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A Circular Dichroism and Fluorescence Spectrometric Assessment of Effects of Selected Chemical Denaturants on Soybean (*Glycine max* L.) Storage Proteins Glycinin (11S) and β-Conglycinin (7S)

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Soybean glycinin (11S) and β-conglycinin (7S) were subjected to select chemical treatments at various concentrations and resulting changes in protein structures were investigated by circular dichroism (CD) and fluorescence spectrometry. Fluorescence quenching results indicated that urea ≥3 M caused significant unfolding of 11S, but not that of 7S. GuHCl was more effective than urea in denaturation of 11S. A two-step transition in 11S structure was observed with a possible existence of a folding intermediate at 2.5 M GuHCl. Sodium dodecyl sulfate (SDS) measurably altered secondary and tertiary structures of 11S and 7S below SDS critical micellar concentration (CMC), possibly due to formation of mixed peptide-SDS micelles. SDS treatment increased α-helical and unordered structures of both proteins at the expense of β-sheet structure. NaCl and $CaCl_2$ caused a significant decrease in fluorescence intensity without shifting emission λ_{max}. Exposure of 7S and 11S to NaSCN respectively at ≥0.3 and ≥0.6 M NaSCN caused a significant increase in fluorescence intensity measured at the corresponding λ_{max} of the protein. β-Mercaptoethanol (β-ME), *N*-ethylmaleimide (NEM), and phytic acid caused variable red shifts, 2.5–4 nm, in the emission λ_{max}.

KEYWORDS: Glycinin (11S); β-conglycinin (7S); soybean; *Glycine max*; CD; fluorescence; quenching; acrylamide; iodide; conformation

INTRODUCTION

Among oilseeds and legumes, soybeans are the largest source of edible proteins (*1*) and are important in human (*2, 3*) and animal nutrition (*4, 5*). In soybeans, two storage globulins, glycinin and β-conglycinin (hereafter referred to as 11S and 7S, respectively), constitute the bulk (∼70%) of the total seed proteins (*6*). The relative proportion of 11S to 7S ranges from 1:3 to 3:1 (*6, 7*), depending on the cultivar and growing conditions (*7, 8*). A cultivar lacking 7S has also been reported recently (*9*). Because of their abundance, 11S and 7S globulins significantly influence functional properties of soybean seeds and protein meals—high protein flours, protein concentrates, isolates, and others. Consequently, soybean 11S and 7S globulins have been extensively investigated to understand relationships between their molecular and functional properties (*10–15*). The native 11S (*16–18*) and 7S (*19, 20*) often consist of several isoforms that may partly account for the observed variations in their functional properties (*7, 21*). A recent investigation using a proteomic approach (*22*) further suggests that differences in subunit composition of these two proteins continue to be of scientific interest. Although molecular heterogeneity in plant proteins increases the difficulty in establishing clear structure–function relationships, it is important to isolate and investigate individual proteins from their natural sources ("native" proteins) as (a) it is the native proteins, including their isoforms, that are encountered in soybean-based foods and (b) specific individual proteins significantly influence specific functional properties, e.g., 7S forms firmer heat-induced gels than 11S (*8*).

During heating, changes in tertiary structure precede changes in secondary structure prior to aggregation and gel formation in 11S (*23, 24*). Genetic variation in soybean 11S subunits (named G1 to G5) has been reported to result in acidic subunits of 11S with differing thermal stabilities at pH 7.6 in the order G2/G3/G1 < A4 < G5 < G4 (lacking acidic subunit A4) (*18*).

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Influence of pH (*25–28*), ionic strength (*25*), heat denaturation (*26*), enzymatic hydrolysis (*32*), and change in pH and ionic strength together with thermal treatments (*26*) on functional properties such as gelation (*26*) have been investigated. However, to date, only a few investigations (*24*, *29*) have attempted to characterize changes in secondary and tertiary structures of purified soybean 11S and 7S subjected to chemical treatments. Certain chemicals such as plasticizers have been investigated for improving functionality of soybean protein-based edible films and/or adhesive coats (*30*, *31*). Protein folding/unfolding studies using chemical agents afford precise control over denaturation conditions, thereby permitting refined understanding of the processes/forces involved. The present study targeted purified soybean 11S and 7S for such investigations.

MATERIALS AND METHODS

Century soybeans were from Indiana Crop Improvement Association, Lafayette, IN.

All chemicals were of reagent or better grade. Acrylamide, GuHCl, and urea were purchased from USB Chemicals, Cleveland, OH. SDS, *N*-ethylmaleimide, sodium phytate, NaSCN, β-ME, $CaCl_2$, NaCl, KI, $Na_2S_2O_3$, Na_2HPO_4, and NaH_2PO_4 were purchased from Fisher Scientific Co, Atlanta, GA.

