Effect of Sodium Dodecyl Sulfate on the Conformation of Soybean Glycinin

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The effect of sodium dodecyl sulfate on the conformation of glycinin from soybean (*Glycine max*) has been studied by the techniques of optical rotatory dispersion, circular dichroism, fluorescence spectrophotometry, and viscosity measurements. The addition of SDS decreased the intensity of bands in the near-UV–CD spectrum of the protein, suggesting disruption of the tertiary structure. In the far-UV–CD spectrum the ellipticity values increased. Calculation of the α-helical content of the protein showed that it increased with increasing SDS concentration. This conclusion was supported by difference CD spectra, ORD, and fluorescence spectral data. The reduced viscosity of the protein did not change significantly with increases in SDS concentration, indicating slight changes in hydrodynamic volume of the protein.

Detergents, ionic as well as nonionic, bring about dissociation and denaturation of proteins even at very low concentrations (Tanford, 1968). SDS, an anionic detergent, has been extensively used in the study of protein denaturation (Reynolds et al., 1967; Tanford, 1968; Lapanje, 1978). Detergents act differently on different proteins. In the case of proteins with high α-helical content, SDS may not affect the helical content of the protein (Reynolds et al., 1967). In the case of proteins that have predominantly aperiodic or β structure in their native state, SDS may increase the helical content of the protein (Jirgensons, 1967, 1969). It has also been found to stabilize several proteins against denaturation by other perturbants like urea, pH, etc. (Lovrien, 1963; Markus et al., 1964; Takagi and Kubo, 1979).

Glycinin, also called the 11S protein of soybean, is one of the major storage proteins of soybean (*Glycine max*) seeds. The molecular weight of the protein is 350 000 Da, and it has 12 subunits (Catsimpoolas et al., 1967). Its properties have been studied by various physicochemical techniques (Badley et al., 1975; Fukushima, 1968; Catsimpoolas et al., 1969, 1970; Diep et al., 1982; Koshiyama, 1972; Peng et al., 1984). It consists essentially of β structure and aperiodic structure and has very little α helix (Koshiyama, 1972; Koshiyama and Fukushima, 1973).

There are very few reports on the effect of detergents on storage proteins of oilseeds that consist mainly of aperiodic structure with a little β structure (Prakash and Nandi, 1976; Prakash et al., 1980; Gururaj Rao and Narasinga Rao, 1979, 1983). In the case of glycinin, SDS has been mainly used to dissociate the protein into its subunits (Kitamura et al., 1976; Moreira et al., 1979). Kato et al. (1984) reported that SDS binding by glycinin increased when the protein was heat denatured. This has been attributed to an increase in the surface hydrophobicity of the protein. Anionic detergent provides a means of increasing the negative charge on the molecule. It also brings about complete conversion of the protein to a 3S protein (Wolf and Briggs, 1958). Information on the effect of SDS on the conformation of glycinin is lacking. This paper describes the results of a study of the effect of SDS on glycinin monitored by CD, ORD, fluorescence spectra, and viscosity measurements.

MATERIALS AND METHODS

Bragg variety soybean seeds, cultivated in a farm in Mysore, India, were purchased locally. SDS from Loba

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Chemie was recrystallized twice from ethanol. β-Mercaptoethanol (β-RSH) from Fluka was used without further purification. All the other reagents were of analytical reagent grade.

Glycinin was isolated by the method of Appu Rao and Narasinga Rao (1977). Protein concentration of the solution was determined by measuring the absorbance of the solution at 280 nm and using a value of 7.9 for $E_{1cm}^{1\%}$ at 280 nm. All the measurements were made in 0.05 M sodium phosphate buffer of 7.8 containing 0.1% β-RSH. β-RSH was omitted for measurements with the spectrofluorimeter.

