The Physicochemical and Functional Properties of Soybean 11S Globulin—A Review[1]

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ABSTRACT

Food scientists and technologists must gain an in-depth understanding of the molecular and functional relationships of soybean proteins. The soybean globulin called 11S protein or glycinin is the major soybean protein in terms of quantity and most importantly, in terms of functionality. The physicochemical characteristics of 11S protein relate directly to its functional properties.

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Soybeans have become an important protein source for the world populace. In addition to traditional Oriental foods, such as tofu and tempeh, soybean proteins can also be used in baked foods, meat, and dairy products. Given the world food shortage, soybean proteins will be used as a food source more frequently.

Before these proteins can be used effectively, however, food scientists and technologists must thoroughly understand their molecular and functional relationships. During the past 30 years, information concerning the physicochemical characteristics and the functional properties of soybean 11S globulin (glycinin) has been accumulating.

Nomenclature

Soybean proteins were initially classified according to ultracentrifugal analysis into 2S, 7S, 11S, and 15S fractions (Naismith 1955, Wolf and Briggs 1956). The 11S fraction consists primarily of a single protein (Wolf and Cowan 1975) and makes up 25–35% of the total soybean protein (Wolf et al 1961). A second major soybean protein class, which constitutes 30–35% of the total soy protein, consists of proteins in the 7S fraction (Wolf and Cowan 1975). In the late-1960s, a different nomenclature for the major soybean globulins, based on an immunochemical system of reference, was developed and proposed (Catsimpoolas and Meyer 1968a, Catsimpoolas and Ekenstam 1969). Four major antigenically different components were isolated and given the names, glycinin, α-, β-, and γ-conglycinin (Catsimpoolas 1969a). Glycinin is identical with 11S globulin, and α-conglycinin is a 2S globulin (Catsimpoolas 1969a). Until recently, the identity of the β- and γ-conglycinin remained unclear. In 1976, Koshiyama and Fukushima (1976a, b) identified β-conglycinin as that portion of the 7S fraction which dimerizes to 9S when the ionic strength of the protein solution is changed from 0.5 to 0.1 at pH 7.6, and γ-conglycinin as that portion of 7S fraction which does not undergo this dimerization at 0.1 ionic strength. Yamauchi et al (1981) recently isolated a new protein in the β-conglycinin category, which was designated B_0-conglycinin and consisted of three subunits. B_0-conglycinin differs from other β-conglycinins in that it forms insoluble aggregates at ionic strengths lower than 0.2. In this paper, the names of 11S globulin and glycinin are used interchangeably.

Laboratory 11S Protein-Rich Preparations

During the process of studying soybean storage proteins, several common laboratory methods of preparing 11S protein-rich fractions (11S PRF) have been devised:

Cold-Insoluble Fraction (CIF). Cooling a concentrated aqueous extract of ground, defatted soybeans results in the precipitation of a cold-insoluble fraction containing 2S, 7S, 11S, and 15S proteins (Briggs and Mann 1950), of which the 11S fraction predominates (5%, 2S; 7%, 7S; 82%, 11S; 6%, 15S) (Briggs and Wolf 1957). Several attempts have been made to purify the protein further. Wolf et al (1962) devised a scheme for further purifying the 11S protein by ammonium sulfate precipitation of the CIF, which yielded 11S protein that had 91–93% purity. Chromatographic purification using gel filtration (Hasegawa et al 1963, Mitsuda et al 1965), hydroxylapatite (Wolf and Sly 1965), or ion exchange (Catsimpoolas et al 1967) has also been reported. Recently, a combination of affinity chromatography on ConA-Sepharose 4B and gel filtration chromatography on Sepharose 6B were used to purify 11S protein; CIF was used as the starting material (Kitamura et al 1974).

Koshiyama's 11S Protein-Rich Fraction (11S PRF). The 11S PRF can also be prepared by taking advantage of the significant differences in acid-induced conformational changes when the ionic strength in the protein solution is changed with sodium chloride (Koshiyama 1972a). Soy 11S globulin completely precipitates in an HCl solution (pH 2) containing NaCl above 0.5M, but 7S globulin precipitates only when the NaCl concentration is 0.8M or greater. In practice, the 11S PRF is prepared by extracting acid-precipitated protein with a pH 2 HCl solution containing 0.6M NaCl. The insoluble fraction contains 11S PRF.

