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Effect of Oxidative Sulfitolysis of Disulfide Bonds of Glycinin on Solubility, Surface Hydrophobicity, and in Vitro Digestibility

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The effect of disulfide bond cleavage on the solubility, surface hydrophobicity, and pepsin and pancreatin digestibility of glycinin and its components was studied by turbidity, 1,8-anilinonaphthalenesulfonate extrinsic fluorescence, ultraviolet absorbance, and polyacrylamide gel electrophoresis measurements. Disulfide bond cleavage increased the solubility of glycinin and its basic and acidic polypeptide components. It increased the surface hydrophobicity of acidic polypeptides and decreased that of glycinin and basic polypeptides. The digestibility of the acidic subunits with pepsin and pancreatin was enhanced whereas glycinin and basic polypeptides showed decreased digestibility following disulfide reduction. The decrease in the surface hydrophobicity and protein digestibility of glycinin might be due to the aggregation of the basic polypeptides primarily through hydrophobic interactions.

INTRODUCTION

Although there is increasing usage, soy proteins have not yet realized their anticipated widespread use in food products. Because functional properties are directly affected by physicochemical properties of proteins, a better understanding of physical characteristics of soy proteins is essential for manipulating their functional properties in foods. (Kinsella et al., 1985; Kinsella, 1985). Two physicochemical properties that may govern many other functional properties are solubility and surface hydrophobicity (Kinsella et al., 1985). The interaction of the food protein with water is manifested by its solubility. The distribution of nonpolar patches on the protein surface determines its surface hydrophobicity, which tends to reduce solubility. Apart from the functional properties, nutritional quality of a protein is also important for the widespread usage of a protein in food formulations. Nutritional quality of a protein depends not only on its amino acid composition but also on the bioavailability of the essential amino acids. This, in turn, is affected to a large extent by the digestibility of the food protein (Del Valle, 1981). Antinutritional factors such as protease inhibitors, lectins etc., may contribute to the low digestibility of these proteins (Liener, 1978). But in addition the tightly folded native conformation of soy proteins may also be responsible for the limited digestibility of these proteins (Wolf, 1978; Rothenbuhler and Kinsella, 1985).

Soy protein is composed of mainly two components, glycinin and conglycinin. Glycinin, the major fraction of soy proteins, has a molecular weight of 350 000 and is made of two identical half-molecules. Each of these consists of three acidic (M_r 37 000–40 000) and three basic (M_r 18 000–20 000) polypeptides. Glycinin has 18–20 disulfide bonds of both inter- and intramolecular nature that contribute to the compact structure of this protein (Kella et al., 1986; Draper and Catsimpoolas, 1978). From the amino acid composition of glycinin's polypeptides it is known that about two-thirds of the S–S bonds of glycinin are contributed by the acidic polypeptides and the rest by basic polypeptides (Iyengar and Ravestein, 1981). The presence of several intramolecular disulfide bonds apparently decreases the digestibility of glycinin, and hence reduction of these should enhance its digestibility. Cleavage of the S–S bonds of glycinin using β-mercaptoethanol in the presence of 6 M GuHCl followed by the blockage of free sulfhydryl groups by iodoacetamide improves its tryptic digestibility (Lynch et al., 1977). However, these chemical modifications are not acceptable for food applications. Since sulfite is permitted in processed foods at certain levels, a method based on sulfite reduction was developed

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to cleave the protein S–S bonds (Kella and Kinsella, 1985). In this paper we describe the effects of cleavage of S–S bonds of glycinin by oxidative sulfitolysis on protein solubility, surface hydrophobicity, and in vitro digestibility by pepsin and pancreatin. The effects of cleavage of the S–S bonds of the acidic and basic polypeptide components of glycinin were also studied to determine which of the components are responsible for the observed changes in the properties of glycinin.