Protein Preparation. Defatted soybean flour was prepared as described earlier (*32*), and 11S and 7S were prepared according to the method of Nagano et al. (*33*). Briefly, petroleum ether defatted Williams 82 soybean flour was extracted with 15 volumes of distilled water adjusted to pH 7.5 with 2 M NaOH at room temperature (~25 °C, RT) for 2 h with constant magnetic stirring. The slurry was filtered through glass wool, the filtrate was centrifuged (12000*g*, 20 min, 4 °C), and to the supernatant was added dry sodium bisulfite (0.98 g/L). The pH of the mixture was adjusted to 6.4 with 2.0 M HCl, kept in a cold room (4 °C) overnight (14 h), and the precipitate (11S) was removed by centrifugation (12000*g*, 20 min, 4 °C). The precipitate was washed five times with distilled water, suspended in a minimum amount of distilled water, dialyzed (72 h, 5 lit each, six changes), and freeze-dried. All subsequent steps were performed at 4 °C. The final NaCl concentration of the supernatant was adjusted to 0.25 M (by adding solid NaCl), the pH was adjusted to 5.0 with 2 M HCl, the mixture was allowed to stand for 1 h and centrifuged (12000*g*, 20 min, 4 °C), and the resulting supernatant was diluted with 2 volumes of cold (4 °C) water. The solution was adjusted to pH 4.8 with 2 M HCl, allowed to stand for 1 h, centrifuged (12000*g*, 20 min, 4 °C), collected the precipitate (7S), washed 5 times with distilled water, suspended in a minimum amount of distilled water, pH adjusted to 7.5 with 2 M NaOH, dialyzed extensively against distilled water (72 h, 5 lit each, 6 changes), and lyophilized. The lyophilized powders were stored in plastic screw-capped bottles at −20 °C until further use.

Electrophoresis. Using the Fling and Gregerson (*34*) procedure, SDS-PAGE was done as described earlier (*32*).

Determination of Soluble Protein. For spectroscopic analyses, fresh protein solutions were made in 2×10^{-2} M sodium phosphate buffer, pH 7.5 (hereafter referred to as "phosphate buffer"), on the day of the experiment. Protein concentrations were determined from absorbance at 280 nm of proteins treated with 8 M GuHCl. Molar extinction coefficients (ε_{280nm}) were calculated from amino acid sequences (11S: NCBI Accession No. P04405, $\varepsilon_{280nm} = 38040\ M^{-1}\ cm^{-1}$; 7S: NCBI Accession No. AAT40424, $\varepsilon_{280nm} = 16640\ M^{-1}\ cm^{-1}$). Fluorescence and CD spectroscopic studies conducted on protein solutions in phosphate buffer (in absence of denaturants or neutral salts) were used as the controls to examine the effects of chemical treatments on protein structure.

Fluorescence Emission Spectra. Fluorescence emission spectra and fluorescence quenching data (Perkin-Elmer fluorometer model LS 50B, Perkin-Elmer Corp., Wellesley, MA) were obtained with 50×10^{-6} g/mL protein solutions at 25 ± 0.1 °C (circulating water bath, Haake Corp, Berlin, Germany) as described earlier (*35*). Briefly, protein stock (~2×10^{-3} g/mL), stock solution of chemical agent (typically, 6 M urea or GuHCl, 24×10^{-3} M SDS, 2 M NaCl or NEM, 0.5×10^{-3}M $CaCl_2$ or phytic acid) and phosphate buffer were mixed in appropriate amounts to obtain the desired concentration of protein and the chemical agent. For quenching studies, suitable aliquots of 2.5 M stock of acrylamide or iodide were added to 2 mL of the protein solution described above. Concentrations of the chemical agents used are the final concentrations of the additive in 3 mL solutions in the quartz cuvette (Perkin-Elmer Corp., Wellesley, MA) and are indicated directly in the figures. Six spectra (three each from two separate samples prepared from a single protein preparation) were averaged and used for analysis.

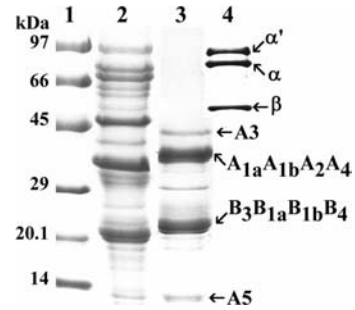

Figure 1. SDS-PAGE for 11S and 7S. Lanes 1, 2, 3, and 4 represent Pharmacia low-MW standards (phosphorylase b 97000, bovine serum albumin 66000, chicken egg white ovalbumin 45000, carbonic anhydrase 30000, trypsin inhibitor 20100, α-lactalbumin 14400), soybean total protein extract, 11S, and 7S, respectively. Protein load in lanes 2, 3, and 4 was respectively 50×10^{-6}, 20×10^{-6}, and 20×10^{-6} g.

CD Spectroscopy. Optically clear solutions in phosphate buffer were used to record CD spectra (190–260 nm) in 1 mm quartz cuvette (Fisher Scientific, Atlanta, GA) with AVIV CD spectrometer (Proterion Corp., Santa Barbara, CA). Six spectra (three each from two separate samples) were averaged and used for analysis.

For SDS-treated proteins, SDS concentrations used were 2×10^{-6}, 4×10^{-6}, 10×10^{-6}, 100×10^{-6}, 500×10^{-6}, 1×10^{-3}, 2×10^{-3}, 4×10^{-3}, 6×10^{-3}, and 10×10^{-3} M, resulting in protein:SDS molar ratios of 1:0.5, 1:1, 1:2.5, 1:25, 1:125, 1:250, 1:500, 1:1000, 1:1500, and 1:2500. Molar ellipticity per amino acid residue was calculated from raw data after correction for buffer and SDS contributions. Secondary structure was interpreted by visual assessment of the spectra and using the computer program CDPro (http://lamar.colostate.edu/~sreeram/CDPro/main.html).

