Comparison of Conformations of 7S and 11S Soybean Globulins by Optical Rotatory Dispersion and Circular Dichroism Studies

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ABSTRACT

Conformational studies of the 7S and the 11S globulins, the major storage proteins of soybean seeds, were made by optical rotatory dispersion (ORD) and circular dichroism (CD). Between 200 and 250 nm., CD spectra of both proteins were fairly similar, and their experimental CD patterns between 210 and 240 nm. agreed well with those calculated from ORD parameters a_0 and b_0. Their secondary structures appeared analogous, and the contents of α-helix, β-structure, and random coil in both proteins were estimated to be approximately 5, 35, and 60%, respectively. However, the formation of α-helix accompanying its dissociation into subunits and changes of molecular ellipticity by treatment with sodium dodecyl sulfate was less in the 11S than those in the 7S globulins. Furthermore, CD spectra between 250 and 320 nm. differed between them. These facts suggested that their tertiary structures differed to some extent, and that the state of tyrosyl residues was different between the two proteins.

The 7S and the 11S globulins[1] are the major proteins in soybean seeds (*Glycine max*). In 1968, Fukushima (2) reported that no appreciable differences exist in the internal structures between the two proteins when crude preparations are used. Catsimpoolas et al. (3) showed that the native 11S globulin exhibits mainly a β-structure by optical rotatory dispersion (ORD) studies and infrared spectra in deuterium oxide. However, recently, Saio et al. (4,5) indicated an interesting result; the physical properties of tofu-gel prepared from crude 7S and 11S components

[1]The 7S and 11S globulins were given the proposed names of γ-conglycinin and glycinin, respectively, by Catsimpoolas (1).

differ remarkably, and the differences are caused mainly by the number of sulfhydryl groups contained in each protein. Moreover, there are significant differences in acid-induced conformation changes between the two proteins when ionic strength is varied with sodium chloride (6). In particular, the 7S quantitatively undergoes a reversible 7S ⇋ 9S isomerization with change of ionic strength from 0.1 to 0.5 μ (7).

In this paper, internal structures of both proteins were further investigated and compared in detail by ORD and circular dichroism (CD) using pure preparations.

MATERIALS AND METHODS

The 7S globulin was prepared as described in previous papers (8,9). The 11S globulin was purified from the cold-insoluble fraction (CIF) of the water-extracted soybean proteins by gel filtration with Sephadex G-100 and G-200 as described previously (10).

Potassium phosphate buffer (0.0325M K_2HPO_4, 0.0026M KH_2PO_4 containing 0.4M NaCl, pH 7.6) (11) was used as the 0.5 μ standard buffer.

Optical Rotatory Dispersion

ORD measurements were made using a Japan Spectroscopic model ORD/UV-5 recording spectropolarimeter equipped with a Xenon arc lamp photomultiplier according to the method reported in a previous paper (12). Measurements were made near 20°C. For measurements in the visible and near-ultraviolet regions, a quartz cell with a light path length of 50 mm. was used. The protein concentration was 0.37% for the 7S and 0.34% for the 11S globulin in the 0.5 μ standard buffer, 0.42% for the 7S and 0.29% for the 11S globulin in 0.01M tris-HCl buffer containing 0.25% sodium dodecyl sulfate (SDS), pH 8.2. The resulting data were treated by the Moffitt-Yang equation (13,14) and expressed in terms of the parameters of a_0 and b_0. The dispersion constant, λ_c, was determined from the one-term Drude equation (13,14).

In the ultraviolet region, a quartz cell with a light path length of 1 mm. was employed with half the protein concentrations used in the visible and near-ultraviolet regions. Data were expressed in terms of reduced mean residue rotation, m'. Mean residue weights M_0, of 107.4 for the 7S (12) and 114.1 for the 11S globulin (10) were used, respectively, and the absorption wavelength associated with the rotation, λ_0, was assumed to be 212 nm.