The ORD and CD measurements were made with a Jasco-J 20C automatic recording spectropolarimeter. The instrument was calibrated with sucrose solution for ORD and d-10-camphorsulfonic acid for CD. Slits were programmed to yield a 1-nm band width at each wavelength. The chart speed, wavelength expansion, and the time constant of the instrument were all set so as to obtain the best signal-to-noise ratio and reproducibility of the spectrum. Scans were repeated to check for reproducibility and to avoid artifacts. Mean residue rotations $(m)_{MRW}$ and mean residue ellipticities $(\theta)_{MRW}$ were calculated according to standard procedures (Adler et al., 1973) and expressed in deg·cm²/dmol. The $(\theta)_{MRW}/(m)_{MRW}$ values were an average of at least three sets of measurements with an error of ±5%. A value of 115 for the mean residue weight (MRW) was used on the basis of the amino acid composition (Sureshchandra, 1984). Quartz cells of path length 1 cm and protein solutions of concentration 1.5–2.0 mg/mL were used for CD measurements in the range 300–250 nm. For measurements in the range 250–200 nm, cells of path length 0.1 cm and protein concentrations 0.2–0.4 mg/mL were used. ORD spectra were recorded between 300 and 200 nm with cells of path length 0.1 cm and protein concentrations in the range 1.0–1.5 mg/mL. All the ORD/CD measurements were made at 30 °C.

The secondary structure of glycinin was calculated by the method of Greenfield and Fasman (1969) and also by the curve-fitting procedure of Provencher and Glockner (1981).

The fluorescence emission spectrum of the protein was measured between 380 and 300 nm with a Perkin-Elmer spectrofluorimeter (Model 203) after excitation at 280 nm; the concentration of the protein solution was 0.2 mg/mL.

Viscosity measurements were made with an Ostwald viscometer having a flow time of 173.3 s for distilled water at 30 °C. Protein solutions (1%) with different concentrations of SDS were used. The blanks for the measurements contained the appropriate concentration of SDS. The temperature of the solutions was maintained constant to ±0.10 °C of the desired temperature by keeping the
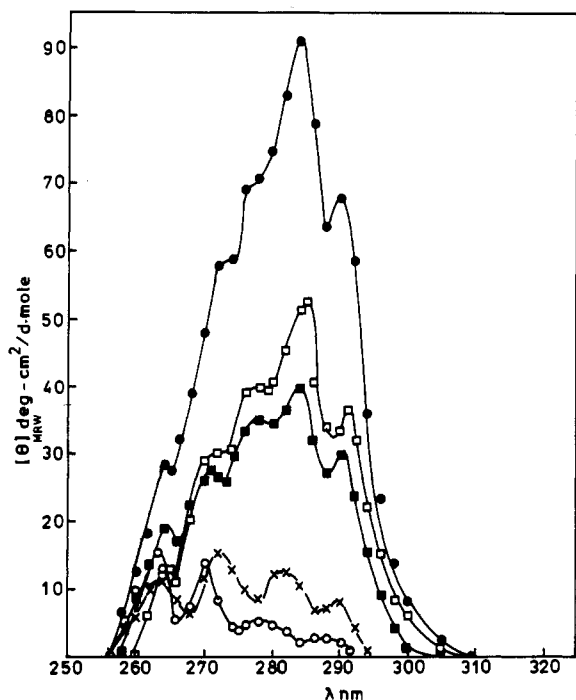
Figure 1. Near-UV–CD spectra of glycinin. SDS, mM: ●, 0.0; □, 0.35; ■, 1.8; ×, 3.5; ○, 18.

viscometer in a constant-temperature bath. Flow time was measured with an accuracy of ±0.1 s after thermal equilibration for 20 min. Reduced viscosity (n_{red}) was calculated according to the standard procedure (Tanford, 1955).

RESULTS AND DISCUSSION

The homogeneity of glycinin was checked by ultracentrifugation. In 0.05 M phosphate buffer of pH 7.8 containing 0.1% β-mercaptoethanol and 0.35 NaCl, the pattern showed a major peak of $S_{20,w}$ value of 11.5S and two minor peaks of 7.3S and 16.7S. Their proportion was 2% and 7% respectively. Further purification of glycinin by gel filtration on Sephacryl gel yielded a material that sedimented as a single peak of 11.5S in the ultracentrifuge. However, on lyophilization, this material gave a sedimentation velocity pattern identical with that obtained with the protein not subjected to gel filtration. Thus, the 7S and 17S protein fractions, which do not constitute more than 9% of the total, were artifacts of the method of preparation and did not represent the true heterogeneity of glycinin. Wolf et al. (1962) and Catsimpoolas et al. (1969) reported the formation of a polymer (15S) from glycinin when it was (1) stored, (2) freeze-dried, (3) precipitated at the isoelectric pH, or (4) heated mildly. The measurements were made with freshly prepared protein solution without further purification on a gel filtration column.

