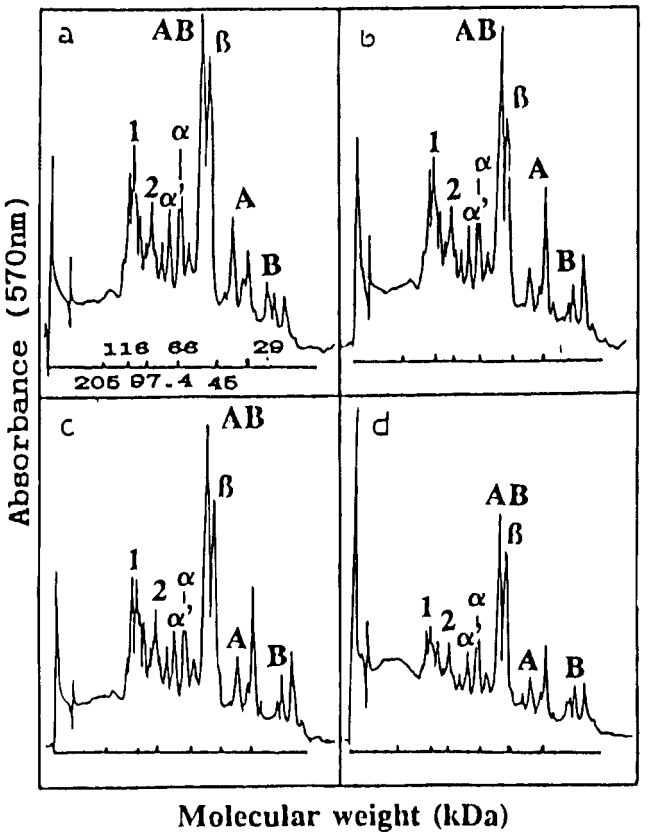

Figure 3. Densitograms corresponding to 5–15% linear gradient nonreducing SDS–PAGE electrophoresis of isolates treated at different pH values at 10% protein concentration at room temperature: a, pH 7; b, pH 8; c, pH 9; d, pH 10.

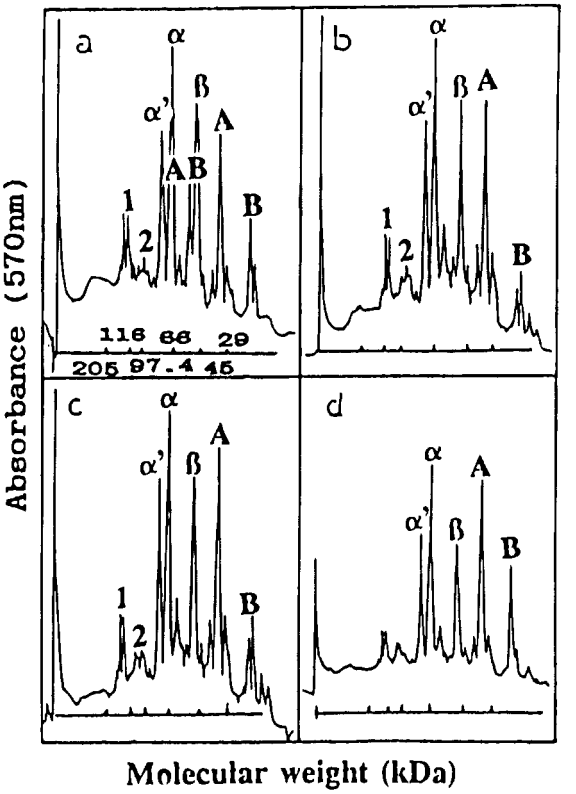

Figure 4. Densitograms corresponding to 5–15% linear gradient nonreducing SDS–PAGE electrophoresis of isolates treated at different pH values at 1% protein concentration at 100 °C for 90 min: a, pH 7; b, pH 8; c, pH 9; d, pH 10.