Saio's 11S Protein-Rich Fraction. The 11S PRF can also be prepared using the method of Saio et al (1973b). The difference in precipitation behavior of 7S and 11S proteins with calcium salt is the basis for separation. Defatted soybean meal is extracted with a dilute calcium salt solution (10 mM $CaCl_2$) at room temperature for 2–3 hr. The supernatant is adjusted to pH 4.5 to precipitate a 7S protein-rich fraction. The residue from the first extraction step is

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suspended in 10 times its weight of warm (approximately 40°C) water, adjusted to pH 8–8.5 and stirred for 1–2 hr. The supernatant is then neutralized and spray-dried to yield 11S PRF.

Okubo's Crude 11S Globulin. Defatted soy meal is extracted with a 0.065*M* NaCl solution containing 10 m*M* 2-mercaptoethanol (MCE) (meal/solvent, 1/100, w/v), adjusted to pH 8 with 1*N* NaOH at 5°C. The extract is filtered through several layers of gauze and centrifuged. The supernatant is then adjusted to pH 6.3 with 1*N* HCl, stirred for 15 min at 5°C, and centrifuged. The precipitate is the Okubo's crude 11S globulin (Okubo et al 1975).

Crude Tris-HCl 11S Protein. Thanh and Shibasaki (1976) published a simple fractionation procedure of major soybean proteins. The difference in solubility of the 11S and the 7S proteins in a dilute Tris-HCl buffer (0.03*M*) is used in the procedure. When the buffer extract (pH 8) is adjusted to pH 6.4, 11S globulin is precipitated. The 7S globulins are then separated from whey proteins by isoelectric precipitation at pH 4.8. Although Thanh and Shibasaki (1976) observed very little cross-contamination among the prepared fractions, the experience in our laboratory indicates that a considerable amount of contaminating proteins still remains in the crude Tris-HCl 11S protein. This procedure is suitable for large-scale preparation of the 11S protein.

Molecular Structure of Soy 11S Protein

Even though the total amino acid sequence of soybean 11S protein has not yet been elucidated, a great deal is known about the primary structure of soy 11S protein.

Molecular Weight. Wolf and Briggs (1959) estimated the molecular weight of 11S protein with sedimentation, diffusion, and light-scattering measurements and concluded that soy 11S protein has a molecular weight of 350,000. Koshiyama (1972b) reported that the molecular weight of 11S globulin was 309,000–322,000 by three separate methods based on different principles. Badley et al (1975) reported a value of 320,000. A molecular weight of 362,000–373,000 was also indicated, based on the intermediary subunit molecular weights and the subunit molecular weights (Kitamura et al 1976). The variation in reported molecular weight is to be expected, because different estimating methods were used, but the reported values seem to hover around 350,000.

Amino-Acid Composition. The amino-acid composition of 11S protein has been determined (Catsimpoolas et al 1971b). 11S protein is low in methionine but high in lysine. The proportion of hydrophobic amino acids (Ala, Val, Ile, Leu, and Phe) and hydrophilic amino acids (Lys, His, Agr, Asp, and Glu) are 23.5 and 46.7%, respectively, as determined by Takagi et al (1979). The isoelectric point of 11S protein was determined to be 4.64 (Koshiyama 1972b).

Sulfhydryl and Disulfide Groups in Soy 11S Protein. The sulfhydryl-disulfide interchange reaction has been found to participate in several reactions involving soy 11S protein. Soy 11S protein contains approximately 48 mol of half-cystine per mole of protein (Shvarts and Vaintraub 1967, Catsimpoolas et al 1969). Approximately 2 mol free sulfhydryl groups are in each mole in the native state, and approximately 2–3 mol of sulfhydryl groups are in each mole after heating (Saio et al 1971). Table I shows the results of a study on the sulfhydryl and the disulfide contents on the surface and interior of an 11S protein molecule using native 11S protein and guanidine-HCl-treated 11S globulin (Simard and Boulet 1978).

