grant-in-aid from Firmenich, Inc. We thank Joan B. Shumsky for her secretarial aid.

Registry No. 2,4,5-Trimethyloxazole, 20662-84-4; 2-methyl-4-ethyloxazole, 53833-20-8; 2,4-dimethyl-5-ethyloxazole, 33318-74-0; 2,5-dimethyl-4-ethyloxazole, 30408-61-8; 2-ethyl-4,5-dimethyloxazole, 53833-30-0; 2,4-dimethyl-5-propyloxazole, 84027-86-1; 2-methyl-4-butyloxazole, 84027-87-2; 2-isopropyl-4,5-dimethyloxazole, 19519-45-0; 2,4-dimethyl-5-butyloxazole, 88300-07-6; 2-methyl-4-ethyl-5-propyloxazole, 88300-06-5; 2,5-dimethyl-4-butyloxazole, 30408-62-9; 2-methyl-4-pentyloxazole, 102586-52-7; 2-ethyl-4-methyl-5-propyloxazole, 102586-53-8; 2-isopropyl-4-methyl-5-ethyloxazole, 102586-54-9; 2-butyl-4,5-dimethyloxazole, 84027-97-4; 2-isobutyl-4,5-dimethyloxazole, 26131-91-9; 2-pentyl-4-methyloxazole, 52713-58-3; 2,4-diethyl-5-propyloxazole, 77311-03-6; 2-pentyl-4,5-dimethyloxazole, 84028-05-7; 2-butyl-4,5-diethyloxazole, 94794-08-8; 2-butyl-4-propyl-5-methyloxazole, 94794-09-9; 2-pentyl-4-methyl-5-ethyloxazole, 84028-13-7; 2-hexyl-4,5-dimethyloxazole, 20662-87-7; 2-hexyl-4-methyl-5-ethyloxazole, 84028-19-3.

LITERATURE CITED

Carlin, J. T. Ph.D. Dissertation, Rutgers University, New Brunswick, NJ 08903, 1983.
Catch, J. R.; Elliot, D. F.; Hey, D. F.; Jones, E. R. H. *J. Chem. Soc.* **1948**, *272*.
Chang, S. S.; Vallese, F. M.; Hwang, L. S.; Hsieh, O. A.-L.; Min, D. B. S. *J. Agric. Food Chem.* **1977**, *25*, *450*.
Coleman, E. C.; Ho, C.-T.; Chang, S. S. *J. Agric. Food Chem.* **1981**, *29*, *42*.
Ho, C.-T.; Hartman, G. J. *J. Agric. Food Chem.* **1982**, *30*, *793*.
Ho, C.-T.; Jin, Q. Z.; Lee, M.-H.; Chang, S. S. *J. Agric. Food Chem.* **1983a**, *31*, *1984*.
Ho, C.-T.; Jin, Q. Z.; Lee, K. N.; Carlin, J. T.; Chang, S. S. *J. Food Sci.* **1983b**, *48*, *1570*.
Jin, Q. Z.; Hartman, G. J.; Ho, C.-T. *Perfum. Flavor.* **1984**, *9*(4), 25.
Maga, J. A. *J. Agric. Food Chem.* **1978**, *26*, *1049*.
Majlat, P.; Erdos, Z.; Takacs, J. *J. Chromatogr.* **1974**, *91*, *89*.
Ohloff, G.; Flament, I. *Forsch. Chem. Org. Naturst.* **1979**, *36*, *231*.
ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Compounds in Foods*; TNO: Zeist, The Netherlands, 1980.
Theilig, G. *Chem. Ber.* **1953**, *86*, *96*.
Thompson, J. A.; May, W. A.; Paulose, M. M.; Peterson, R. J.; Chang, S. S. *J. Am. Oil Chem. Soc.* **1978**, *55*, *897*.
Vitzthum, O. G.; Werkhoff, P. J. *J. Food Sci.* **1974a**, *39*, *1210*.
Vitzthum, O. G.; Werkhoff, P. J. *Z. Lebensm. Unters.-Forsch.* **1974b**, *156*, *300*.

Received for review June 24, 1985. Revised manuscript received October 14, 1985. Accepted April 7, 1986.