The behavior of the isolates when they were adjusted to different pH values without and with thermal treatment was also studied. Figures 2 and 4 show the densitometer scans corresponding to isolates obtained by adjusting the pH to 7, 8, 9, and 10, at 1% (w/v) protein concentration. No differences of protein composition are observed in unheated isolates neutralized at pH 7, 8, and 9. An increase of fraction A-11S is observed at pH 10 (the height of the A-11S peak—which was 50% of the height of peak β-7S at pH 7–9—reaches 75% of that height) (Figure 2). An increase of one of the fractions (having the highest molecular weight) of polypeptide B-11S is also noticed. This increase could arise from the separation of free A and B polypeptides that had been associated in glycinin. Thermal treatments at this protein concentration (1%) (Figure 4) lead to the disappearance of AB subunits and the appearance of A and B polypeptides. The disappearance of AB-11S subunits is mainly produced by the aggregate formation through disulfide bonds; meanwhile, exchange reactions between free sulfhydryl groups and disulfide bonds would be responsible for the small increase of fractions A and B (Sathe et al., 1989; Shiga and Nakamura, 1987). A decrease of the aggregates of intermediate molecular weight (aggregate 2) also takes place (Figure 4).

The effects of Na_2SO_3, Na_2SO_4, and NaCl on the stability of aggregates 1 and 2 were studied by analyzing the changes occurring both in the electrophoretic profiles and in the viscosity. These salts were added prior to the SDS–PAGE and viscosity measurements. Figure 5 shows the densitograms of isolates treated at two protein concentrations (10% and 1% (w/v)) with 0.01%, 0.1%, and 1% (w/v) Na_2SO_3 for 30 min. An almost

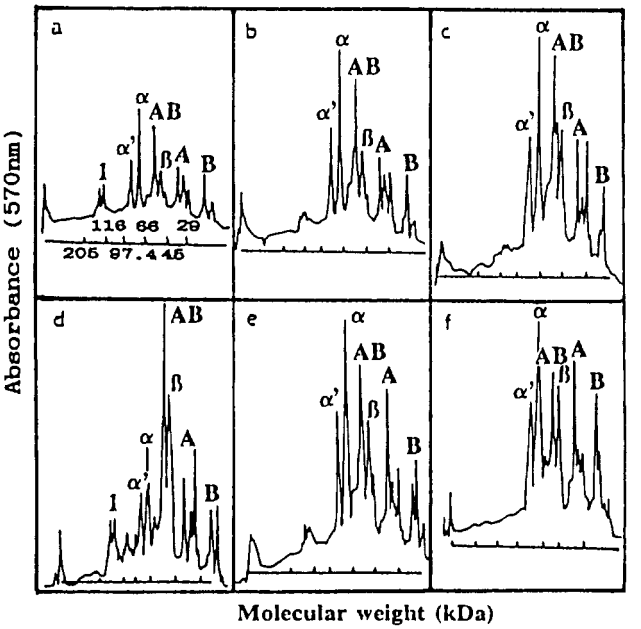

Figure 5. Densitograms corresponding to 5–20% linear gradient SDS–PAGE electrophoresis: 10% isolates (a–c) and 1% isolates (d–f) treated with 0.01% (a and d), 0.10% (b and e), and 1% (c and f) Na_2SO_3.

complete disappearance of aggregate 1 and all of aggregate 2 was noted when Na_2SO_3 was present in the medium (Figure 5b); removal of Na_2SO_3, in turn, allows re-formation of aggregates (Figure 6b). The reaggregation caused by Na_2SO_3 removal is also evidenced by the increased viscosity exhibited by these isolates in comparison with those containing Na_2SO_3; viscosity of a sulfite-treated isolate (30%) with regard to an untreated isolate (100%) increases until it reaches 50%