Draper and Catsimpoolas (1978) studied the rate of cleavage of SS bonds in 11S protein with dithiothreitol (DTT) at various urea concentrations and found that SS bonds reached a maximum of 20 in 8*M* urea. Soy 11S protein contains 1.7 SH groups per mole in 6*M* urea at pH 7.6; at pH 11.9, it contains 9.2 SH groups per mole, which is the maximum achieved by alkaline treatment. This is because the SS groups are mostly buried inside the protein molecule and alkaline treatment cleaves some of the susceptible SS bonds into SH groups (Draper and Catsimpoolas 1978).

N-Terminal Amino Acids of Soy 11S Protein. N-terminal analysis of the purified 11S protein revealed that 11S globulin has three kinds of N-terminal amino acids: glycine, phenylalanine, and leucine (isoleucine) (Mitsuda et al 1965). This result was confirmed by Catsimpoolas et al (1967), who further showed that the molar ratio of N-terminal Gly, Phe, and Leu (Ile) was 8:2:2 in 11S globulin.

In studying the secondary structure of an 11S protein molecule, Koshiyama (1972b) measured the ordered structure of 11S globulin with the optical rotary dispersion (ORD) method and concluded that 11S protein has 5.2% α-helix, 34.8% β-structure, and 60% random coil. The Moffitt-Yang's parameters are $a_o = -246$ and $b_o = -33$ at $\lambda = 223$ μm. The figures obtained by Yamauchi et al (1979) are 6, 40, and 55%, respectively. Ishino and Kudo (1980a) used a different calculating method and reported that the α-helical, β-structure, and random coil contents of an 11S protein molecule are 20, 17, and 63%, respectively, in the native state. Soy 11S protein clearly has a relatively low content of ordered structure and a high content of random coil structure.

The ordered structure of 11S globulin can be changed by several factors. Alkali treatment decreases ordered structures, especially the β-structure, and adding ethanol to the alkali-denatured 11S protein increases the ordered structures (particularly α-helix) and decreases the random coil content (Ishino and Kudo 1980a). On the other hand, acetylation of 11S globulin decreases the β-structure and increases the random coil contents (to 24% and 68%, respectively) at 90% acetylation (Yamauchi et al 1979). Ishino and Kudo (1980a) showed that ordered structure, especially α-helix, is related to the formation of a three-dimensional structure in the gels obtained by alkali-alcohol treatment.

Fukushima (1968) used ORD, ultraviolet (UV) difference spectra, infrared absorption, and other methods to study the higher conformational structure of soy 11S protein. Urea denaturation of the protein caused an increase in a_o value in the negative direction without accompanying changes in b_o values. The far-U.V. ORD curve indicated a positive peak at 200–210 μm and a negative trough at 233–235 μm, but the shoulder near 210 μm was not observed. Levorotation near the negative trough increased on urea denaturation. U.V. difference spectra indicated that tyrosine and tryptophan are buried in the hydrophobic regions of the native molecule. Catsimpoolas et al (1969) confirmed that tyrosine (287 nm) and tryptophan (293 nm) are buried in the hydrophobic regions of the native glycinin—ie, 11S protein (Catsimpoolas 1969a)—and are exposed by treatment of glycinin with different concentrations of urea and with acid (pH 2). The native 11S protein has a very compact molecular conformation with neutral and basic groups buried in the interior (Catsimpoolas et al 1971a).