Effects of Reduction with Dithiothreitol on Some Molecular Properties of Soy Glycinin

Seung H. Kim and John E. Kinsella*

The reduction of soy glycinin with 5 and 10 mM dithiothreitol (DTT) caused rupture of the disulfide bonds linking the acidic and basic subunits and the intramolecular bonds, respectively. The surface hydrophobicity as determined by *cis*-parinaric acid increased fourfold following treatment with 5 mM DTT while specific viscosity was doubled. Further reduction of disulfide bonds had little effect. The UV absorbance spectra decreased while the ionization of tyrosines above pH 9.5 was enhanced following reduction. The intrinsic fluorescence of glycinin was accentuated following reduction, and a red shift was observed, suggesting exposure of tryptophan to a more polar environment. The data are consistent with the initial dissociation of glycinin with 5 mM DTT while further reduction, with 10 mM DTT, resulted in some reassociation of the component subunits.

The major globulins of soybeans are comprised of glycinin (11S) and conglycinin (7S). Glycinin contains six disulfide-linked acidic (37 kDa) and basic (20 kDa) subunits, and both are transcribed as a single polypeptide (Neilsen, 1985). the component polypeptides show a high degree of homology (Nielsen, 1984). The subunits are packed as two identical apposed hexagons, each composed of three pairs of alternating acidic and basic subunits mostly joined by one disulfide bond with hydrophobic forces holding the adjacent pairs of subunits together. The opposing hexagonal layers associate by electrostatic forces and/or hydrogen bonding (Kinsella et al., 1985; Badley et al., 1975; Kitamura et al., 1976). Soy 11S globulin has about 5% α-helix and about 35% β-structure with the remainder being random coil (Catsimpoolas et al., 1970; Koshiyama and Fukushima, 1973; Jacks et al., 1973). Soy 11S contains 38–42 half-cysteine residues per molecule including two free sulfhydryl groups (Saio et al., 1971; Kella and Kinsella, 1985). In addition to the six intersubunit disulfide bonds linking the acidic and basic subunits, there are from zero to two intramolecular disulfide bonds in acidic subunits and from zero to one disulfide bond in basic subunits on the basis of amino composition (cysteine) of the subunits (Moreira et al., 1979; Utsumi, 1981; Iyengar and Ravenstein, 1981; Catsimpoolas et al., 1971; Okubo et al., 1969).

Molecular flexibility is an important attribute governing some functional properties, e.g. the surface-active properties of proteins (Graham and Phillips, 1976; Kinsella, 1981; Halling, 1981). Soy glycinin is a compactly folded molecule, the rigidity of which is stabilized by the disulfide bonds, and as such it has limited surface-active properties (Kinsella, 1979). However reduction of some of the disulfide bonds by "loosening" the tertiary structure of glycinin making it more flexible may improve its functional properties. In this study we report the effects of reduction

Institute of Food Science, Cornell University, Ithaca, New York 14853.

0021-8561/86/1434-0623$01.50/0

by dithiothreitol (DTT) on some structural properties of glycinin. The effcts on surface properties will be reported subsequently.

MATERIALS AND METHODS

Chemical reagents were purchased from Sigma Co. (St. Louis, MO) and Eastman Kodak (Rochester, NY). Doubly distilled water was used in all solutions.

Preparation of Soy Glycinin. Soy glycinin (11S) was prepared from defatted, low-heat-treated soy flour (Central Soya, Chicago, IL; 4 Bank White Flakes) by the procedure of Thanh and Shibasaki (1976). The defatted flour (50 g) was suspended in 1 L of 0.03 M Tris-HCl buffer (pH 8.0) containing 2 mM 2-mercaptoethanol. The solution was stirred for 1 h at 24 °C and then centrifuged at 15000*g* for 20 min at 20 °C. The supernatant was adjusted to pH 6.4 with 2 N HCl and centrifuged at 15000*g* for 20 min at 2 °C. The 11S precipitate was washed with Tris-HCl buffer (pH 6.4) and then dispersed in Tris-HCl buffer (pH 8.0). Aliquots of 2 N NaOH were added while stirring until the protein was fully dissolved (pH 8.0). The protein solution was dialyzed against water at pH 8.0 at 4 °C and lyophilized. This preparation contained 95% glycinin (11S) as determined by gel electrophoresis (Thanh and Shibasaki, 1976).