Circular Dichrosim

A Japan Spectroscopic model J-20 and a model ORD/UV-5 recording spectropolarimeter with a CD attachment were used for CD measurements. A 0.2-mm. quartz cell was used with 0.10% for the 7S and 0.13% for the 11S in the 0.5 μ standard buffer for CD spectra between 200 and 250 nm., and a 10-mm. quartz cell with 0.18% for the 7S and 0.15% for the 11S in the 0.5 μ standard buffer between 250 and 320 nm. Protein concentrations of 0.024% for the 7S and 0.029% for the 11S in 0.01M tris-HCl buffer containing 0.25% SDS were used with a 1-mm. quartz cell for CD spectra between 210 and 250 nm., and 0.060% for the 7S and 0.073% for the 11S in 0.1N NaOH with a 10-mm. quartz cell between 250 and 320 nm., respectively. The CD data were expressed in terms of molecular

ellipticity, θ. In the calculation of θ, average residue weight was also used instead of molecular weight. The units of θ are degrees decimole^{-1} cm.^{2}

RESULTS AND DISCUSSION

Circular Dichroism of the 7S and the 11S Globulins between 200 and 250 nm.

Figure 1 shows CD spectra of 7S and 11S globulins in the 0.5 μ standard buffer. Comparing CD spectra of both proteins revealed only slight differences in detail. For example, they clearly show troughs near 210 nm. Shoulders or troughs at 208

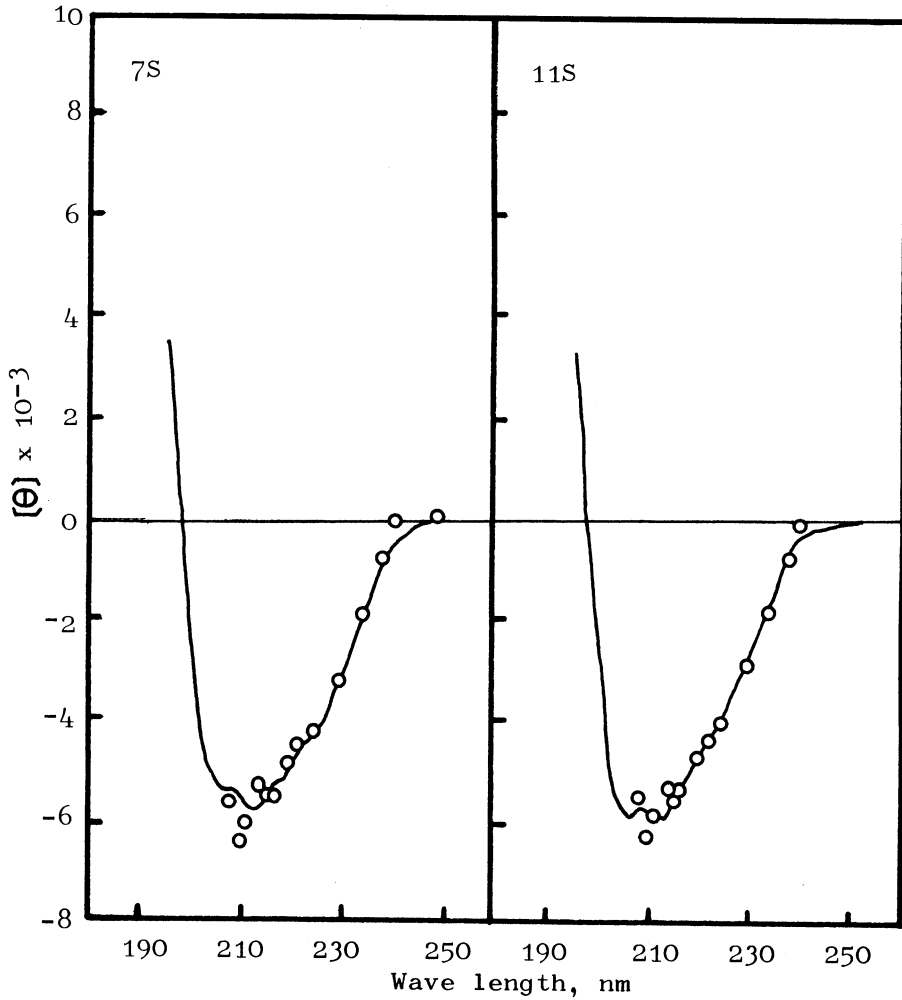

Fig. 1. The CD spectra of native 7S and 11S globulins in 0.5 μ standard buffer. Open circles are molecular ellipticities calculated from Moffitt's parameters of a_0 and b_0 for poly-L-lysine reference spectra in water using 6% α-helix, 34% β-structure, and 60% random coil computed for the 7S, and 5% α-helix, 35% β-structure, and 60% random coil for the 11S.