Factors affecting the conformation of 11S globulin include pH, urea, guanidine HCl (Gu HCl), salt concentration, temperature, and alcohol. Hydrogen ion titration of 11S globulin in the presence or absence of 6*M* urea or 6*M* Gu HCl at various pH values showed that acid denaturation of 11S protein with subsequent dissociation into subunits starts at pH 3.75 and reaches a maximum at pH 2. Between pH 3.75 and 6.5, 11S protein exists in the form of an undissociated swollen sphere. Conformational changes accompanying alkaline denaturation start at pH 10, but proceed more rapidly at pH values of more than 11. Six molar (6*M*) urea-induced denaturation differs in nature and is more effective in unfolding the 11S protein than is alkaline denaturation. However,

Table I
Contents of the Sulfhydryl and the Disulfide Groups in a Soy 11S Protein Molecule According to Location[a]

	SH		SS	
Location	μ mol/g N	mol/mol protein	μ mol/g N	mol/mol protein
Surface[b]	20	1.1	177	10.1
Interior[c]	79	4.5	477	27.2
Total	···	5.6	···	37.3

[a] Reprinted from Can. Inst. Food Sci. Technol. J. 11:45, 1978. With permission.
[b] Determined on native protein.
[c] Determined on Gu HCl denatured protein. Molecular weight of 11S protein = 356,000.

there are still some unavailable neutral and basic side groups in 6*M* urea, due to the burying of these groups in areas of the molecule adjacent to disulfide bonds (Catsimpoolas et al 1971a, Koshiyama 1972c). The concentration of urea is important in unfolding the 11S molecules; 5*M* urea incompletely unfolds 11S protein, but 8*M* urea completely unfolds the 11S molecule (Kamata et al 1979b). The denaturing ability of various alcohols depends completely on the hydrophobicity of the alcohol used. The 11S molecules are quite compact and are not hydrolyzed by proteinase without disrupting the internal structure (Fukushima 1968). When 11S protein is dissolved in low-ionic strength buffer ($\mu = 0.1$) and subjected to acid treatment, dissociation and simultaneous unfolding of the polypeptide chains are observed, whereas in high-ionic strength buffer ($\mu = 0.5$), the acid treatment causes less denaturation. This denaturing effect due to the ionic strength is even more pronounced at $\mu = 0.01$ (Koshiyama 1972c, Wolf et al 1958).

Type and Number of Subunits. Soy 11S protein molecules contain 12 polypeptide chains (Catsimpoolas et al 1967, Catsimpoolas 1969b, 1970). There are two types of subunits: acidic and basic. The 11S protein molecule consists of six acidic and six basic subunits (Catsimpoolas et al 1971b, Catsimpoolas 1969b, Draper and Catsimpoolas 1977, Badley et al 1975). Monomeric glycinin consists of six subunits. The dimer (mol wt 350,000) contains 12 subunits. The dimer is generally thought to be composed of two identical annular-hexagonal monomers stacked on top of each other, yielding a hollow, oblate cylinder of 110 × 110 × 75 Å. Some or all of the subunits are nonspherical, resulting in a partial blocking of the central hole (Fig. 1). The acidic and basic subunits alternate in the same layer and are held together by hydrophobic and disulfide bonds, forming three different intermediary subunits (IS') when subjected to a dissociating buffer without reducing agents (eg, MCE). The two layers are held together by hydrophilic bonds (electrostatic and/or H-bondings) (Catsimpoolas 1969b, Badley et al 1975, Yamauchi et al 1979). Although it is generally accepted that the native 11S molecule is composed of two layers of proteins stacked on top of each other, ambiguities still exist as to the number of different acidic or basic subunits in the molecule and whether the two layers are identical.

Previous work (Catsimpoolas et al 1971b, Catsimpoolas 1969b) indicated that there are three kinds of acidic subunits and three kinds of basic subunits (A1, A2, A3, and B1, B2, B3, respectively). In contrast, Kitamura and Shibasaki (1975) and Kitamura et al (1976) reported that four kinds of acidic and four kinds of basic subunits (A1, A2, A3, A4, and B1, B2, B3, B4, respectively) exist in the 11S protein molecule. The acidic subunits are present in the approximate molar ratio of 1:1:2:2 (A1:A2:A3:A4); the basic subunits, 1:1:2:2 (B1:B2:B3:B4). Moreover, Mori et al (1979a) found five acidic subunits (AS1+2, AS2+3, AS4, AS5, and AS6) and four basic subunits (BS) in the native 11S protein molecule.