Statistical Analysis. Data were analyzed for significance (One-way ANOVA) using SPSS 11.0 (SPSS Inc., Chicago, IL). Fisher's protected LSD ($p = 0.05$) values were calculated for appropriate data.

RESULTS AND DISCUSSION

Protein Purification. SDS-PAGE analysis of 11S and 7S (**Figure 1**) indicated the proteins to be at least 90% pure.

Tertiary Protein Structure. Three observations suggest that tryptophan fluorophores in 7S control sample were in a more hydrophilic, surface-accessible environment than those in 11S: (1) 7S had a $\lambda_{max} = 344$ nm while 11S λ_{max} was at 340 nm (**Figure 2**); (2) a higher K_{SV} for 7S than for 11S with acrylamide quenching (**Table 1**), indicative of overall higher tryptophan accessibility; (3) using iodide quencher, tryptophan accessibility was 65.04% for 7S and ~25.72% for 11S (**Table 1**). Hydrodynamic studies (*42*) in conjunction with the published amino acid sequences of α and α′ 7S subunits and spectroscopic studies (*38*) indicate soybean 7S tryptophan residues to be on the surface of 7S molecule and therefore easily accessible to quenchers.

In iodide quenching, Stern–Volmer (SV) plots for both proteins showed a slight downward curvature (**Figures 4E** and **5E**, lines labeled "control"), indicating that at least a fraction of tryptophanyl fluorophores were completely accessible (*36*).

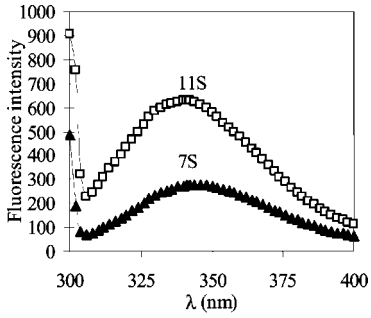

Figure 2. Fluorescence emission spectra for soybean 7S and 11S controls. Protein concentration for both = 50×10^{-6} g/mL.

Secondary Protein Structure. CD spectra of both 11S and 7S controls (**Figure 8**) had a strong maximum at 195 nm, a minimum at 208 nm, and a weak shoulder at ~222 nm. Estimation of secondary structure from the recorded CD spectra indicated predominance of β-strands (38.6% in 11S and 36.6% in 7S), with ~10% α-helices. In general, our findings are in agreement with the literature (*37–40*). From the CD spectra, we conclude that native soybean proteins belong to the β-I class of proteins; i.e., the β-sheet content of soybean proteins is significantly higher than random secondary structure content (*41*).

Effect of Chemical Treatments on Protein Structure. *Urea.* A red shift in λ_{max} of both 7S and 11S (**Figure 3C**) at ≥3 M urea was recorded. Quenching of intrinsic fluorescence of urea-treated proteins with either acrylamide or iodide resulted in SV plots with downward curvature in ≤3 M urea and a weaker downward curvature in 4 M urea (**Figures 4A**,**D** and **5A**,**D**). A statistically significant gain in tryptophan accessibility for iodide quencher was noted for 11S (2.58× over the control) but not 7S (1.1× over the control) when exposed to 4 M urea (**Table 1**). Tryptophan accessibility to acrylamide in both proteins treated with 4 M urea was close to 100% (Fa, **Table 1**).

Based on bimodal tryptophan accessibility upon urea treatment, tryptophan fluorophores in 11S appear to be located on the surface as well as in the interior of the molecule. At >3 M urea concentration, 11S unfolds in a two-step fashion as indicated by significantly higher K_{SV} for both acrylamide and iodide quenching. Analytical ultracentrifugation investigations have shown 11S dissociation to a 2S component at ≥3 M urea (*39*). In the case of 7S, urea denaturation did not increase tryptophan accessibility to iodide quenching (**Table 1**), indicative of minimal change in 7S conformation upon urea exposure.

GuHCl. A two-step transition in protein structure was observed when 11S was exposed to increasing concentrations of GuHCl (**Figure 4B**,**D** insets). SV plots of acrylamide quenching of both proteins changed from a straight line (controls and proteins treated with ≤2 M GuHCl) to a slightly upward curvature when treated with ≥3 M GuHCl (**Figures 4B** and **5B**). According to Eftink and Ghiron (*36*), the upward curvature suggests either all fluorophores being equally accessible or a single fluorophore being predominant. To choose between these two possibilities, increments in K_{SV} upon GuHCl treatments were compared. If only a single fluorophore existed, increments in K_{SV} would not be significant. However, a 3.14-fold increase in K_{SV} suggested multiple tryptophan fluorophores in 11S with different acrylamide accessibilities. Further, when GuHCl concentration was raised from 2 to 3 M, these fluorophores became equally accessible to acrylamide. The red shift to 351 nm at ≥2.5 M GuHCl (**Figure 3D**, hollow square markers) is characteristic of fully exposed tryptophan residues (*43*). A gradual shift in emission λ_{max}, indicative of cooperative protein unfolding, was not observed in case of 11S. Instead, at 2.5 M GuHCl, an abrupt but significant increase in fluorescence intensity at λ_{max} was observed, suggestive of existence of folding intermediate(s) of 11S (**Figure 3B**). Clearly, more studies are needed to determine the biochemical nature of these intermediates. Taken together, these results demonstrate that significant denaturation of 11S occurred at ≥2 M GuHCl. At ≥2 M GuHCl ~90% glycinin is reported to dissociate from its native 11S form into a 2S form (*44*). Such dissociation may expose tryptophan residues that are buried due to intersubunit contacts for 11S formation. Of the four tryptophan residues in glycinin monomer (amino acid residue numbers 52, 146, 147, and 343), number 343 is a part of binding region IV (numbers 320–380) that is involved in hexamer formation of glycinin (*40*). At ≥2 M GuHCl, W343 appears to become accessible for fluorescence quenching possibly due to disruption of the hexameric state. As 55% amino acid residues in binding region IV are hydrophobic (*40*), 11S dissociation at ≥2 M GuHCl may be the result of direct interaction of GuHCl with hydrophobic side chains, rather than rearrangement of hydration structure of 11S. At ≥2 M, GuHCl seems to break hydrophobic interaction forces stabilizing 11S. Neutron diffraction experiments on aqueous GuHCl solutions have demonstrated that, owing to its poor hydration, GuHCl is able to interact preferentially with poorly hydrated hydrophobic side chains on protein's surface, leading to protein denaturation (*45*). GuHCl-induced two-step protein denaturation has also been reported for sunflower 11S (*46*), plant protease procerain (*47*), cAMP receptor protein (*48*), thyroglobulin (*49*), and D-glyceraldehyde-3-phosphate dehydrogenase (*50*).