Reduction of Soy 11S. Soy 11S globulins were reduced with two levels of dithiothreitol (DTT), and the sulfhydryl (SH) groups were blocked with iodoacetamide to prevent subsequent disulfide interchange (Konigsberg, 1972). The 11S (1 g) was dissolved in 20 mL of water, and 80 mL of buffer containing 10 M urea, 0.5 M Tris-HCl (pH 8.0), and 0.22 M EDTA was added. The solution was saturated with nitrogen, stoppered, and heated at 50 °C for 30 min, after which DTT was added at 5 and 10 mM, and the N_2-saturated solution was heated at 50 °C for 4 h and then cooled. The blocking agent, iodoacetamide, was then added at twice the molarity of the added DTT, and the solution was held for 20 min, then dialyzed against water (pH 8.0) at 4 °C, and lyophilyzed.

Determination of Disulfide Bonds. The number of disulfide bonds was determined with 2-nitro-5-thiosulfobenzoic acid (NTSB) by the procedure of Thannhauser et al. (1984) as applied by Kella et al. (1985). Aliquots of the protein solution (10% w/v) were mixed with the NTSB assay solution (1:1, v/v) held at 25 °C in the spectrophotometer (Cary 219) and the absorbance read at 412 nm after 20 min. For converting the absorbance to disulfide bond concentration, an extinction coefficient of 13600 M^{-1} cm^{-1} at 412 nm was used.

Electrophoresis. A modification of the method of Laemmli (1970) was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Linear gradient separating gels were prepared with equal volumes of 5% and 20% acrylamide solutions. Each solution contained 0.38 M Tris-HCl (pH 8.8), 0.1% SDS, and 0.025% (w/v) TEMED, and 0.046% ammonium persulfate was added prior to mixing. The 5% solution contained 0.067% bis(acrylamide), and the 20% solution contained 0.267% bis(acrylamide) plus 9.7% (v/v) glycerol. The stacking gels contained 0.125 M Tris-HCl, (pH 6.8), 0.1% SDS, 0.025% (w/v) TEMED, 0.046% ammonium persulfate, and 4% acrylamide with 0.146% bis(acrylamide). The gel solutions were poured immediately after addition of ammonium persulfate and allowed to polymerize.

Protein samples were prepared in a dissociating buffer containing 1% SDS, 8 M urea, 0.005% bromophenol blue, and 10 mM Tris-HCl (pH 8.0). Prior to loading, the samples (50 μg) were incubated at 40 °C for 20 min. The gels were run at 12-mA constant current for about 4 h at 23 °C with 0.025 M Tris-HCl (pH 8.3), 0.192 M glycine, and 0.1% SDS as running buffer. Gels were fixed in 10% glacial acetic acid plus 30% methanol for at least 12 h, stained with 0.0125% Coommassie Brilliant Blue R-250 in 10% glacial acetic acid plus 10% methanol, and destained with 10% glacial acetic acid in 20% methanol.

Specific Viscosity. The specific viscosity of protein solution (0.1% in 20 mM phosphate buffer (pH 8.0)) was determined on an Oswald-type viscometer (25). The glass viscometer was cleaned with chromic acid, rinsed twice with buffer (20 mM phosphate buffer (pH 8)), distilled water, and acetone, and air-dried. The viscometer was immersed in a water bath and securely clamped. Exactly 8 mL of protein solution was introduced into the viscometer and allowed to equilibrate at 25.0 °C. The sample was gently sucked through the capillary tube into the reservoir. The time for the sample to flow through the capillary tube under gravity was used to calculate specific viscosity according to the equation

$$\text{sp visc} = t_s/t_b - 1$$

where t_s and t_b are the flow times for the sample and the buffer solution, respectively.

Measurements of Surface Hydrophobicity. The surface hydrophobicity of proteins was determined by a fluorescence probe, *cis*-parinaric acid (*c*-PNA) as described by Sklar et al. (1977) and Kato and Nakai (1980). Fluorescence intensity (FI) measurements were determined with a Perkin-Elmer fluorescence spectrophotometer, Model 650-40. Freshly prepared (10 μL) *cis*-parinaric acid (0.0036 M) was added to 2 mL of protein solution (0–0.1%), which were then excited at 330 nm, and the emission measured at 420 nm (slit width 5 nm). The peak value of FI was plotted against protein concentration, and the hydrophobicity of the protein was calculated from the initial slope of the binding curve of FI vs. percent protein concentration (Kato and Nakai, 1980).