TABLE I. COMPARISON OF CONFORMATION CHANGES BETWEEN 7S AND 11S GLOBULINS DERIVED FROM SDS TREATMENT BY ORD[a]

	a_0 nm.	b_0 nm.	λc nm.	α-Helix %	β-Structure %	Random Coil %
7S						
Native	−246	−37	226	6	34	60
SDS	−371	−74	227	12	14	75
11S						
Native	−246	−33	223	5	35	60
SDS	−353	−52	224	8	19	73

[a]SDS treatment was performed for 16 hr. at 4° C.

nm. and near 222 nm. were considered to be contributed by α-helix. The helical contents of about 6% for the 7S and the 11S globulin in the 0.5 μ standard buffer were calculated from the values of molecular ellipticity at 208 nm. according to the method of Greenfield and Fasman (15). These helical contents agree well with those calculated from the Moffitt's parameters of b_0 in previous papers (10,12) as shown in Table I. These results indicate that α-helical structure is not the main structure in either protein as reported by Fukushima (2).

Fukushima (2,16) has shown by infrared (IR) absorption measurements that both proteins clearly have the amide V band at 698 $cm.^{-1}$ characteristic of the β-form, and has suggested that they contain rather large amounts of β-structure. Catsimpoolas et al. (3) have also recognized that the 11S globulin has mainly a β-conformation with some unordered regions in the molecule by IR spectroscopy. Imahori (17) and Arai et al. (18) have shown typical CD patterns of β-structure with a large absorption minimum at 218 nm. for soybean proteins and CIF, respectively. However, the only large absorption minimum in CD spectra obtained in our experiments with both proteins was near 210 nm. with a small shoulder at 218 nm. (Fig. 1). This discrepancy cannot be explained, but the contribution based on β-structure must be contained in this large trough.

Greenfield and Fasman (15) have shown that computed CD curves using poly-L-lysine are useful in predicting protein structure. If the protein possesses a high degree of secondary structure, the agreement between the calculated and the structure determined by X-ray diffraction is extremely good. In particular, between 208 and 240 nm., the calculated CD spectra agrees well with the experimental spectra.

Open circles in Fig. 1 show the molecular ellipticities calculated from poly-L-lysine reference spectra in water (15) based on the degrees of α-helix, β-structure, and random coil obtained from the Moffitt parameters of a_0 and b_0 in the 7S (12) and the 11S (10), assuming values of a_0=680 and b_0=−630, a_0=800 and b_0=0, a_0=−560 and b_0=0 for 100% helix, 100% β-structure, 100% random coil, and 100% random coil and 0% helix, respectively (19,20). The results obtained gave a good estimate in both proteins as shown in Fig. 1. Obviously, the secondary structures of both proteins are similar.

Effect of SDS on ORD and CD Spectra of the 7S and 11S Globulins

In a previous paper (12), it was suggested that the 7S globulin dissociated into

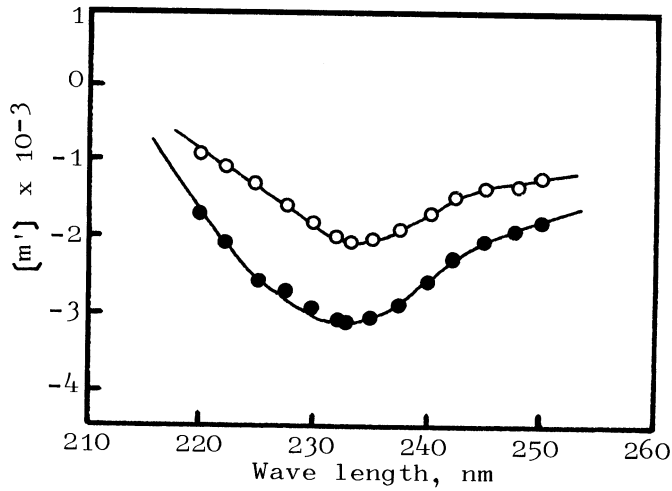

Fig. 2. Ultraviolet ORD spectra of 11S globulin in 0.5 μ standard buffer (open circles) and in 0.01M tris-HCl buffer containing 0.25% SDS, pH 8.2 (closed circles).