The near-UV–CD spectrum of glycinin between 330 and 250 nm consists of a major peak at 285 nm, smaller peaks at 290 and 265 nm, and shoulders at 273 and 278 nm (Figure 1). These CD bands in the near-UV region arise from tryptophanyl, tyrosyl, phenylalanyl, cystinyl, and certain prosthetic groups; and they reflect the tertiary structure of the protein (Strickland, 1974).

There was a decrease in the intensities of all the bands in the near-UV–CD spectrum of glycinin with the addition of SDS (Figure 1). The decrease in intensity increased with increasing SDS concentration. This suggested disruption of the tertiary structure of the protein. At 18 mM SDS most of the tertiary structure of glycinin appears to have
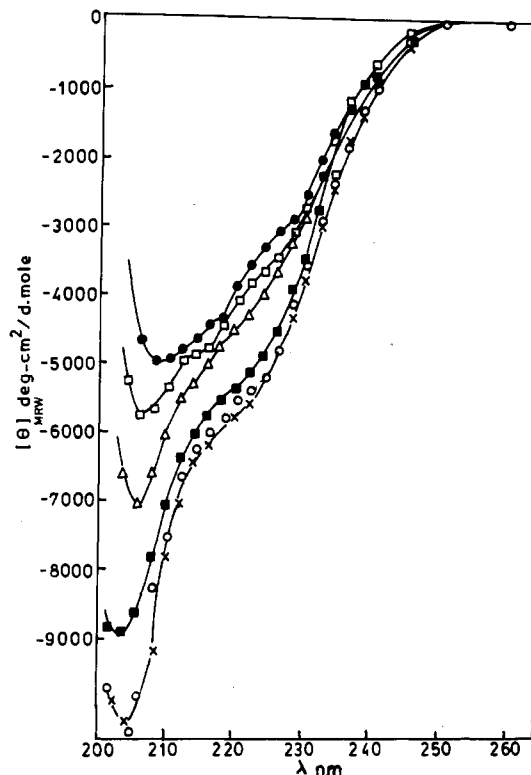

Figure 2. Far-UV–CD spectra of glycinin. SDS, mM: ●, 0.0; □, 0.35; △, 0.7; ■, 1.8; ×, 3.5; ○, 18.

been disrupted. By sedimentation velocity experiments, it has been shown that glycinin is completely dissociated at this SDS concentration (Sureshchandra, 1984).

The far-UV–CD spectrum of glycinin in the region 250–200 nm is characterized by a trough at 208 nm and shoulders around 222 and 215 nm (Figure 2). These shoulders are known to arise from the n–π* transition of the peptide chromophore and may be due to the presence of antiparallel β and cross β structure (Stevens et al., 1968; Raghavendra and Ananthanarayanan, 1981). However, the absence of very fine structure in the spectrum possibly indicates the presence of a large proportion of aperiodic structure in the native conformation.

Addition of SDS up to 3.5 mM increased the ellipticity value, and the negative peak observed at 208 nm in the native state shifted to 203–204 nm (Figure 2). There was no further change in the spectrum by increasing the SDS concentration further.

Addition of SDS increased the $(m)_{MRW}$ values of glycinin (Figure 3). Basically the ORD spectral shape remained unaltered, but the rotational values at 232 nm increased, suggesting that the helical content of the protein increased with increasing SDS concentration (Chen and Yang, 1971).

Increases in the ellipticity values with increases in SDS concentration would suggest an increase in the ordered structure of the protein. The secondary structure of the protein at different SDS concentrations was calculated by the method of Greenfield and Fasman (1969) and of Provencher and Glockner (1981). These data are given in Table I.