Turbidity. The solubilty of the protein was estimated by turbidometry. The absorbance of 0.05% protein solutions at 600 nm dispersed in 20 mM citrate for pH 3–5 and phosphate buffer pH 6–8 was determined with appropriate buffer as control.

Ultraviolet Absorbance. The UV spectra of proteins (0.1% in 20 mM phosphate buffer (pH 8.0)) was monitored in the range 230–330 nm with 1-cm path-length-matched cuvettes and 1-nm band width on a Cary 219 spectrophotometer.

Spectrophotometric pH Titration. Spectrophotometric titrations of proteins at various pHs were conducted in a Cary 219 spectrophotometer to determine the dissociation of tyrosine. The absorbance at 290 nm of protein (0.1% concentration) dispersed in 50 mM borate buffers ranged from pH 8.0 to pH 12.5. The percent tyrosines titrated at a particular pH x was calculated according to the equation (Yang, 1961)

$$\%\ \text{Tyr titr} = \frac{A_{295}(\text{pH } x) - A_{295}(\text{pH } 8)}{A_{295}(\text{pH } 12.5) - A_{295}(\text{pH } 8)} \times 100$$

Fluorescence Spectra. The intrinsic emission fluorescence spectra of native and reduced glycinin were measured in a Perkin-Elmer 650-40 fluorescence spectrophotometer. The protein solutions (0.02% in 20 mM phosphate buffer (pH 8.0)) were excited at 285 nm and the emission intensities measured from 300 to 400 nm (slit width 5 nm).

RESULTS AND DISCUSSION

The unmodified native glycinin contained 20 S–S bonds while glycinin treated with 5 and 10 mM DTT contained

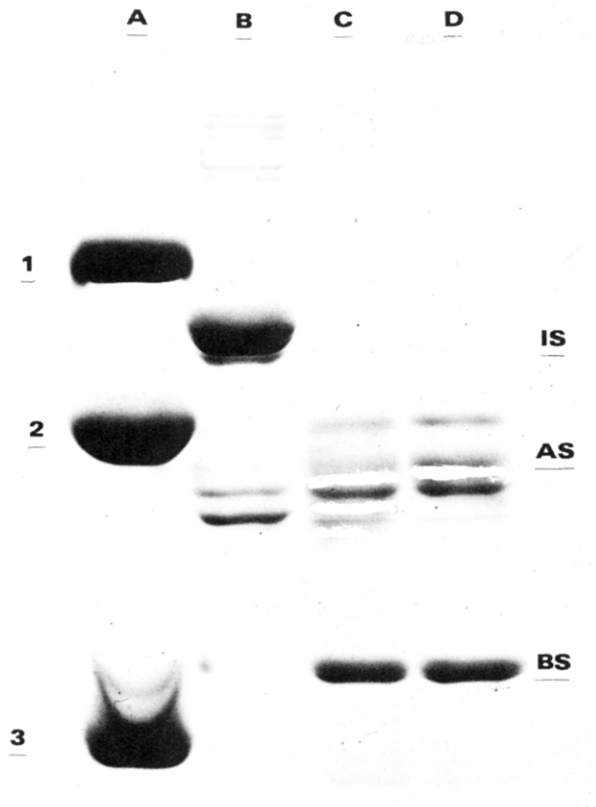

Figure 1. Sodium dodecyl sulfate (SDS) gel electrophoresis patterns of native and reduced glycinin. Track A (standard pure protein markers): 1 bovine serum albumin, 66 kDal; 2, ovalbumin, 45 kDa; 31 lysozyme 14.3 kDa. Track B: 1S denotes intermediate dimeric subunit of soy glycinin. Tracks C and D: soy glycinin reduced to its constituent acidic (AS) and basic subunits (BS) by 5 (C) and 10 (D) mM dithiothreitol, respectively.