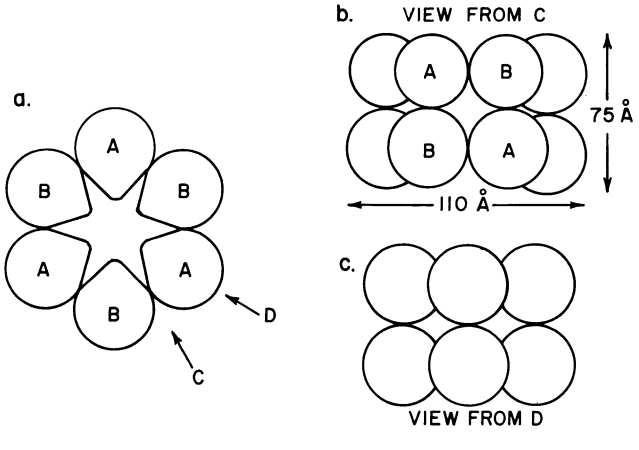


Fig. 1. Schematic diagram of the soy 11S dimer showing position of the acidic (A) and basic (B) subunits. View from above the central hole. (Reproduced from Biochim. Biophys. Acta 412:214, 1975. With permission.)

Staswick et al (1981) found six acidic (Ala, Alb, A2, A3, A4, and F2(2)) and five basic (Bla, Blb, B2, B3, and B4) subunits in their 11S protein preparation. However, Iyengar and Ravestein (1981) showed the existence of a "free" acidic subunit not covalently bound to a basic subunit and postulated that the two "free" acidic subunits probably occupy the central hole of each half-molecule. The difference in the number and kinds of 11S protein subunits may be due to the different varieties of soybeans from which the 11S protein was prepared (Wolf 1976).

Based on the subunit-composition information obtained, Kitamura et al (1976) proposed that 11S globulin consists of two similar but not identical monomers and that each monomer contains three intermediary subunits of the following compositions: IS1-A1 or A2 with B3; IS2-A3 with B1 or B2; IS3-A4 with B4. The acidic and basic subunits in an intermediary subunit are held together by a disulfide bond. Therefore, the dissociation of the 11S globulin was proposed as:

$$11S \xrightarrow{\text{urea or SDS}} \text{Intermediary Subunits}$$

$$(A1B3 + A2B3 + A3B1 + A3B2 + 2A4B4) \xrightarrow[+\ \text{MCE}]{\text{urea or SDS}}$$

$$(A1 + A2 + 2A3 + 2A4) + (B1 + B2 + 2B3 + 2B4)$$

Molecular Weights and Isoelectric Points of 11S Subunits. The molecular weights of subunits vary according to the different methods of estimation. Catsimpoolas et al (1971b) reported that A1, A2, and A3 subunits have a molecular weight of 37,200 and that B1, B2, and B3 have a molecular weight of 22,300. Ochiai-Yanagi et al (1977) reported molecular weight values of 28,000 and 18,000 for acidic and basic subunits, respectively, using gel filtration in 6*M* guanidine-HCl. Kitamura and Shibasaki (1975) indicated that A1, A2, and A3 have a molecular weight of 37,000 and that A4 has a molecular weight of 45,000, based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Draper and Catsimpoolas (1977) gave 42,000 for A1, A2, and A3 and 19,000 for B1, B2, and B3. Badley et al (1975) obtained figures of 34,800 and 19,600 for acidic and basic subunits, respectively. Mori et al (1979a) determined the molecular weights at 34,000 for AS1+2, AS2+3, AS4, and AS6; 38,000 for AS5; and 19,000, 18,300, and 17,900 for the basic subunits. These figures are all close to the values of 37,000 for acidic subunits and 20,000 for basic subunits.

The isoelectric points for A1, A2, and A3 are 5.15, 5.40, and 4.75, respectively. Those for B1, B2, and B3 are 8, 8.25, and 8.5, respectively (Catsimpoolas et al 1971a, Catsimpoolas 1969b).

Primary Structure and Amino-Acid Composition of 11S Subunits. The complete amino acid sequences of the 11S acidic and basic subunits have not yet been determined, but the amino-acid compositions of acidic and basic subunits as a group (Ochiai-Yanagi et al 1977) and the amino-acid compositions of the individual subunits of soy 11S protein (Catsimpoolas et al 1971b) are known.