The α-helical content of the protein increased with increases in SDS concentration; the values by both the procedures agreed fairly well. However, the agreement in the β structure and aperiodic structure content by the two procedures is not satisfactory. These calculations suggest that SDS causes the formation of ordered structure. It should be emphasized that such calculations are subject to considerable uncertainty, especially when the protein
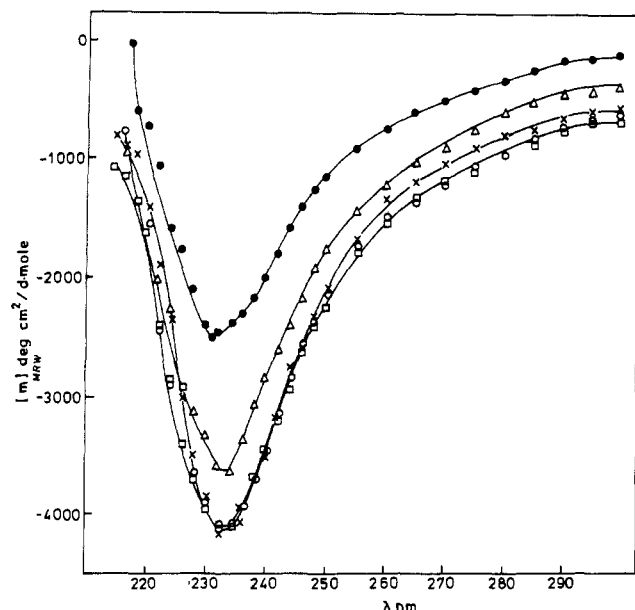
Figure 3. ORD spectra of glycinin. SDS, mM: ●, 0.0; ▲, 0.7; □, 1.8; ×, 3.5; ○, 18.

Table I. Secondary Structure (%) of Glycinin at Different Concentrations of SDS

SDS, mM	Greenfield and Fasman (1969)			Provencher and Glockner (1981)		
	α helix	β struct	aperiodic struct	α helix	β struct	aperiodic struct
0.00	4	33	63	6	60	34
0.35	6	30	64	13	41	46
0.70	8	28	64	13	44	43
1.80	13	24	63	15	38	47
3.50	18	16	66	20	40	40

has a high content of aperiodic structure.

Other types of evidence also seem to suggest the formation of a more ordered structure (α helix) on addition of SDS to glycinin. The difference CD spectrum of the protein in 3.4 mM SDS against native protein in the range 260–200 nm shows a crest at 218 nm and a trough at 222 nm (Figure 4). These may be due to the higher α-helical content of the protein in SDS solution. The minimum around 220 nm in the far-UV–CD spectrum of proteins is characteristic of α helix.

Glycinin had a fluorescence emission maximum at 325 nm (Figure 5). Addition of SDS to the protein decreased the fluorescence intensity. The decrease increased with increasing SDS concentration. However, above 3.5 mM SDS, there was no further decrease in intensity. Also, a blue shift ($\sim$5 nm) was observed at and above 3.5 mM SDS. Denaturation of a protein leads to fluorescence quenching and a red shift in the emission maximum (Teale, 1969). The observed blue shift, admittedly small, would also suggest an increase in the ordered structure of the protein.

Thus, both far-UV–CD and fluorescence measurements seem to suggest that addition of SDS increases the α-helical content of glycinin. Such a phenomenon in the case of other proteins has been termed as "reconstructive denaturation" (Jirgensons, 1982). Thus, vegetable proteins also seem to exhibit this phenomenon.