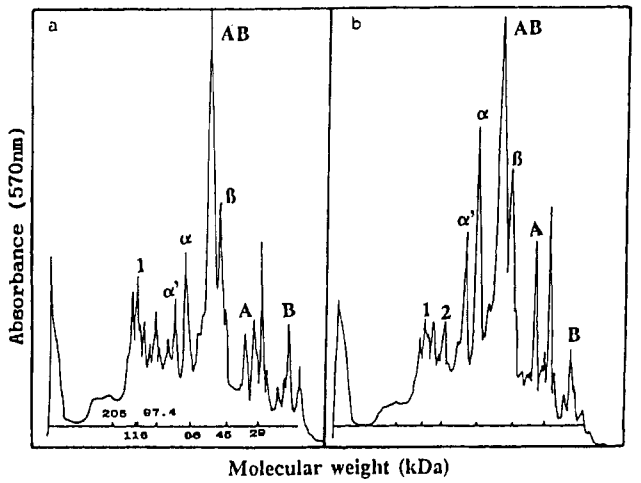

Figure 6. Densitograms corresponding to 5–20% linear gradient SDS–PAGE electrophoresis: a, nonreduced isolate; b, reduced (0.1% Na_2SO_3) isolate, from which sulfite had been removed by serial washings and isoelectric precipitations.

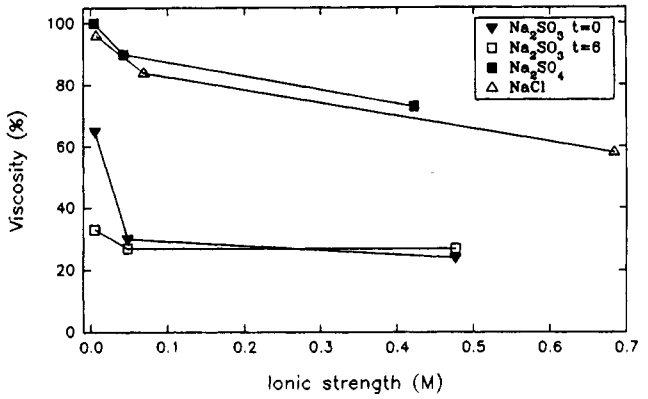

Figure 7. Viscosity of a native isolate at different ionic strengths. Curves correspond to addition of different amounts of Na_2SO_3, immediately after the addition ($t = 0$) and 6 h later ($t = 6$), Na_2SO_4, and NaCl.

when the Na_2SO_3 is removed. These aggregates are fewer in number or have smaller hydrodynamic volumes than those found in untreated isolates.

Viscosity of 10% (w/v) solutions of an isolate to which different amounts of Na_2SO_3 [0.01%, 0.1%, and 1% (w/v)] had been added was also studied; viscosity was determined immediately and 6 h after the addition of Na_2SO_3. No differences of viscosity were found at the higher concentrations of sulfite with the different treatment times; however, at 0.01% (w/v) concentration (Figure 7), the initial viscosity was approximately 30% higher than the final. Figure 7 also shows the viscosity variations caused by the addition of NaCl and Na_2SO_4; there is a slight decrease of viscosity, probably resulting from a decrease of the water imbibing capacity caused by the salt action (Yao et al., 1988) rather than from changes in the size of the aggregates already present.

Comparison of Figures 2a and 3a with Figure 8 shows the effects of Na_2SO_4 on SDS–PAGE analysis. Figure 8 corresponds to an isolate to which 0.01%, 0.1%, or 1% (w/v) Na_2SO_4 was added, at two protein concentrations: 10% (w/v) (a–c) and 1% (w/v) (d–f). When the treatment takes place at 10% protein concentration, the following changes occur: there is a decrease of the intermediate molecular weight aggregate 2 and of the β-7S subunits and an increase of A and B-11S polypeptides. These changes also result from sulfate treatments at 1% protein concentration, but they are less