In case of 7S, gain in K_{SV} was 1.24-fold, suggesting a single fluorophore being predominant and surface-accessible. X-ray crystallography studies indicate that tryptophan residues in 7S are located at the N-terminal extensions of α and α′ subunits (2 in α subunit and 3 in α′ subunit) that are rich in hydrophilic, negatively charged amino acids (41% of α and 10% of α′ subunit N-terminal extension) (*51*).

Iodide quenching of proteins treated with GuHCl also suggested complete exposure of buried tryptophan residues in both proteins as SV plots showed a downward curvature in ≥2 M GuHCl (**Figures 4E** and **5E**). K_{SV} values (**Table 1**) indicate changes in conformation were significantly more in 11S than in 7S.

SDS. SDS exposure of 11S and 7S did not change λ_{max} of 7S and caused a small but significant red shift in 11S λ_{max} at SDS > 2×10^{-3} M (**Figure 6A**,**B**). At 2×10^{-3} M SDS concentration, the red shift in λ_{max} of both 7S and 11S did not indicate complete exposure of tryptophan residues to a polar environment. On a per unit weight basis, SDS typically binds with most proteins in a ratio of 1.4–1.5:1 (*55*). At 2×10^{-3} M SDS (and typically, at 4×10^{-3} M protein) there was enough SDS for protein denaturation. This suggests that although both proteins are denatured by 2×10^{-3} M SDS, hydrophobic cores in both proteins remained unchanged relative to the corresponding controls. Increase in SDS concentration from 0.5×10^{-3} to 2×10^{-3} M caused almost twice the rise in K_{SV} (**Table 1**), further confirming presence of two populations of tryptophan fluorophores in 11S. Similarly, lack of major change in K_{SV} in 7S indicated single fluorophore population.

SDS-induced ($(0.5–2) \times 10^{-3}$ M) structural changes (**Figure 8**) in CD spectra included (a) disappearance of maximum at 195 nm, (b) shift in the minimum from 208 to

Table 1. Effect of Interaction with Denaturant on Various Fluorescence Parameters Obtained by Fitting Fluorescence Quenching Data into Stern–Volmer and Modified Stern–Volmer Equations[a]

		11S				7S			
quenching agent	parameter	control	4 M urea	4 M GuHCl	10×10^{-3} M SDS	control	4 M urea	4 M GuHCl	10×10^{-2} M SDS
acrylamide	K_{SV} (M^{-1})	2.31	6.72	8.18	7.13	6.70	4.49	6.75	10.13
				3.42				*4.28*	
	Fa (%)	73.50	100.00	100.00	100.00	91.26	100.0	100.00	100.00
				12.63				*2.94*	
iodide	K_{SV} (M^{-1})	0.78	3.27	4.49		2.13	2.65	4.29	
			0.67				*2.45*		
	Fa (%)	25.72	66.42	85.42		65.04	71.58	87.62	
			10.35				*4.92*		

[a] Fisher's LSD values ($p = 0.05$) are indicated in italics.