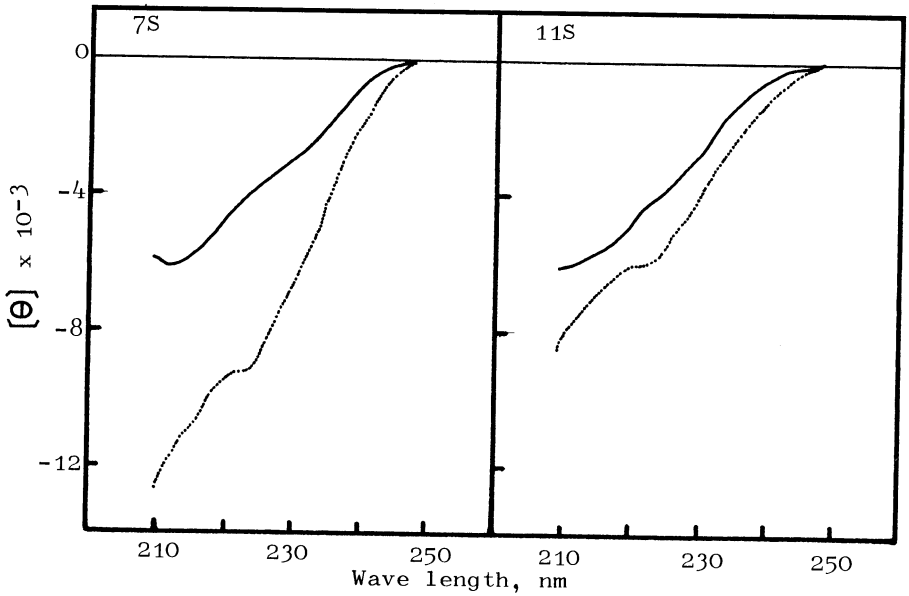

Fig. 3. The CD spectra of 7S and 11S globulins in 0.5 μ standard buffer (—) and in 0.01M tris-HCl buffer containing 0.25% SDS, pH 8.2 (.....).

subunits with a new partial formation of α-helix by SDS treatment. The 11S globulin also underwent the same reaction in the SDS treatment.

In the 220- to 250-nm. region of the ORD curve, the depth of a trough resulting from the negative Cotton effect of α-helix in the protein at 222 nm., the reduced

mean residue rotation, m′, at 233 nm. was clearly more negative on SDS treatment than in the native state as shown for the 11S protein in Fig. 2. The same result was also obtained in the visible and near-ultraviolet regions between 300 and 600 nm. as shown in Table I. The more negative b_0 value by SDS treatment was less in the 11S than that in the 7S. Therefore, a new partial formation of α-helix was estimated to be more pronounced in the latter than in the former.

Furthermore, a new partial formation of α-helical structure in both proteins by SDS treatment was clearly proved by CD. As shown in Fig. 3, a clear shoulder at 222 nm., the second ellipticity band for the α-helix, was found in both proteins by SDS treatment. But changes of molecular ellipticity values obtained by SDS treatment were very different for the two proteins. This fact suggested differences in their tertiary structures. Hydrophobic and hydrogen bonds might participate to maintain the tertiary structure in the 7S, though other binding forces, as for example, disulfide bonds, might participate in the 11S.