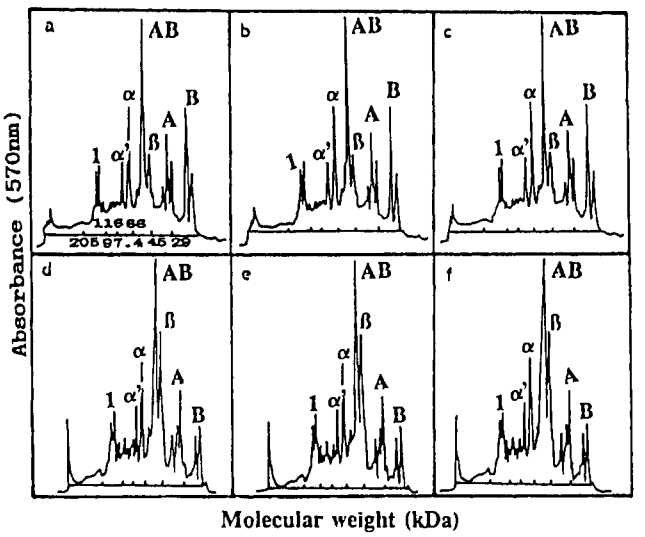

Figure 8. Densitograms corresponding to 5–20% linear gradient SDS–PAGE electrophoresis of isolates at pH 7 treated at 10% (a–c) and 1% protein concentrations (d–f) with 0.01% (a and d), 0.10% (b and e), and 1% (c and f) Na_2SO_4.

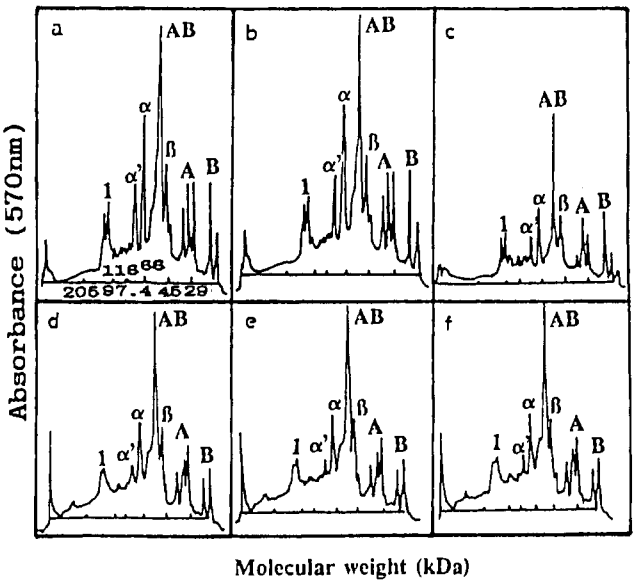

Figure 9. Densitograms corresponding to 5–20% linear gradient SDS–PAGE electrophoresis of isolates at pH 7 treated at 10% (a–c) and 1% protein concentrations (d–f) with 0.02% (a and d), 0.20% (b and e), 2.0% and (c and f) NaCl, respectively.

important with the exception of the increase of A and B-11S polypeptides that in this case was not observed. No changes of subunit composition are observed at a given protein concentration as a result of changes in sulfate concentration.

Figure 9 shows the effect of NaCl. Treatments at 1% (w/v) produce an aggregation of the β-7S subunit which is more pronounced than that seen with Na_2SO_4. At 10% (w/v) the aggregation of β, α', and α-7S subunits is more intense. At variance with the changes produced by sulfate, there are no increases of either A or B-11S polypeptides.

It can be assumed that treatment with NaCl and Na_2SO_4 leads to greater changes of β-conglycinin, which, in turn, produce an increase of aggregation of β subunit and also a decrease of intermediate molecular weight aggregate 2; this aggregate fraction is affected both by ions and by thermal treatment. Consequently, it could be assumed that even though these 1 and 2 aggregates

exhibit disulfide bonds, they also remain joined by other interactions of the ionic type; this is in accordance with the high polarity in the NH_2-terminal end shown by α and α′ subunits (Iwabuchi et al., 1991a,b). Interactions of the hydrophobic type have not been evaluated, since all changes were studied by electrophoresis in the presence of SDS.