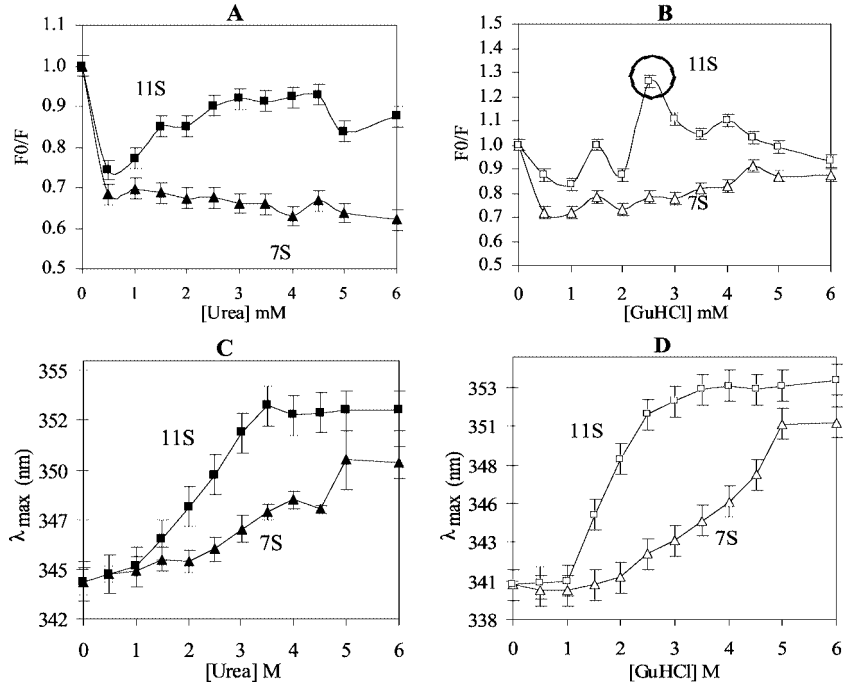

Figure 3. Changes in fractional fluorescence intensity (F_0/F) and λ_{max} as a result of urea and GuHCl. Data reported as mean ± SEM ($n = 6$). The existence of a possible folding intermediate for 11S at 2.5 M GuHCl has been marked with a circle.

205 nm with considerable gain in signal, and (c) appearance of shoulder at 222 nm. The maximum at 195 nm is attributed to β-sheets; the minimum at 205 nm in CD spectra is believed to arise from random structure while the shoulder at 222 nm is attributed to helical structure (*52*). It appears from CD spectra that SDS treatment increases α-helical and disordered structure in both proteins at the expense of β-sheet structure. Similar observation (shift from β-sheets to α-helical/disordered structure as a result of denaturation) has been made in soybean 11S (*53*) and in oat globulin (*54*). CDPro analyses suggested that helical structure was gained at the expense of strands in both proteins (**Figure 8A**,**B** insets). Further, no significant changes in secondary structure occurred below 0.5×10^{-3} M and above 2×10^{-3} M. Results of fluorescence quenching and CD spectroscopy of SDS-treated proteins are in agreement.

SDS–protein binding interactions are influenced by physical state of SDS. Monomeric SDS promotes β-strands while micellar SDS stabilizes helical structures (*57*). The concentration of SDS that caused major changes in CD spectra (2×10^{-3} M) is slightly below the typical CMC (2.6×10^{-3} M) of SDS in 10×10^{-3} M sodium phosphate buffer, pH 7.5 at 25 °C (*56*). This apparent decrease in CMC may be expected as a consequence of formation of mixed peptide–SDS micelles (*57*). As a result, the hydrophobic core of these micelles may preferentially interact with hydrophobic amino acid side chains on the protein, causing changes in the secondary structure. X-ray crystallographic evidence has shown acidic amino acids to be on the surface of both 11S (*40*) and 7S (*51*). Under the conditions used in the present investigation, acidic amino acid side chains were expected to be negatively charged, resulting in electrostatic repulsion between the sulfate groups of SDS and unprotonated carboxyl groups on the proteins, thus preventing SDS–protein binding (*57*).

NaCl and $CaCl_2$. In both 7S and 11S, NaCl treatments up to 1 M did not cause a shift in the λ_{max}, suggesting minimal change in the polarity of tryptophan residues (**Figure 7A**,**C**). Fluorescence intensity at λ_{max} increased significantly at the lowest salt concentration (0.05 M) tested and then reached a plateau as NaCl concentration was increased further (**Figure**