It is clear that the six subunits have different amino-acid compositions. Acidic subunits have higher contents of glutamic acid, proline, and half cystine than do basic subunits. Moreover, basic subunits have a higher hydrophobic amino acid content (Leu, Ala, Val, Tyr, and Phe) than do acidic subunits. The ratios of acidic amino acids:basic amino acids for A1, A2, and A3 are 1.9, 2.2, and 2.5, respectively. Those for B1, B2, and B3 are 2.2, 2.4, and 1.8, respectively. This means that acidic amino acids in basic subunits are in the amide form. In fact, 55% of the total acidic amino acids in 11S protein are in the amide form (Catsimpoolas et al 1971b).

As a group, the acidic subunits have similar amino acid composition, but some differences are noted in the contents of basic amino acids (Lys, Arg, and His), serine, and proline. Relatively large differences in the contents of amino acids with stable side chains, such as Lys, Arg, Ala, Ile, and Pro, are noted between A3 and A1 and/or A2 (Kitamura and Shibasaki 1975).

The content of the sulfur-containing amino acids (methionine plus cysteine) varies among the component subunits. The reported percentages of these two amino acids in A1, A2, A3, A4, B1, B2, B3, and B4 are 2.4, 3, 1.6, 0.6, 2.3, 2.4, 0.6, and 1.6, respectively (Moreira et al 1979).

Acidic subunits A1 and A2 contain phenylalanine as the N-terminal amino acid, whereas A3 and A4 contain leucine (isoleucine) as the N-terminal amino acid (Kitamura and Shibasaki 1975). However, in addition to phenylalanine and leucine (isoleucine), arginine was found to be the N-terminal amino acid for an acidic subunit in one report (Moreira et al 1979). For basic subunits, glycine is the N-terminal amino acid (Gilroy et al 1979, Moreira et al 1979).

Although the complete amino acid sequences of the individual subunits have not been determined, some information about the primary structure of the subunits is available. Various techniques have shown that there are sequential homologies among A1, A2, and A3 subunits and that the primary structure of A4 subunit is relatively different from those of A1–A3. Methionines are located at homologous positions in the polypeptide chains of A1 and A2. The primary structure of A3 may be more similar to those of A1 and A2 than to that of A4 (Kitamura and Shibasaki 1975, 1977). A1 and A2 are two distinct polypeptide chains with very similar primary structures. A4 is serologically distinct from A1, A2, and A3, although there are some similarities of amino acid sequences (Kitamura and Shibasaki 1977). Partial N-terminal (Moreira et al 1979, Iyengar and Ravestein 1981) and partial internal (Moreira et al 1981) amino acid sequence analyses also showed a close similarity in the primary structures of these acidic subunits. Partial N-terminal amino acid sequences (17 and 29 amino acid residues) of the basic subunits of 11S protein have been determined (Gilroy et al 1979, Moreira et al 1979). The heterogeneity of the basic subunits, which was observed by Catsimpoolas and Wang (1971), was not seen in these partial sequences, and it is postulated that the remaining subunit structures may contain more radical amino acid substitutions than does the N-terminal fragment.

Miscellaneous Properties of Soy 11S Subunits. The acidic subunits have a $S°_{20,w}$ value of approximately 3S at 1% protein concentration (Kitamura and Shibasaki 1975). The $E^{0.1\%}_{280nm}$ values for A1, A2, A3, and A4 are 0.8, 0.8, 0.72, and 0.74, respectively (Kitamura and Shibasaki 1977). Acidic and basic subunits have been found to have different susceptibilities towards trypsin degradation. Acidic subunits degrade faster than do the basic subunits in the native state, especially under high-ionic strength ($\mu = 0.5$) condition. Trypsin digestion of 11S protein has been reported to yield peptides called P fragments (mol wt = 9,600) (Kamata et al 1980).