As β-conglycinin has very low contents of cysteine and cystine (Sebastiani et al., 1990; Doyle et al., 1986), we studied whether other proteins are capable of forming aggregates. To this end, a two-dimensional electrophoresis of a native isolate was performed. A SDS–PAGE gel was used in the first dimension, in a 5–15% linear polyacrylamide gradient in the absence of β-mercaptoethanol; for the second dimension a 5–15% polyacrylamide gel was used as well, but in the presence of mercaptoethanol (Figure 10a). A straight line at 45° is expected in the absence of disulfide bonds, since the breakdown of interchain bonds will give rise to lower molecular weight components. It can be seen that aggregate 1 is formed by the α and α′ subunits of β-conglycinin, whereas aggregate 2 contains in addition A polypeptide of glycinin. The aggregates are 192 000 and 179 000 (corresponding to the two most intensive bands of aggregate 1) and 123 000 and 115 000 (corresponding to the two proteins of aggregate 2 having the highest intensity). Bearing in mind that the α and α′ subunits are 87 000 and 71 000, the aggregates could be dimers or trimers of those subunits. It is necessary to clarify that the molecular weights used in this analysis are subject to error because the glycoprotein molecular weight determined by electrophoresis is overestimated (Poduslo, 1981). As reported in the literature, β-conglycinin can exist in two types lacking subunit β, namely B_5 formed by $\alpha_2\alpha'$ and B_6 formed by α_3 (Thanh and Shibasaki, 1976; Yamauchi et al., 1981; Sykes and Gayler, 1981). Nevertheless, the forces responsible for the formation of these trimers are, according to these authors, of the electrostatic type.

Figure 10b shows an isolate that had been subjected to a reducing treatment [0.1% (w/v) Na_2SO_3]; the two-dimensional run was performed as described earlier. It can be noted that reduction produces an increase of the α and α′-7S subunits, the intensity of which is greater than those of the aggregates, a phenomenon that was not seen in the native isolate. It can also be seen that aggregate 2 is the most affected by reduction.

The results obtained suggest that aggregate 1 is a trimer or dimer of α and α′-7S subunits; these subunits appear to interact, at least partially, through interchain disulfide bonds. At variance with this, aggregate 2 is formed by α and α′-7S subunits, as well as by A polypeptide of glycinin. The stability of aggregate 2 is lower than that of aggregate 1, since either the addition of Na_2SO_3, Na_2SO_4, or NaCl or the thermal treatment leads to their disappearance. The presence of SDS has no effect on the aggregate structure, since interactions of the hydrophobic type are not included in the forces which permit the formation and stability of their structure. Such aggregates are also found in soy flour.

Figure 10a indicates also the presence of other components with interchain disulfide bonds: one is a protein of an approximate molecular weight of 56 000, which is located between the AB subunits (58 000) and the β-7S subunit (54 000), and upon reduction gives rise to a 37 000 component and a 30 000 component; the other is a protein of a molecular weight lower than that