14 and O disulfide bonds, respectively. The glycinin treated with 5 mM DTT had six fewer disulfide bonds, suggesting that the six intermolecular disulfide bridges linking the acidic and basic subunits were reduced by 5 mM DTT. This was supported by the SDS gel electrophoresis patterns, which showed that following treatment of 11S with 5 mM DTT the band for the dimeric intermediate subunits disappeared and the bands for acidic and basic subunit appeared (Figure 1). However, recently Kella et al. (1986) reported that sulfite at low concentrations cleaved both inter- and intramolecular disulfide bonds. Treatment with 10 mM DTT resulted in complete reduction of all the disulfide bonds including the intramolecular disulfide bonds.

The surface hydrophobicity of native and reduced glycinin as estimated with *cis*-parinaric acid is shown Figure 2. The glycinin reduced with 5 mM DTT had a substantially higher surface hydrophobicity than the native protein whereas the fully reduced glycinin showed a slightly lower surface hydrophobicity than that treated with 5 mM DTT. Thus, upon reduction of the intersubunit disulfide bonds with 5 mM DTT some hydrophobic sites, previously inaccessible to *cis* parinaric acid in the native oligomeric structure, became exposed. However, upon further reduction of intrasubunit disulfide bridges, conformational changes resulted in some of the hydrophobic sites becoming buried or occluded, resulting in a decreased binding of *cis*-parinaric acid. This suggests that either fully reduced glycinin adopted a more compact structure than the partially reduced globulin or increased protein–protein interaction via hydrophobic association occurred following extensive reduction.

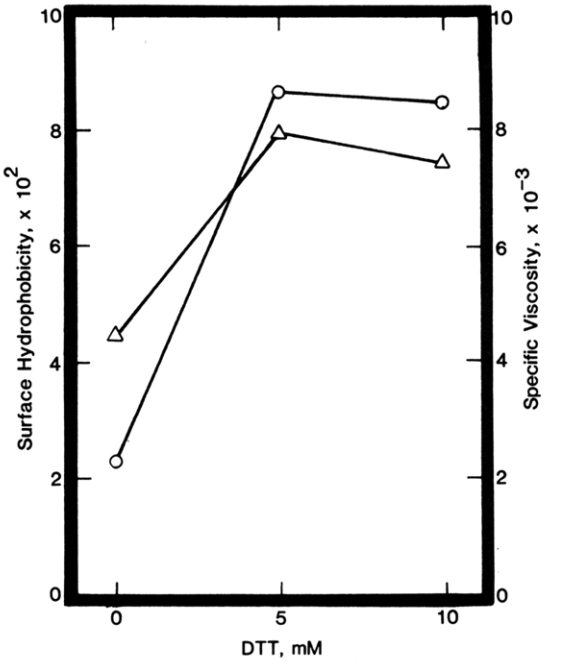

Figure 2. Surface hydrophobicity (O) and specific viscosity (Δ) of native and reduced glycinin. For specific viscosity, the protein concentration was 0.1% (w/v) in 20 mM phosphate buffer (pH 8); for surface hydrophobicity, the protein concentration was 0–0.1% (w/v) in 20 mM phosphate buffer (pH 8). Reduction of disulfide bonds in the native 11S was performed with 5 and 10 mM dithiothreitol.

To further assess the effects of reduction on protein conformation the specific viscosities of native and reduced glycinin were determined (Figure 2). Reduction with 5 mM DTT substantially increased viscosity whereas treatment with 10 mM DTT resulted in a slightly lower viscosity. The reduction of intersubunit disulfide bridges with 5 mM DTT resulted in an increase in the number of protein particles and may result in a change in the shape of the free subunits, thereby increasing viscosity. However, further reduction of the intramolecular disulfide bridges caused the viscosity to decrease slightly, indicating that the fully reduced glycinin had a more compactly folded structure and that some aggregation, possibly of the dissociated basic subunits, occurred (Damodaran and Kinsella, 1982; German et al., 1982; Utsumi and Kinsella, 1985).