MATERIALS AND METHODS

Materials. Defatted soy flakes were obtained from Central Soya, Fort Wayne, IN. DTNB and GuSCN were purchased from Kodak Organic Chemicals, Rochester, NY. Sodium sulfite was obtained from Specialty Chemicals, Morristown, NJ. Cupric sulfate was purchased from Mallinckrodt, St. Louis, MO. High-grade urea (with very low amounts of cyanate) was obtained from Pierce Chemical Co., Rockford, IL. ANS (magnesium salt), DEAE-Sephadex A50, β-mercaptoethanol, pepsin, pancreatin, and BSA were all purchased from Sigma Chemical Co., St. Louis, MO. ANS was recrystallized twice prior to use. All the other chemicals used were of reagent grade.

Methods. *NTSB.* NTSB was synthesized according to the method of Thannhauser et al. (1984).

Preparation of Glycinin and Its Polypeptides. Glycinin was prepared from defatted soy flour by the method of Thanh and Shibasaki (1976). After isolation, the protein was dialyzed extensively against water and lyophilized. Acidic and basic polypeptides were isolated from glycinin by a slight modification of the method of Utsumi (1981). Glycinin (1.5 g) was added to a preswollen suspension of DEAE-Sephadex A50 gel (10 g equilibrated in 1 L of 0.1 M phosphate buffer containing 6 M urea, 0.02 M β-mercaptoethanol, and 0.02% sodium azide). The suspension was stirred for 2 h and filtered through a sintered-glass funnel under vacuum to recover the unbound basic polypeptides. The gel was washed 3 times with 50 mL of 0.1 M phosphate buffer of pH 6.3 containing 0.5 M NaCl and 0.02% sodium azide for 2 h to release all the bound acidic polypeptides. It was filtered through a sintered-glass funnel. Filtrates containing acidic and basic polypeptides were separately dialyzed extensively against distilled water and lyophilized.

Preparation of S–S Bond Cleaved Glycinin Fractions. For the sulfitolysis of glycinin and the basic polypeptides, 50 mg of each was dissolved in 50 mL of 0.1 M phosphate buffer of pH 7.0 containing 4 M urea and 0.1 M sodium sulfite. In the case of acidic polypeptides urea was omitted from the buffer. The solutions were incubated at 40 °C for 15 min after which ammoniacal cupric sulfate (pH 9.0; at 400 μM level) was added and molecular oxygen was bubbled through a gas dispenser slowly without producing too much frothing. The kinetics of the sulfitolysis were followed as described previously (Kella and Kinsella, 1985). When the sulfitolysis reaction was complete, glycinin and acidic polypeptides were dialyzed extensively against water containing 40 mM EDTA and the basic polypeptides against 0.1 N acetic acid (pH 3.0) containing 40 mM EDTA. All three fractions were finally dialyzed extensively against distilled water and then lyophilized.

Protein Estimations. These were done by the microbiuret method using crystalline BSA as the standard (Bailey, 1961).

Solubility. Solubility was determined by measuring the turbidity of the protein solution in the range pH 1–11 according to Kitabatake et al. (1985) using 10 mL of protein solutions (1 mg/mL). In all the cases pH was first adjusted to 11 by 1 N NaOH and then decreased gradually with 1 N HCl. Transmittance at 500 nm of the protein solutions as a function of pH was noted using a Spectronic 700 and 1-cm path-length cuvette.

Surface Polarity. Polarity of the hydrophobic binding sites on the protein surface was determined according to Turner and Brand (1968) using ANS and Perkin-Elmer Model 650-40 fluorescence spectrophotometer. Protein solutions (0.1 mg/mL) containing 20 mM ANS were excited at 350 nm, and the emission was automatically recorded at right angles to excitation, in the range 400–500 nm using a 5-nm excitation band width at 25 °C (Kella et al., 1984). The fluorescence spectra in all cases were corrected for the emission of ANS blank. ν_F, the reciprocal of the emission maximum, was calculated in each case. From these values the surface polarity on Kosowar's Z scale was calculated from a standard curve (Turner and Brand, 1968).