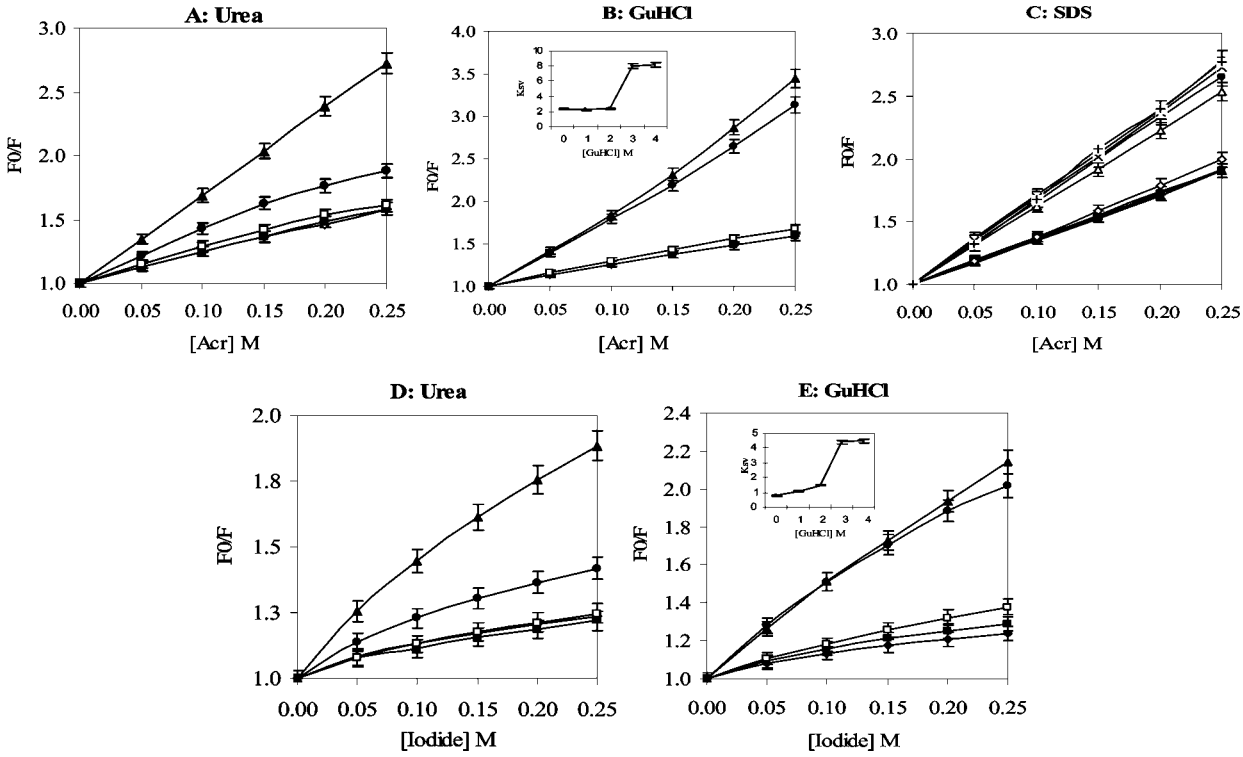

Figure 4. Stern–Volmer plots for 11S exposed to urea, GuHCl, and SDS. (A, B, D, E) control = ♦, 1 M = ■, 2 M = □, 3 M = ● and 4 M = ▲. (C) Control = ■, 1 × 10^{-6} M = □, 2 × 10^{-6} M = ∗, 4 × 10^{-6} M = ▲, 10 × 10^{-6} M = ♦, 100 × 10^{-6} M = ○, 500 × 10^{-6} M = ◊, 2 × 10^{-3} M = △, 4 × 10^{-3} M = □, 6 × 10^{-3} M = ●, 8 × 10^{-3} M = × , 10 × 10^{-3} M = +.

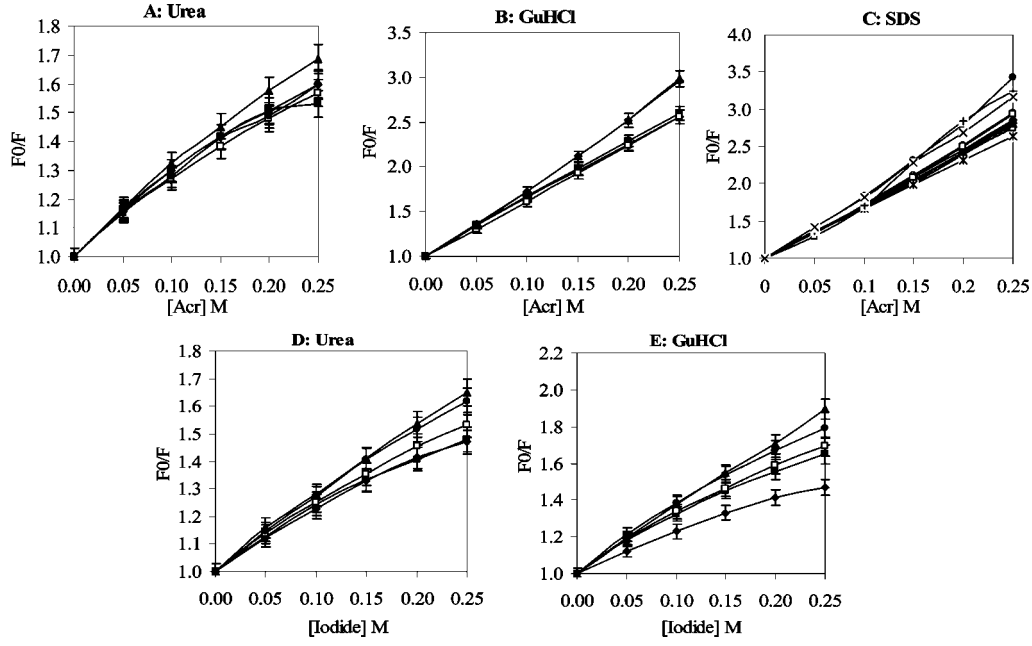

Figure 5. Stern–Volmer plots of fluorescence intensity data obtained from quenching of 7S treated with urea, GuHCl, and SDS. (A, B, D, E) Control = ♦, 1 M = ■, 2 M = □, 3 M = ●, and 4 M = ▲. (C) Control = ■, 1 × 10^{-6} M = □, 2 × 10^{-6} M = *, 4 × 10^{-6} M = ▲, 10 × 10^{-6} M = ♦, 100 × 10^{-6} M = ○, 500 × 10^{-6} M = ◊, 2 × 10^{-3} M = △, 4 × 10^{-3} M = □, 6 × 10^{-3} M = ●, 8 × 10^{-3} M = × , 10 × 10^{-3} M = +.

7B,**D**). Lakemond et al. (*25*) similarly found no significant change in λ_{max} for 11S at different ionic strengths.

Since 11S precipitates on $CaCl_2$ addition, effects of $CaCl_2$ on 11S samples could not be evaluated. In case of 7S, a red shift in λ_{max} was observed at ≥2 M $CaCl_2$ (**Figure 6A**), accompanied with a significant decrease in fluorescent intensity at λ_{max} (**Figure 6C**).