The UV spectra of native and reduced glycinin (Figure 3) revealed a slight shift of λ_{max} to shorter wavelength (blue shift) (277–276 nm) with partial reduction with no further change following full reduction with DTT. The absorbance decreased most dramatically following partial reduction. The λ_{max} around 274 nm is due to uncharged tyrosine at pH 8 (Timasheff, 1970). According to empirical rules for the interpretation of the absorption spectra of proteins (Freifelder, 1982), the blue shift and the decrease of absorbance indicate that the chromophore (i.e., tyrosine) and tryptophan were shifted to a more polar environment. Interpretation of UV spectra using λ_{max} as a parameter is difficult because of the small change in the λ_{max}. However, the results suggest that the chromophores become exposed to more polar environment due to dissociation of the oligomeric structure of the native glycinin following reduction of the intermolecular disulfide bonds. The change in absorbance is consistent with the findings from viscosity and surface hydrophobicity, i.e. with partial reduction of disulfide bonds: glycinin dissociated to expose tyrosine and tryptophan residues to the polar environment. However, with full reduction of intramolecular disulfide bonds

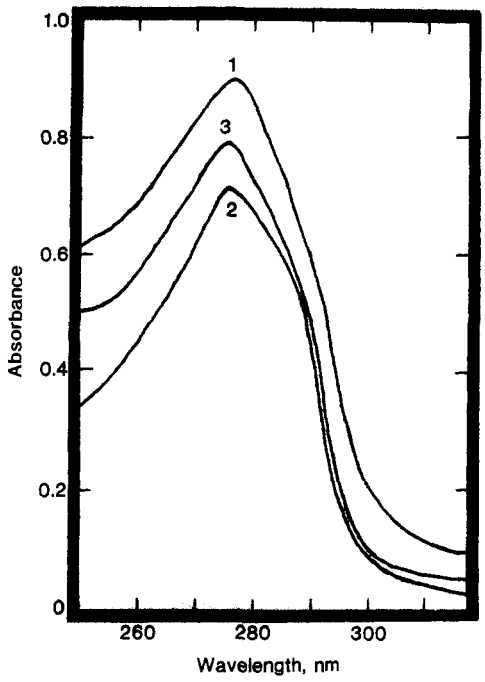

Figure 3. Ultraviolet spectra of native and reduced glycinin: (1) native; (2) 5 mM; (3) 10 mM DTT-reduced glycinin. Protein concentration was 0.1% (w/v) in 20 mM phosphate buffer (pH 8). Reduction of disulfide bonds was performed with dithiothreitol.

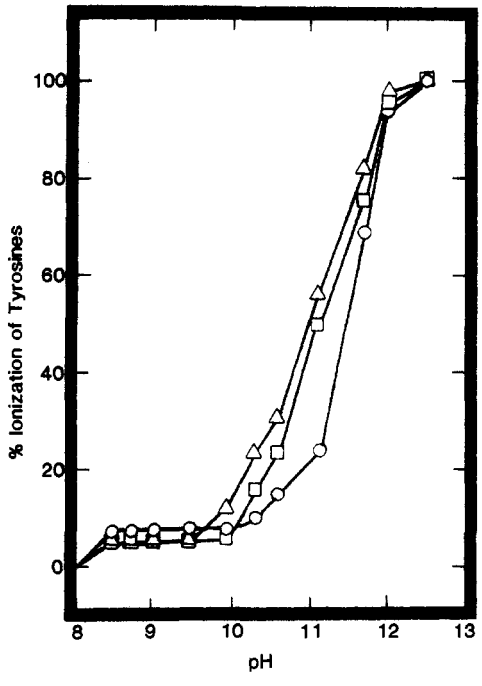

Figure 4. Spectrophotometric pH titration at 295 nm of native and reduced glycinin: native (○); 5 mM DTT-reduced glycinin (△); 10 mM DTT-reduced glycinin (□). Protein concentration was 0.1% (w/v) in 20 mM borate buffer (pH 8–12.5).

the dissociated subunits adopted a more compact structure and associated to partly bury these apolar aromatic residues but to a lesser extent than in native glycinin.