Pepsin and Pancreatin Digestibility. Digestibility was assessed by the TCA precipitation method adopted from Shyamasunder and Rao (1984). Protein samples (12 mL) of 1 mg/mL concentration (in 0.1 N HCl) were equilibrated in a water bath at 37 °C for 15 min. To these was added 60 μL of 0.1% pepsin solution (in 0.1 N HCl; at 1:200 enzyme to protein ratio) by a microsyringe. To 1-mL aliquots (that were removed at regular intervals from the protein sample) was added 1 mL of 20% TCA solution and the resultant mixture allowed to stand for 10 min. After centrifuging for 10 min, at 10000 rev/min, the supernatant was collected and its absorbance at 280 nm was read from a Cary-219 double-beam spectrophotometer. Analyses were done in triplicate.

Following the 2-h pepsin digestion, the remaining sample (7 mL) in each case was adjusted to pH 8.0 by adding 0.1 equiv of sodium hydroxide, 58 mg of sodium bicarbonate, and 2 mg of sodium carbonate (to get 0.1 M carbonate buffer). The protein solutions were equilibrated at 37 °C in a water bath for 15 min. Pancreatin (at pH 8.0, in water) was added to these at 1:200 enzyme to protein ratio, and proteolysis (up to 6 h) was monitored by TCA precipitation as described above.

SDS–PAGE. Changes in proteins accompanying pepsin and pancreatin hydrolysis were monitored by SDS–PAGE by carrying out separate sets of experiments as described above. At regular intervals of time, 0.2-mL aliquots of the hydrolyzates were removed and the proteolysis terminated by diluting 1:4 (v/v) with SDS–PAGE sample buffer (0.0625 M Tris; pH 6.8, containing 8 M urea, 1% SDS, 10 mM DTT, and 0.025% bromophenol blue). Electrophoretic experiments were performed on 5–20% acrylamide slab gels using a BioRad system according to the method of Laemmli (1970). Native glycinin (20 μg/channel) and hydrolyzates (100 μg/channel) were loaded, and electrophoresis was carried out at a constant current of 24 mA for about 4 h. The gels were fixed and stained in 10:10:80 methanol–acetic acid–water containing Coomassie brilliant blue R-250 (0.0125%) and destained in the same solution without the dye.

RESULTS

Purity. Glycinin prepared by the method of Thanh and Shibasaki (1976) gave six bands on the SDS–PAGE (Figure 1, lane A). Bands 1–4 correspond to acidic and bands 5 and 6 to basic polypeptides as judged by their respective molecular weights (Moreira et al., 1981). The isolated acidic polypeptides gave four bands corresponding to the four acidic polypeptides (Figure 1, lane B). The basic polypeptide preparation showed two fast-moving bands that correspond to basic polypeptides (Figure 1, lane C). The electropheretic pattern shows that the prepared acidic

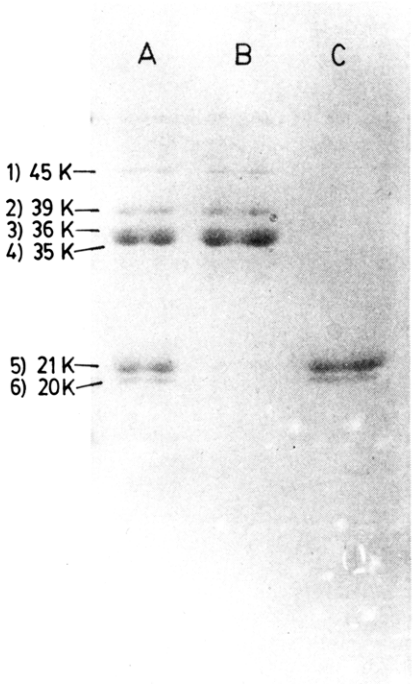

Figure 1. SDS–DTT polyacrylamide gel electrophoresis of prepared glycinin (lane A) and acidic (lane B) and basic (lane C) polypeptides.