Salts typically disrupt the ionic interactions between protein molecules, break hydrogen bonds, and indirectly promote hydrophobic interactions (*58*), thereby unfolding proteins, often

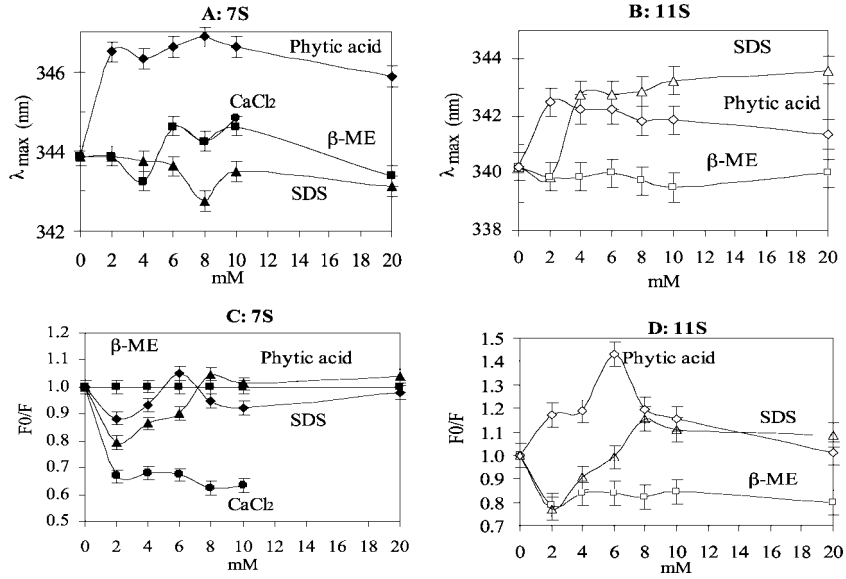

Figure 6. Effects of β-ME, SDS, phytic acid, and $CaCl_2$ on fluorescence spectra of 7S and 11S. Data are reported as mean ± SEM ($n = 6$).

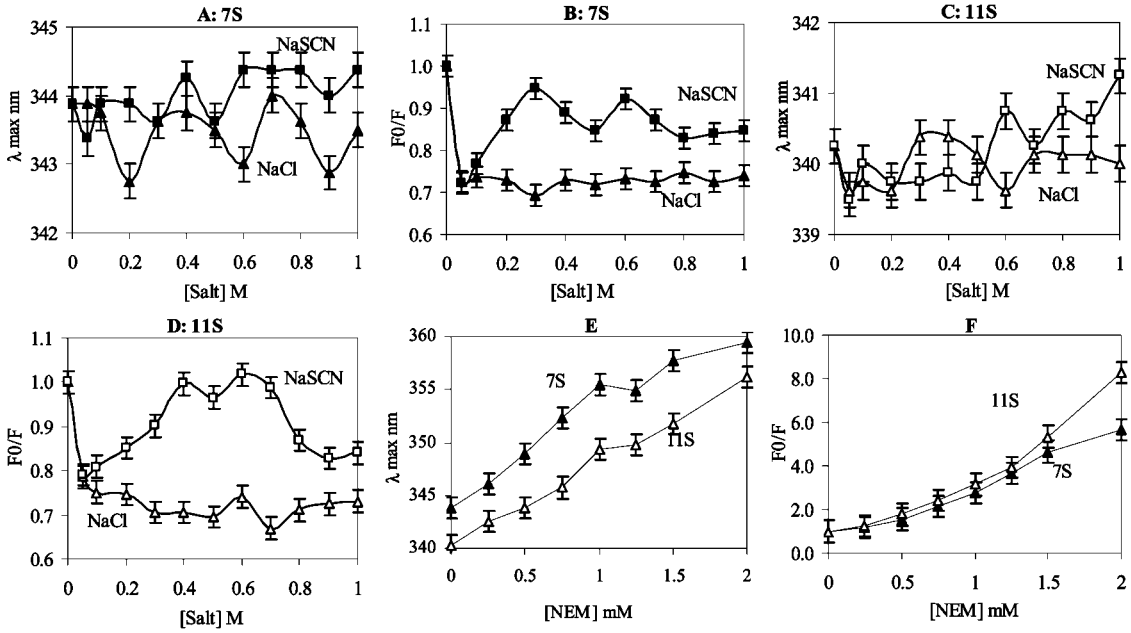

Figure 7. Effects of NaCl, NaSCN, and NEM on 7S and 11S fluorescence λ_{max} and fluorescence intensity. Data are reported as mean ± SEM ($n = 6$).

reflected in an increase in the fluorescence intensity due to exposure of the buried tryptophan residues.

NaSCN. Fluorescence intensity at λ_{max} abruptly increased at 0.5×10^{-3} M NaSCN and then decreased at higher NaSCN concentrations (0.3 M for 7S and 0.6 M for 11S) (**Figure 7B**,**D**). At 1 M NaSCN, gain in fluorescence intensity was significant in both proteins. However, shifts in λ_{max} in case of both 11S and 7S were not significant with increasing NaSCN concentrations (**Figure 7A**,**C**). Both S and N atoms in thiocyanate anion can form hydrogen bonds with lysyl and arginyl side chains (*45*). Crystal structures of both 7S and 11S show lysine and arginine residues forming salt bridges at crucial points at intersubunit contacts (*40*, *51*). NaSCN may break these salt bridges (*59*, *60*) to expose buried tryptophan(s), thus causing an increase in fluorescence intensity. As discussed earlier, tryptophan residue number 343 is located at the contact interfaces in hexameric 11S (*40*). These subunit contacts may be disrupted by NaSCN with a resultant increase in exposure of tryptophan 343, which is reflected in increased fluorescence intensity. Upon NaSCN (1 M) exposure of 7S there was a gain in fluorescence intensity. The gain may be partly attributed to the increased exposure of buried tryptophan residues, suggestive of compact folding structure for the hydrophilic extension region on α and α′ subunits in the native molecule (*51*).