The increase in helical content of the protein due to the action of SDS can be explained by the "necklace model" proposed by Shirahama et al. (1974) and Takagi et al. (1975). According to them, SDS binds to the protein chain forming micella-like clusters, and the protein SDS complex chain is essentially flexible; due to the extended hydro-
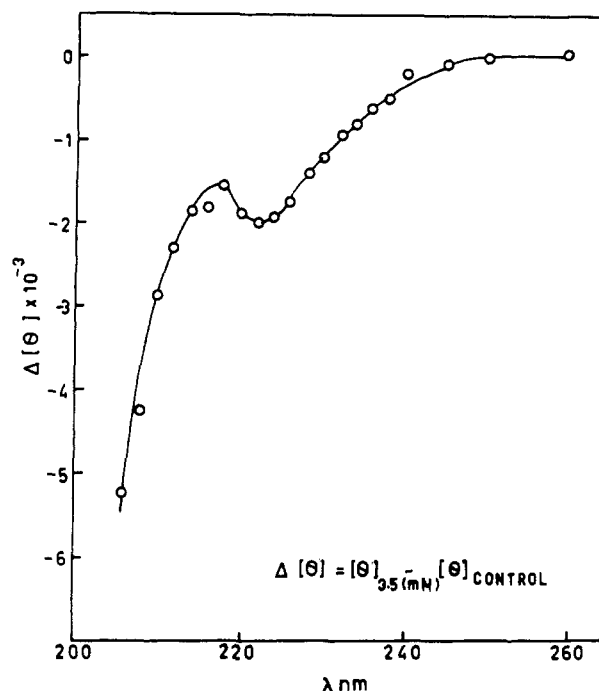

Figure 4. Difference CD spectrum of glycinin in 3.5 mM SDS.
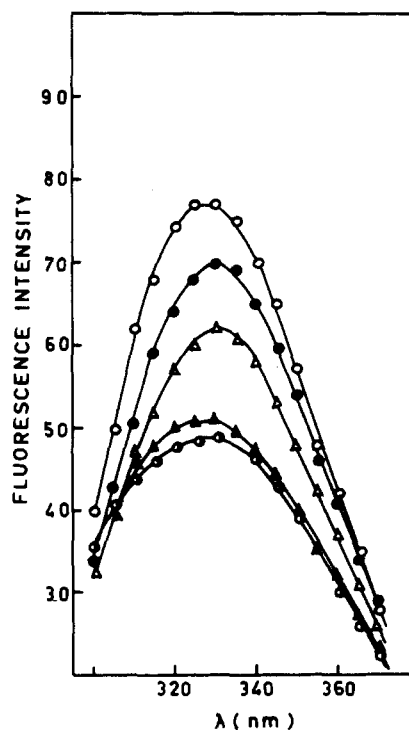

Figure 5. Fluorescence emission spectra of glycinin. SDS, mM: ○, 0.0; ●, 0.35; △, 0.7; ▲, 1.8; ◑, 3.5, 9.0, and 18.

phobic environment of the bound SDS molecules, the protein chain locally assumes α-helical structure.

The reduced viscosity (n_{red}) of glycinin at 30 °C was 7.93 mL/g. The higher viscosity value obtained is consistent with the reported value (Diep et al., 1982). Addition of SDS did not alter significantly the reduced viscosity values (Figure 6), suggesting that SDS does not alter the hydrodynamic volume of the protein.

From these measurements it appears that the glycinin polypeptide chain of low α-helix content is reorganized by SDS in such a way that the polypeptide chain has more helix than the native protein. The ability of SDS to increase the helix content in glycinin suggests the availability
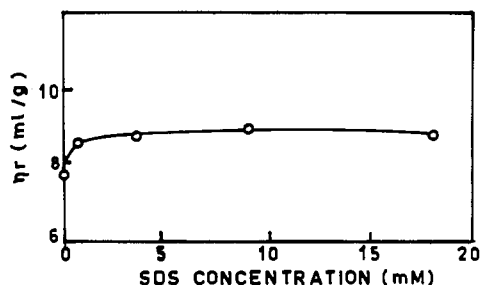
Conformation of Glycinin in SDS Solution

J. Agric. Food Chem., Vol. 35, No. 2, 1987 **247**

Figure 6. Effect of SDS concentration on the reduced viscosity at 30 °C.

of potential helicogenic sequences in the polypeptide chain. Thus, certain amino acid sequences can assume several conformations depending upon the macroenvironment.

ABBREVIATIONS

Sodium dodecyl sulfate, SDS; optical rotatory dispersion, ORD; circular dichroism, CD; β-mercaptoethanol, β-RSH.

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Registry No. SDS, 151-21-3.

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