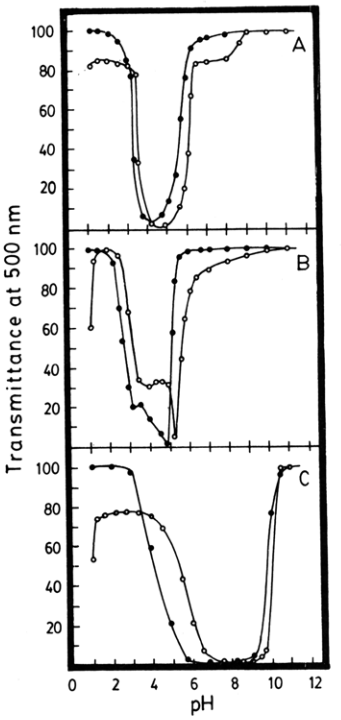

Figure 2. Solubility patterns of unmodified and S–S bond cleaved glycinin and its polypeptides: (a) (○) unmodified glycinin and (●) S–S bond cleaved glycinin; (B) (○) unmodified acidic polypeptides and (●) S–S bond cleaved acidic polypeptides; (C) (○) unmodified basic polypeptides and (●) S–S bond cleaved basic polypeptides.

and basic polypeptides are pure without any cross contamination.

S–S Bond Cleaved Glycinin and Its Polypeptides. Unmodified glycinin contained 18 S–S bonds as determined by NTSB method (Thannhauser et al., 1984). Isolated acidic and basic polypeptides contained about 13 and 5 S–S bonds, respectively. None of the three protein fractions contained free sulfhydryl groups as estimated by DTNB method (Ellman, 1959) in 8 M urea. In glycinin and its polypeptides that were subjected to oxidative sulfitolysis, no residual S–S bonds were detectable by NTSB method, indicating that all the S–S bonds are cleaved completely.

Solubility Measurements. Unmodified glycinin revealed an insoluble region in the range pH 3.5–6.5 with solubility minimum around pH 4.8 in water (Figure 2A, ○). The protein also showed two small solubility minima around pH 2–3 and 7–8. After S–S bond cleavage, the solubility minima at pH 2–3 and 7–8 disappeared (Figure 2A, ●). Unmodified acidic polypeptides had two solubility minima at pH 4.0 and 5.2, the minimum at pH 4.0 being lesser in magnitude than the latter (Figure 2B, ○). In the case of S–S bond cleaved acidic polypeptides, both the minima shifted to pH 3.2 and 4.8, respectively. Above pH 5.5 the solubility of S–S bond cleaved acidic polypeptides increased as compared to the unmodified one (Figure 2B, ●). Unmodified basic polypeptides had a broad solubility minimum in the range of about pH 6.5–9.8 (Figure 2C, ○). The S–S bond cleaved basic polypeptides also had a broad solubility minimum that slightly shifted to the range pH 5.7–9.2, but the protein solubility increased in the range pH 1–3 as compared to the unmodified polypeptides (Figure 2C, ●).

Surface Polarity. The extrinsic fluorescence emission spectra of ANS conjugates of unmodified glycinin and acidic and basic polypeptides (corrected for the fluorescence of the ANS blank) had emission maxima around 474, 476, and 460 nm, respectively. After complete S–S bond cleavage, the emission maxima of the ANS bound to glycinin, acidic polypeptides, and basic polypeptides shifted to 470, 472, and 465 nm, respectively. From the emission maxima, ν_F values (which are the measure of emission transition energy) and Z (surface polarity) values were calculated according to the method of Turner and Brand (1968) (Table I). Unmodified glycinin, acidic polypeptides, and basic polypeptides gave Z values of 90.7, 90.0, and 85.0. With the S–S bond cleavage a decrease in the Z values of both glycinin and acidic polypeptides was observed whereas in the case of basic polypeptides the value increased (Table I).

Table I. Surface Polarity of Unmodified and S–S Bond Cleaved Glycinin and Its Polypeptides as Estimated from the Emission Maxima of the Bound ANS according to the Method of Turner and Brand (1968)

protein sample	ν_F, $\times 10^4$ cm^{-1}	Z
glycinin	2.100	90.7
S–S bond cleaved glycinin	2.128	89.0
acidic polypeptides	2.105	90.0
S–S bond cleaved acidic polypeptides	2.155	87.5
basic polypeptides	2.174	85.0
S–S bond cleaved basic polypeptides	2.128	89.0

Pepsin and Pancreatin in Vitro Digestibility. The kinetics of peptic hydrolysis of both unmodified and S–S bond cleaved glycinin and acidic and basic polypeptides up to 2 h is shown in Figure 3. The rate of pepsin hydrolysis of all these samples increased rapidly up to 30 min after which it reached a constant value. The rates of proteolysis of the protein fractions followed the order S–S bond cleaved acidic polypeptides > acid polypeptides > unmodified glycinin > S–S bond cleaved glycinin > basic polypeptides > S–S bond cleaved basic polypeptides.