With an increase in pH from 8 to 12, the λ_{max} gradually changed from 274 to 295 nm with an increase in absorbance reflecting the ionization of tyrosine residues (Donovan, 1973). The percent ionization of tyrosines as a function of pH of the unmodified and DTT-treated glycinin is shown in Figure 4. In all the cases the increase

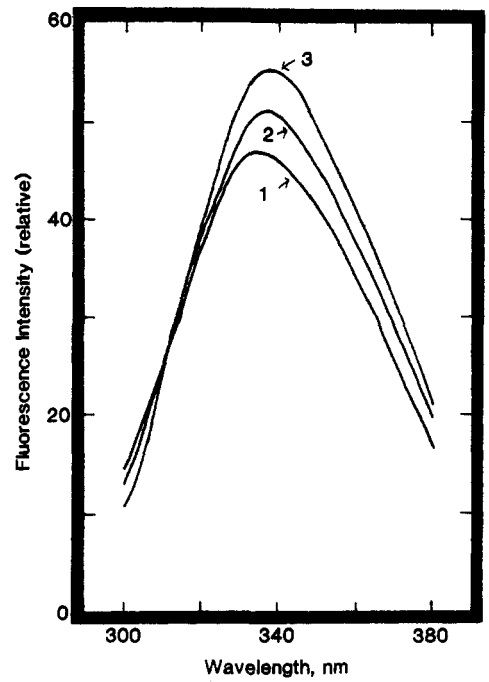

Figure 5. Intrinsic fluorescence spectra of native (1) and reduced glycinin. Protein concentration was 0.02% (w/v) in 20 mM phosphate buffer (pH 8). Reduction of disulfide bonds in glycinin was performed with 5 mM (2) and 10 mM (3) dithiothreitol, respectively.

in ionization of tyrosine residues was small in the pH 8–10 range but was more marked between pH 10 and 12. This pattern indicated that most of the tyrosine residues were folded in the nonpolar protein interior and became available for titration in the higher pH range. The titration curves indicated that tyrosine in the native glycinin had an apparent ionization pK of 11.45, which is abnormal, and this may arise from the interaction of tyrosine residues with neighboring amino acid residues in the protein interior as well as in the subunit interface. Tyrosine in glycinin reduced with 5 mM DTT gave a pK of 10.9. This lower pK value (as compared to that of the native glycinin) may result from the perturbation of the molecular interactions between tyrosine and contiguous amino acid residues in the intersubunit interface resulting from cleavage of the intersubunit S–S bonds. However, at 10 mM DTT the value increased to a pK_a 11.15. This may arise from the increased interactions of tyrosines with other amino acid residues due to refolding and/or aggregation of the fully S–S-bond-cleaved glycinin components.

The intrinsic fluorescence spectra of native and reduced soy glycinin (Figure 5) revealed that λ_{max} shifted slightly from 335 to 337nm (red shift) with reduction of the six intersubunit disulfide bridges but was only slightly changed with further reduction of intrasubunit disulfide bonds. The relative fluorescence intensity increased with reduction of disulfide bonds. The fluorescence spectra reflect mostly the contribution of the tryptophan residues, which have a high quantum yield compared to phenylalanine and tyrosine (Freifelder, 1982). The shift in emission λ_{max} to longer wavelength reflects exposure of some of the 16 tryptophan residues in glycinin to a more polar environment upon reduction of the disulfide bonds. However, the increased emission intensity indicates that some of the chromophores were being relocated into a less polar environment. This may reflect the disposition of the tryptophan residues in the glycinin polypeptides and the fact that while some segments containing tryptophan were being exposed to the polar environment, others may have

Table I. Effects of Reduction of Disulfide Bonds on the Solubility of Soy Glycinin

pH	native glycinin	abs at 600 nm of glycinin reduced with dithiothreitol	
		5 mM	10 mM
6	0.705	0.004	0.470
7	0.010	0.002	0.090
8	0.008	0.002	0.010

[a] Protein concentration was 0.05% (w/v) in 20 mM phosphate buffer (20 °C).

been associating in an apolar region due to folding or protein–protein association.

Because solubility is an important parameter in determining the functional applications of soy proteins (Kinsella, 1979; Damodaran and Kinsella, 1982), the solubility of glycinin and reduced glycinin was determined at pH 6, 7, and 8 (Table I). Reduction of the intermolecular disulfide bonds improved solubility, but further reduction of intramolecular disulfide bonds resulted in some loss in solubility particularly around pH 6.0.

These analyses revealed the effects of reduction of both inter- and intramolecular disulfide bonds on the structure and conformation of glycinin. At the lower level of DTT, i.e. 5 mM, the disulfide bonds linking the acidic and basic subunits appear to be preferentially reduced though some intramolecular bonds are probably also cleaved. Reduction disrupts the oligomeric structure of glycinin and also the tertiary structure of the acidic and basic subunits. These changes should enhance the flexibility of these molecules and thereby improve certain functional properties. The effects on surface activity and foaming properties will be reported in a subsequent paper.