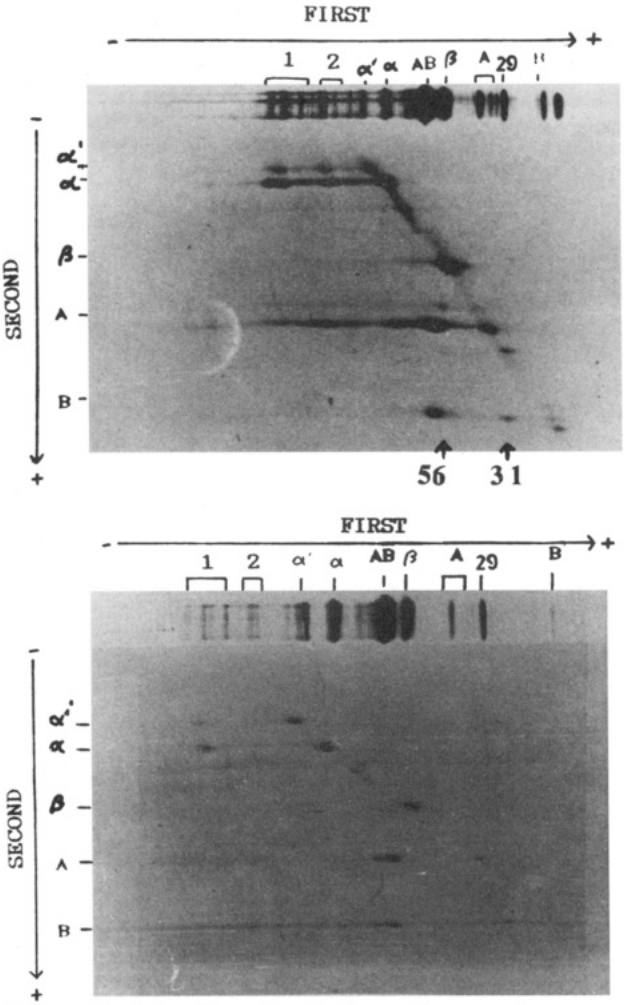

Figure 10. Two-dimensional electrophoresis of a native isolate (a, top) and of an isolate reduced with Na_2SO_3 (b, bottom). First dimension: SDS–PAGE in a 5–15% polyacrylamide linear gradient. Second dimension: SDS–PAGE in a 5–15% polyacrylamide linear gradient in the presence of β-mercaptoethanol. Migration is from top to bottom. Proteins 1 and 2 correspond to low molecular weight aggregates (100 000–200 000; α′, α, and β subunits of β-conglycinin; AB subunits and A and B polypeptides of glycinin.

of A polypeptide of 11S, which gives rise to an 18 000 component.

CONCLUSIONS

Aggregates formed by α and α′ subunits of β-conglycinin exhibit both ionic interactions and disulfide bonds. Higher molecular weight aggregates (180 000–190 000) consist of trimers or dimers of α′ and α subunits, whereas those of intermediate molecular weight (115 000–120 000) are formed by α′,α subunits of β-conglycinin and A polypeptides of glycinin. The latter exhibit a higher sensitivity toward changes of ionic strength and thermal treatments. The proportion of α′ and α subunits of the isolate which is included in these aggregates is highly dependent on the freeze-drying conditions. These conditions, in turn, determine the number of high molecular weight components of 100 000–200 000 that will appear in the isolate (range varies between 4 and 10). These aggregates are readily reduced in the presence of Na_2SO_3, even at low concentrations and in the absence of denaturing agents, thus suggesting that the disulfide bonds involved are accessible. Another feature of these aggregates is that once the reducing

agent has been removed, reoxidation takes place, leading again to aggregate formation. These aggregates are stable at different pH values in the presence of both SDS and urea. This result is not in accordance with those of Iwabuchi et al. (1991a,b), who reported that aggregates of β-conglycinin formed solely by α′ and α subunits are the most sensitive to changes of ionic strength and that those subunits undergo dissociation under the electrophoresis conditions. This high stability implies that interactions between α′ and α subunits in those aggregates take place at least partially through disulfide bonds.

Increase of an isolate pH from 7 to 9 causes changes in the conformation of A polypeptide. If the pH is increased further—to pH 10—an increase of A and B-11S polypeptides is also observed.

LITERATURE CITED

Badley, R. A.; Atkinson, D.; Hauser, H.; Oldani, D.; Green, J. P.; Stubbs, J. M. The structure, physical and chemical properties of the soybean protein glycinin. *Biochim. Biophys. Acta* **1975**, *412*, 214–228.

Beveridge, T.; Toma, S. J.; Nakai, S. Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *J. Food Sci.* **1974**, *39*, 49–51.

Davies, C. S.; Coates, J. B.; Nielsen, N. C. Inheritance and biochemical analysis of four electrophoretic variants of β-conglycinin from soybean. *Theor. Appl. Genet.* **1985**, *71*, 351–358.

Doyle, J. F.; Schuler, M. A.; Dean Godette, W.; Zenger, V.; Beachy, R. N. The glycosylated seed storage proteins of Glycine max and Phaseolus vulgaris. *J. Biol. Chem.* **1986**, *261*, 9228–9238.

George, A. A.; de Lumen, B. O. A novel methionine-rich protein in soybean seed—Identification, amino acid composition, and N-terminal sequence. *J. Agric. Food Chem.* **1991**, *39*, 224–227.

Hirano, H.; Kagawa, H.; Kamata, Y.; Yamauchi, F. Structural homology among the major 7S globulins subunits of soybean seed storage proteins. *Phytochemistry* **1987**, *26*, 41–45.

Iwabuchi, S.; Yamauchi, F. Determination of glycinin and β-conglycinin in soybean proteins by immunological methods. *J. Agric. Food Chem.* **1987a**, *35*, 200–205.

Iwabuchi, S.; Yamauchi, F. Electrophoretic analysis of whey proteins present in soybean globulin fractions. *J. Agric. Food Chem.* **1987b**, *35*, 205–209.