Peptic hydrolysates collected at 5 min were analyzed by SDS–PAGE. Native glycinin (not treated with enzyme) gave six bands (Figure 4, lane A). As described earlier, bands 1–4 correspond to acidic and bands 5 and 6 to basic polypeptides. Peptic hydrolysates of the unmodified and S–S bond cleaved glycinin gave four more bands in ad-

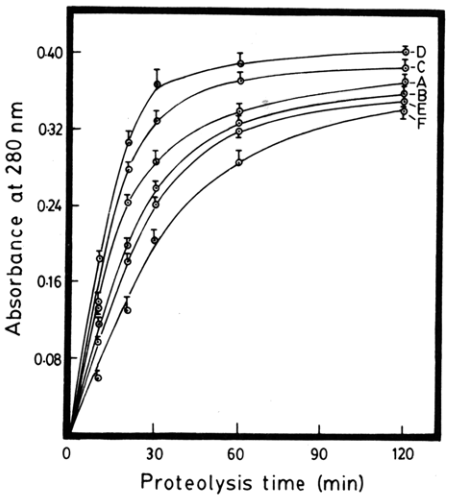

Figure 3. Kinetics of peptic hydrolysis of unmodified and S–S bond cleaved glycinin and its polypeptides as followed by the TCA precipitation method: (A) unmodified glycinin; (B) S–S bond cleaved glycinin; (C) acidic polypeptides; (D) S–S bond cleaved acidic polypeptides; (E) basic polypeptides; (F) S–S bond cleaved basic polypeptides.

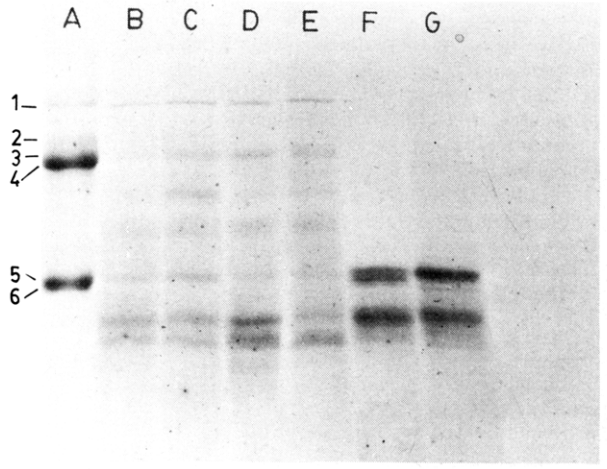

Figure 4. SDS–DTT polyacrylamide gel electrophoresis of the peptic hydrolysates of unmodified and S–S bond cleaved glycinin components: (A) unmodified and untreated glycinin; (B) 5-min peptic digest of unmodified glycinin; (C) 5-min peptic digest of S–S bond cleaved glycinin; (D) 5-min peptic digest of unmodified acidic polypeptides; (E) 5-min peptic digest of S–S bond cleaved acidic polypeptides; (F) 5-min peptic digest of unmodified basic polypeptides; (G) 5-min peptic digest of S–S bond cleaved basic polypeptides.

dition to the six observed in native glycinin (Figure 4, lanes B and C). Two of the extra bands had mobilities that were intermediate as compared to the smallest acidic polypeptide (band 4) and larger basic polypeptide (band 5). This indicates that they are peptic fragments arising from the cleavage of acidic polypeptides. The other two extra bands had higher electrophoretic mobilities compared to the basic polypeptides. These proteolytic fragments might have originated from either acidic or basic polypeptides. Further inspection of the digests of both the acidic and basic polypeptides (Figure 4 lanes D–G) indicated that the first of the electrophoretic bands is contributed by the digestion of both acidic and basic polypeptides whereas the second one arises mainly from the acidic polypeptides. In the case of glycinin and acidic polypeptides, the S–S bond cleavage enhanced the intensity of all the new bands while in the case of basic polypeptides it decreased the intensity of all the proteolytic fragments.