β-ME and NEM. β-ME red-shifted the emission λ_{max} by 3 nm and slightly decreased fluorescence intensity at λ_{max} for 11S but not that of 7S (**Figure 6**), indicating minor alterations in the microenvironments of tryptophan residues. Kim et al. (*53*) have

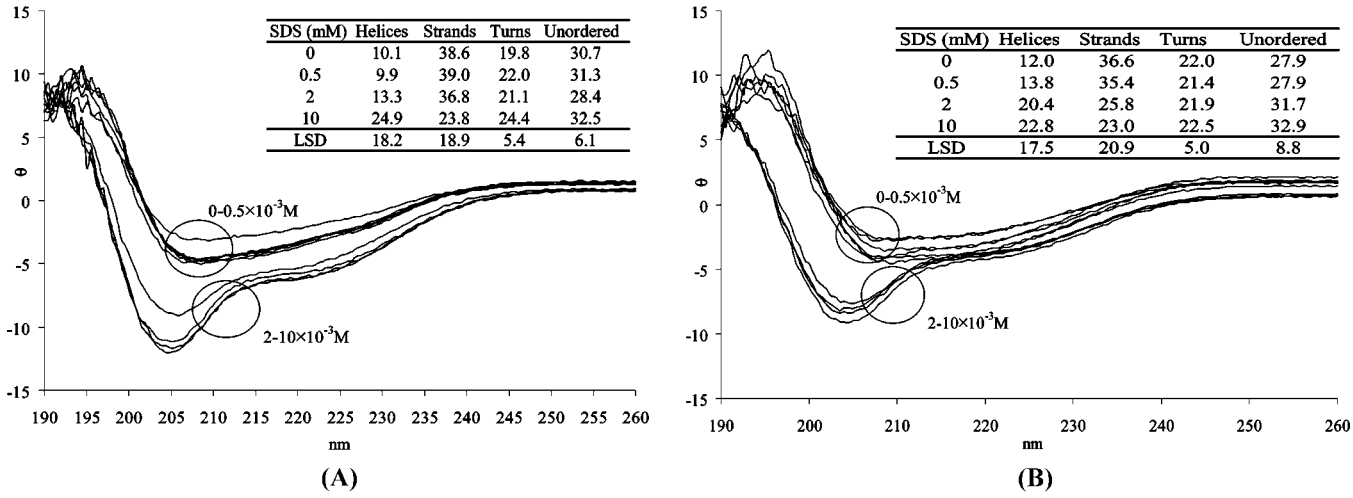

Figure 8. CD spectra: 11S (A) and 7S (B) exposed to SDS. Inset tables: CDPro estimates of secondary structures. θ (*Y*-axis) values expressed as $[\theta]_{MRW} \times 10^{-3}$ deg cm^2 dmol^{-1}. Depending on the SDS concentration, CD spectra were segregated in two clusters (indicated by circles) for a specific protein. Note the significantly higher unordered structure in both proteins as SDS concentration was raised above 5×10^{-3} M.

reported insignificant changes in 11S thermogram when treated with β-ME. Thus, it appears that although β-ME treatment may potentially break disulfide bonds in 11S, overall conformational structure of 11S is not significantly altered because of involvement of noncovalent interactions (hydrophobic interactions and salt bridges) in stabilizing the protein.

Sulfhydryl blocking agent NEM red-shifted λ_{max} of emission for both 7S and 11S (**Figure 7E**) coupled with a significant decrease in the signal intensity at λ_{max} (**Figure 7F**). NEM thus has a shielding effect on tryptophan fluorescence while exposing the tryptophan residues to more polar environment. The shielding effect of NEM on fluorescence intensity may be due to the ability of NEM to absorb the emission energy since NEM does have two ketone groups and a single >C═C< in its structure. Owing to >C═C<, maleimides are known to engage in fluorescence quenching through intermolecular charge-transfer interactions (*61*).

Phytate. Phytate addition at 2×10^{-3} M caused a red shift of 2.5 nm in the λ_{max} of both proteins (**Figure 6A**,**B**). The signal intensity (at λ_{max}) for 11S significantly decreased at $\geq 6 \times 10^{-3}$ M phytate (**Figure 6D**). The variable nature of soybean protein–phytate complexation (*62*) may be responsible for such mixed nature of the spectra.

CONCLUSIONS

Tryptophan fluorophores in 7S appeared to be in more hydrophilic and surface-accessible environment compared to those in 11S. 11S was more easily denatured by GuHCl and urea than 7S. GuHCl denaturation revealed a two-step mechanism for 11S denaturation. At submicellar concentrations ($<2 \times 10^{-3}$ M) SDS increased α-helix at the expense of β-sheet structures in both 7S and 11S. On the basis of fluorescence results, NaCl, $CaCl_2$, NaSCN, β-ME, NEM, and phytic acid caused variable structural changes in both proteins.

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