Iwabuchi, S.; Watanabe, H.; Yamauchi, F. Thermal denaturation of β-conglycinin—Kinetic resolution of reaction mechanism. *J. Agric. Food Chem.* **1991a**, *39*, 27–33.

Iwabuchi, S.; Watanabe, H.; Yamauchi, F. Observations on the dissociation of beta-conglycinin into subunits by heat treatment. *J. Agric. Food Chem.* **1991b**, *39*, 34–40.

Kitamura, K.; Takagi, T.; Shibasaki, K. Renaturation of soybean 11S globulin. *Agric. Biol. Chem.* **1976**, *40*, 1837–1844.

Lei, M. G.; Tyrell, D.; Bassette, R.; Reeck, G. R. Two-dimensional electrophoretic analysis of soybean proteins. *J. Agric. Food Chem.* **1983**, *31*, 963–968.

Nielsen, N. C. The structure and complexity of the 11S polypeptides in soybeans. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1680–1686.

Pearson, A. M. Soy proteins. In *Developments in Food Proteins—2*; Hudson, B. J. F., Ed.; Elsevier: London, 1982; pp 67–108.

Plietz, P.; Damaschum, G.; Muller, J. J.; Schwenke, K. D. The structure of 11S globulins from sunflower and rape seed. A small angle x-ray scattering study. *Eur. J. Biochem.* **1983**, *130*, 315–320.

Poduslo, J. F. Glycoprotein molecular-weight estimation using sodium dodecyl sulfate-pore gradient electrophoresis: Comparison of tris-glycine and tris-borate-EDTA buffer systems. *Anal. Biochem.* **1981**, *114*, 131–139.

Sathe, S. K.; Mason, A. C.; Weaver, C. M. Thermal aggregation of soybean (Glycine max L.) sulfur-rich protein. *J. Food Sci.* **1989**, *54*, 319–323, 342.

Sebastiani, F. L.; Farrel, L. B.; Schuler, M. A.; Beachy, R. N. Complete sequence of cDNA of alpha subunit of soybean beta-conglycinin. *Plant Mol. Biol.* **1990**, *15*, 197–201.

Shiga, K.; Nakamura, Y. Relation between denaturation and some functional properties of soybean protein. *J. Food Sci.* **1987**, *52*, 681–684, 690.

Sykes, G. E.; Gayler, K. R. Detection and characterization of a new β-conglycinin from soybean seeds. *Arch. Biochem. Biophys.* **1981**, *210*, 525–530.

Thanh, V. H.; Shibasaki, K. Heterogeneity of β-conglycinin. *Biochim. Biophys. Acta* **1976**, *439*, 326–338.

Thanh, V. H.; Shibasaki, K. Beta conglycinin from soybeans proteins. Isolation and inmunological and physicochemical properties of the monomeric forms. *Biochim. Biophys. Acta* **1977**, *490*, 370–384.

Thanh, V. H.; Shibasaki, K. Major proteins of soybeans seeds. Reversible and irreversible dissociation of β-conglycinin. *J. Agric. Food Chem.* **1979**, *27*, 805–809.

Utsumi, S.; Inaba, H.; Mori, T. Heterogeneity of soybean glycinin. *Phytochemistry* **1981**, *20*, 585–589.

Utsumi, S.; Damodaran, S.; Kinsella, J. E. Heat-induced interactions between soybean proteins: preferential association of 11S basic subunits and β subunits of 7S. *J. Agric. Food Chem.* **1984**, *32*, 1406–1412.

Yamauchi, F.; Sato, M.; Sato, W.; Kamata, K.; Shibasaki, K. Isolation and identification of a new type of β-conglycinin in soybean globulins. *Agric. Biol. Chem.* **1981**, *45*, 2863–2868.

Yao, J. J.; Wei, L. S.; Steinberg, M. P. Water-imbibing capacity and rheological properties of isolated soy proteins. *J. Food Sci.* **1988**, *53*, 464–467.

Received for review August 15, 1994. Revised manuscript received February 21, 1995. Accepted March 21, 1995.[⊗] This investigation was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. S.P. is a Research Fellow of the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), and M.C.A. is Member of the Researcher Career of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

JF940003T

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1995.