ACKNOWLEDGMENT

Supported in part by NSF Grant No. CBT 8506243.

Registry No. DTT, 3483-12-3.

LITERATURE CITED

Badley, R. A.; Atkinson, O.; Oldani, D.; Green, J. P.; Stubbs, J. M. *Biochim. Biophys. Acta* **1975**, *412*, 214.
Catsimpoolas, N.; Bert, T.; Meyer, E. W. *Int. J. Peptide Protein Res.* **1970**, *2*, 277.
Catsimpoolas, N.; Kenny, J. A.; Meyer, E. W.; Szuhaj, B. F. *J. Sci Food agric.* **1971**, *22*, 150.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1982**, *30*, 812.
Donovan, J. W. In *Methods Enzymol.* **1973**, *27*, 525.
Freifelder, D. *Physical Biochemistry*; W. H. Freeman: San Francisco, 1982.
German, B.; Damodran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1982**, *30*, 806.
Graham, D. E.; Phillips, M. C. In *Foams*; Akers, R. J., Ed.; Academic: New York, 1976; p 233.
Halling, P. J. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, 155.
Iyengar, R. B.; Ravenstein, P. *Cereal Chem.* **1981**, *58*(4), 325.
Jacks, T. J.; Barker, R. H.; Weigang, O. E., Jr. *Int. J. Peptide Protein Res.* **1973**, *5*, 289.
Kato, A.; Nakai, S. *Biochim. Biophys. Acta* **1980**, *624*, 13.
Kella, N. K. D.; Barbeau, W. E.; Kinsella, J. E. *Int. J. Peptide Protein Res.* **1986**, in press.
Kella, N. K. D.; Kinsella, J. E. *J. Biochem. Biophys. Methods* **1985**, *11*, 251.
Kinsella, J. E. In *Food Proteins*; Fox, P. F., Condon, J. J., Eds.; Applied Science: New York, 1981; p 51.
Kinsella, J. E.; German, B.; Damodran, S. In *New Protein Foods*; Altschul, A., Wilcke, H., Eds.; Academic: New York, 1985; p 108.
Kinsella, J. E. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242.
Kitamura, K.; Takagi, T.; Shibasaki, K. *Agric. Biol. Chem.* **1976**, *40*, 1837.
Konigsberg, W. In *Methods Enzymol.* Hirs, C. H. W., Timasheff, S. N., Eds.; Academic: New York, 1972; Vol. 25, p 185.
Koshiyama, I.; Fukushima, D. *Cereal Chem.* **1973**, *50*, 114.
Laemmli, U. K. *Nature* **170**, *227*, 68.
Moreira, M.; Hermodson, M.; Larkings, B.; Nielsen, N. *J. Biol. Chem.* **1979**, *254*, 9921.
Neilsen, N. In *World Soybean Research Conference III Proceedings*; Shibles, R., Ed.; Westview: Boulder, CO, 1985; p 281.
Nielsen, N. C. *Phil. Trans. R. Soc. London* **1984**, *304*, 287.
Okubo, K.; Asano, M.; Kimura, Y.; Shibasaki, K. *Agric. Biol. Chem.* **1969**, *33*, 463.
Saio, K.; Kajikawa, M.; Watanabe, T. *Agric. Biol. Chem.* **1971**, *35*, 890.
Sklar, L. A.; Huson, B. S.; Simoni, R. D. *Biochemistry* **1977**, *16*, 5100.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1976**, *24*, 1117.
Thannhauser, T. W.; Konishi, Y.; Scheraga, H. A. *Anal. Biochem.* **1984**, *138*, 181.
Timasheff, S. N. In *The Enzymes*, 3rd ed.; Boyer, P., Ed. Academic: New York, 1970; Vol. 2, p 371.
Utsumi, S. Ph.D. Thesis, Kyoto University, 1981.
Utsumi, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1985**, *33*, 297.
Yang, J. T. *Adv. Protein Chem.* **1961**, *16*, 323.

Received for review December 19, 1985. Accepted March 26, 1986.