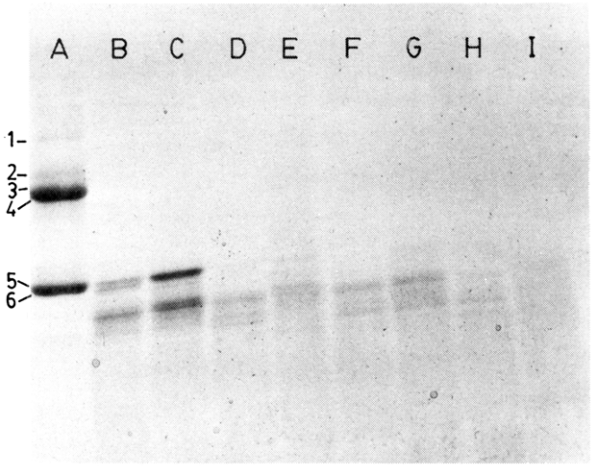

Figure 5. SDS–DTT polyacrylamide gel electrophoresis of the peptic hydrolysates of unmodified and S–S bond cleaved basic polypeptides: (A) unmodified and untreated glycinin; (B) and (C) 5-min digests of unmodified and S–S bond cleaved polypeptides; (D) and (E) 10-min digests of unmodified and S–S bond cleaved polypeptides; (F) and (G) 20-min digests of unmodified and S–S bond cleaved polypeptides; (H) and (I) 30-min digests of unmodified and S–S bond cleaved polypeptides respectively.

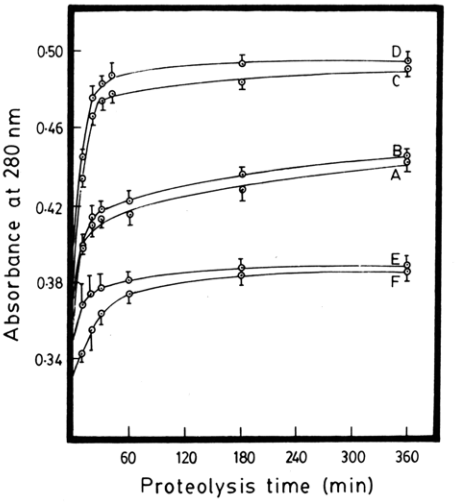

Figure 6. Kinetics of tryptic hydrolysis of unmodified and S–S bond cleaved glycinin and its polypeptides as followed by the TCA precipitation method: (A) unmodified glycinin; (B) S–S bond cleaved glycinin; (C) unmodified acidic polypeptides; (D) S–S bond cleaved acidic polypeptides; (E) unmodified basic polypeptides; (F) S–S bond cleaved basic polypeptides.

Since the basic polypeptides were relatively more resistant to pepsin digestion (as shown in Figures 3 and 4), the proteolytic degradation of these was followed by SDS–PAGE with time. The S–S bond cleaved basic polypeptides were more resistant to peptic digestion than the unmodified ones (Figure 5). However, the proteolytic resistant band of higher intensity present in the S–S bond cleaved basic polypeptides (Figure 5, lane C) disappeared completely from the 30-min peptic digest (Figure 5, lane I), indicating that it was completely cleaved to smaller fragments.

Pancreatin digestibility of both unmodified and S–S bond cleaved glycinin and acidic and basic polypeptides are shown in Figure 6. In all instances, the rate of proteolysis by pancreatin increased up to 60 min beyond which the values changed only slightly. Almost the same order to digestibility as in the case of pepsin was observed except for the fully cleaved glycinin, which was greater than the unmodified one. In the pancreatin digests, no

bands could be observed by SDS–PAGE possibly because of the small size of the resulting peptides (M_r <5000).