Circular Dichroism of the 7S and the 11S Globulins in the 250- to 320-nm. Region

Figure 4 illustrates representative results of the CD spectra recorded between 250 and 320 nm. for the 7S and the 11S globulins. There were significant

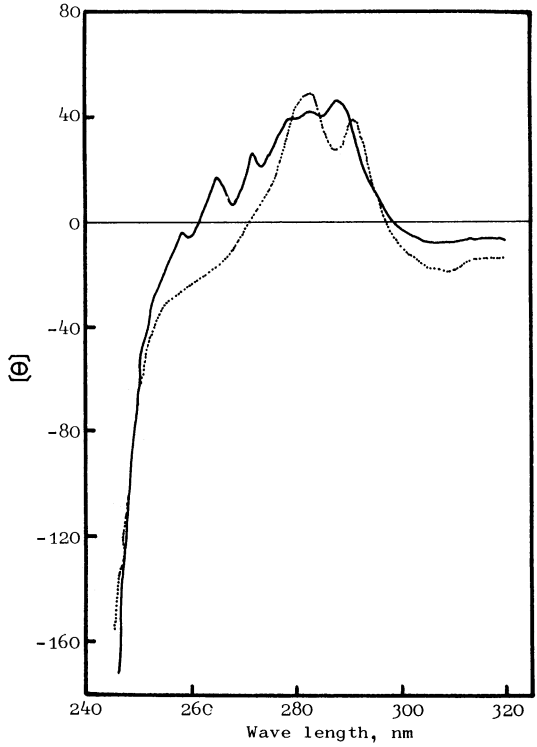

Fig. 4. The CD spectra of 7S (—) and 11S globulins (.....) from 250 to 320 nm. in 0.5 μ standard buffer.

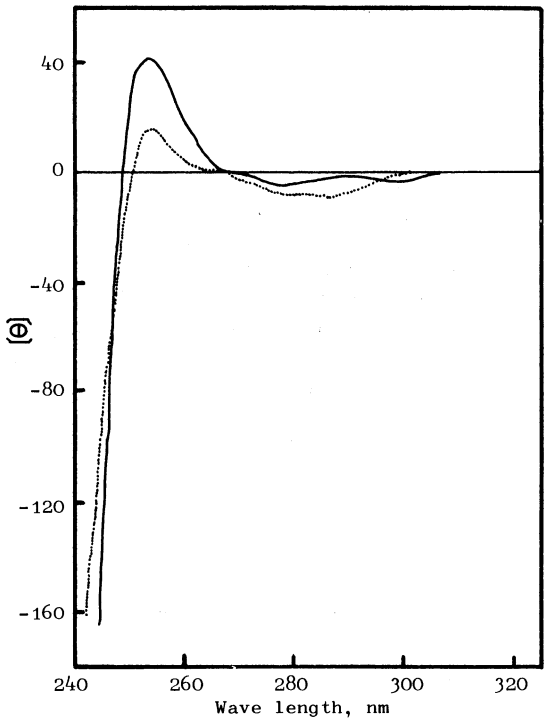

Fig. 5. The CD spectra of 7S (—) and 11S globulins (.....) in 0.1N NaOH.

differences between the CD spectra for the two proteins, with that of the 7S exhibiting some broad positive bands extending over the wavelengths between 265 and 295 nm. and the spectrum of the 11S characteristically exhibiting two positive bands at 283 and 291 nm. These CD bands may orginate from tyrosyl and tryptophyl residues. Therefore, the state of the aromatic amino acid side-chain residues in the protein molecules was presumed to be different for the two proteins.

These positive ellipticities disappeared immediately after solution of both proteins in 0.1N NaOH with appearance of a new positive peak at 253 nm. as shown in Fig. 5. This new peak remained practically the same after the solution had been allowed to stand overnight. The same observation was made for stem bromelain (21) and lyzozyme (22). The magnitude of the CD band at around 250 nm. depends mainly on the number of ionized tyrosyl residues and involves no significant contribution from protein conformation. Therefore, the difference of molecular ellipticities at 253 nm. indicates that the state of tyrosyl residues is different in the two proteins. Further comparisons of the state of tyrosyl residues in the 7S molecule with those in the 11S must await future studies. Detailed investigation of tyrosyl residues in the 11S protein has been described by Catsimpoolas et al. (23).

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