DISCUSSION

Solubility is desirable to achieve optimal functionality in foods where gelation, emulsification, foaming, etc., are involved (Kinsella, 1976). The solubility of a protein results from an equilibrium between protein–solvent and –protein interactions (Kuntz and Kauzmann, 1974). Conditions that favor protein–solvent interactions could generally increase the protein solubility. For example, increase in net negative or positive charge on the protein leads to increased electrostatic repulsive forces between the protein molecules, thus favoring protein–solvent interactions. Although an increase or decrease in pH of the medium toward alkalinity or acidity can be used to increase the protein solubility by charge solvation effects, this is not always practical for many reasons. In this study, an attempt was made to increase the solubility of glycinin by increasing the net charge following the introduction of negatively charged $-SO_3^-$ group by sulfitolysis of protein S–S bonds. For each of the S–S bonds, two $-SO_3^-$ groups were introduced. Cleavage of the protein S–S bonds by the sulfitolysis method altered the solubility pattern of glycinin and its components and caused a shift in the pI of the protein fractions toward acidic side. This also resulted in a slightly better solubility of the protein around the neutral and alkaline pH range. Since the pK_a of the $-SO_3^-$ group is around 6.8 (McPhee, 1956), the total shift in the pI in each of the S–S bond cleaved fractions was not significant.

During the protein folding process, most nonpolar residues occupy the protein interior to avoid their contact with water while the polar (charged) residues occupy the protein surface and favorably interact with water (Anfinsen and Scheraga, 1975). But when the content of the nonpolar residues in a protein is more than normal (one-third of the total residues), the excess residues appear on the protein surface in the form of hydrophobic patches (Schulze and Schirmer, 1979). Surface hydrophobicity of the protein has considerable impact on the functional properties such as fat binding, emulsification, etc. (Kato and Nakai, 1980). There are several methods for measuring the surface hydrophobicity of proteins such as those utilizing the binding of ANS (Turner and Brand, 1968), *cis*-parinaric acid (Sklar et al., 1977), SDS (Kato et al., 1984) etc., to the protein surface hydrophobic patches. Because of fewer problems with the method and the availability of a clearly defined polarity scale (Kosowar's Z scale), the ANS binding method was utilized to calculate the polarity of the surface hydrophobic patches of both the unmodified and the S–S bond cleaved glycinin fractions. S–S bonds maintain the structural integrity of a protein (Cantor and Schimmel, 1980). Hence, their cleavage may increase the number of nonpolar residues on the protein surface due to the exposure of many nonpolar residues from the protein interior to the polar exterior following the unfolding of the protein. In the present study, S–S bond cleavage altered the surface hydrophobicity (decrease in surface polarity) of only the acidic polypeptides. The observed decrease in the surface hydrophobicity (as observed by the increase in surface polarity) of S–S bond cleaved glycinin and basic polypeptides might result from the aggregation of the basic polypeptides (Kella et al., 1986; Utsumi and Kinsella, 1985; Damodaran and Kinsella, 1982).

Protein digestibility, the extent to which a protein can be hydrolyzed by a proteolytic enzyme, can affect the nutritional quality of a protein. Changes in native conformation of a protein often termed as denaturation affect protein digestibility (Mihalyi, 1978). The effects of several physical and chemical factors such as heat, pH, urea, GuHCl, SDS, etc., which denature proteins, on the digestibility of glycinin has been studied (Kamata et al., 1979a,b; Richardson and Catsimpoolas, 1979; Lynch et al., 1977). Most denaturant treatments decreased the digestibility of glycinin. In the present study, cleavage of protein S–S bonds, which causes the loosening of the protein structure, was explored to improve the protein digestibility. In vitro digestibility test of a protein using one or a combination of proteolytic enzymes is quicker method than time-consuming animal tests (Akeson and Stahman, 1964; Buchanan and Byers, 1969). In the present investigation to simulate in vivo conditions, pepsin followed by pancreatin digestion was conducted for about same duration as occurs in vivo in physiological buffer systems. With the exception of basic polypeptides, both pepsin and pancreatin digestibility of the S–S bond cleaved protein fractions increased. The decrease of the protein digestibility in the case of the basic polypeptides (as compared to the unmodified ones) could be due to the aggregation of these components (Kella et al., 1986; German et al., 1982), which may decrease the number of susceptible peptide bonds for the proteolysis (Mihalyi, 1978).

The present investigation revealed that, although the cleavage of S–S bonds increased the solubility of glycinin, its overall surface hydrophobicity and digestibility decreased after the S–S bond cleavage. S–S bond cleaved acidic polypeptides contribute to the overall increase in the surface hydrophobicity and digestibility of glycinin, whereas the basic polypeptides after S–S bond cleavage had the opposite effect. Basic polypeptides are more hydrophobic than the acidic ones as judged by their amino acid compositions (Iyengar and Revestein, 1981) and higher average hydrophobicity values, as compared to those of acidic polypeptides (Kinsella et al., 1985). Cleavage of S–S bonds could expose many of the nonpolar groups that were buried in the protein interior to the exterior, due to protein unfolding. The exposed nonpolar groups might favorably interact, leading to protein–protein interactions especially at the higher salt concentrations and temperatures (Kauzmann, 1959) that were used in digestibility experiments. These interactions involving basic polypeptides in turn may contribute to overall decrease in the surface hydrophobicity and digestibility of glycinin.

ABBREVIATIONS USED

Key: ANS, 1,8-anilinonaphthalenesulfonate; BSA, bovine serum albumin; DEAE, (diethylamino)ethyl; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, 1,4-dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GuHCl, guanidinium hydrochloride; GuSCN, guandinium thiocynate; NTSB, nitro-2,5-thiosulfobenzoate; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; Tris, tris[(hydroxymethyl)amino]-methane.

Registry No. Pepsin, 9001-75-6; pancreatin, 8049-47-6.

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Effect of Sodium Chloride on the Extractability of Proteins from Sesame Seed (*Sesamum indicum* L.)

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The effect of various concentrations of sodium chloride on the extractability of sesame seed proteins has been investigated. The extractability of total protein increases to nearly 80% until 5% sodium chloride concentration after which it remains constant. The extractability is also investigated as a function of pH in all the sodium chloride concentrations. The results indicate that the pH of minimum extractability drifts toward acidic pH as the sodium chloride concentration increases from 0.05 to 2.0 M. The observed results are explained due to preferential extractability of the various protein fractions with increasing concentration of sodium chloride and also due to the binding of sodium ions to these protein fractions.

Sesame seed (*Sesamum indicum* L.) contains nearly 25% protein, and the defatted meal contains nearly 50% protein. The earliest work on sesame seed proteins was by Ritthausen (1880) who extracted the protein from the sesame seed cake under variable conditions of alkali, NaCl, and temperature. Later Adolph and Lin (1936) determined the solubility of sesame seed proteins from the fat-free meal in NaCl, NaOH, and Na_2CO_3 solution. Basu and Gupta (1947) carried out similar solubility studies in water at various pH values and in the presence of NaCl and $NaHSO_3$. Nath and Giri (1957 a,b) carried out peptization studies of sesame seed proteins in NaCl and in acidic and alkaline pH media. Later, Guerra and Park (1975) carried out similar extractability studies of the protein in $CaCl_2$, Na_2SO_3, and Na_2HPO_4. They observed that high salt concentration increased the solubility of the proteins. Prakash and Nandi (1978) extracted the total protein in 1 M NaCl in order to isolate the major fraction α-globulin. Even though work on the protein α-globulin has advanced considerably (Prakash, 1985; Prakash and Narasinga Rao, in press), it is felt that no systematic work is available on the effect of different concentrations of NaCl on the extractability of total proteins. In most of the seed proteins it is an established fact that during extraction if more of NaCl is present, the point of minimum extractability is generally shifted toward acidic pH (Prakash and Narasinga Rao, in press). The explanation given is only empirical, and no systematic data are available, both experimentally as well as the analysis of data to explain the above phenomenon.

In this study data are presented on the extractability of sesame seed total proteins in various concentrations of NaCl. The shift in the pH of minimum extractability of

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