Chemical Reactions of 11S Protein

Molecular Aggregations. During cryoprecipitation, 11S protein polymerizes via disulfide (SS) bonds to form aggregates of various sizes, insoluble precipitates, or 14-22S soluble aggregates. Reducing agents (10 m*M* MCE, 10 m*M* cysteine, NaCN, etc.) increase the solubility of the 11S component in the cryoprecipitate (CIF). During isoelectric precipitation (pH 4.8), maximal polymerization of 11S protein occurs when 11S protein is in the reduced (ie, reactive) state (Briggs and Wolf 1957, Nash et al 1971). Hydrogen bonds are implied to be involved in the aggregate formation during acid precipitation of 11S protein at room temperature (Catsimpoolas et al 1970). If the isoelectric forms of freeze-dried soybean protein isolate are stored in the dark at room temperature, solubility is lost, primarily because of the formation of disulfide-crosslinked polymers involving mainly the 7S and 11S proteins (Nash and Wolf 1980). In addition to acid precipitation and cryoprecipitation, cold storage of the purified 11S protein as a damp $(NH_4)_2SO_4$ precipitate and precipitation of 11S globulin by dialysis against water followed by freeze-drying also convert 11S protein to 15S or faster sedimenting polymers (Wolf et al 1962). Storage of concentrated 11S protein solution (>10 mg/ml) at low temperature (4° C) enhances the formation of polymers in the form of precipitates (Hashizume et al 1971, personal observation).

Tofu is a soybean protein gel with a well-defined three-dimensional network of protein macroaggregates. The physical properties of tofu made from 11S protein are closely related to the formation of intermolecular bonds, such as SS bonds, and a tofu gel from 11S protein becomes firmer as the quantity of SH groups increases (Saio et al 1971). When 11S protein is frozen and stored at −1–5° C, the protein becomes partially insoluble after thawing The partial insolubility is due to the formation of aggregates, presumably through SS bonds, because *N*-ethylmaleimide (NEM)-11S is not insolubilized by frozen storage (Hashizume et al 1971).

Association-Dissociation Reaction. This reaction is perhaps the most important one that has a bearing on the functional properties of 11S protein. Several factors affect this reaction, including:

pH. Behavior of the 11S protein in acid solutions was studied with the ORD method by Wolf et al (1958). Low pH and low-ionic strength convert the 11S protein into a slow sedimenting component (2S) as a result of dissociation of the protein into subunits. An intermediate dissociation product ($\overline{7S}$) was observed under certain conditions. The symbol, $\overline{7S}$, denotes that this dissociation product of 11S protein is not the same as the other major soy protein fraction, the 7S globulins. The dissociation of 11S protein was suggested to be due to electrostatic repulsion among the subunits. 11S protein dissociates into subunits, which then form associated products at acidic (pH 2) and alkaline (pH 11) conditions as detected by disk electrophoresis (Catsimpoolas et al 1969). Acid denaturation of the protein and subsequent dissociation into subunits starts at pH 3.75 and reaches a maximum at pH 2 (Catsimpoolas et al 1971a, Koshiyama 1972c), whereas alkali-induced denaturation and the accompanying conformational changes start at pH 10 and proceed more rapidly at pH values of more than 11.4 (Koshiyama 1972b). At low-ionic strength and slightly alkaline pH values, 11S protein readily dissociates into subunits—eg, in a 0.03*M* Tris-HCl buffer and at pH 8.6, 11S extensively dissociates into 2S and $\overline{7S}$ components (Eldridge and Wolf 1967). The conformational changes induced by pH were initially thought to be irreversible, but Hachiya et al (1972) showed that the secondary and tertiary structures of 11S protein were partially recovered from the alkaline denatured protein by neutralization.

Temperature. Soy 11S protein in a 0.5 ionic strength buffer appears to be stable to temperatures of up to 70° C. Above 70° C, the soy 11S protein becomes increasingly turbid; it precipitates at 90° C. Disk gel electrophoresis showed that 11S protein heated to 70–90° C exhibits significant dissociation. With mild heat treatment, 11S protein solution contains monomer, dimer, and polymers (Catsimpoolas et al 1969, 1970). Heating 11S protein solution at 100° C for 30 min results in the formation of precipitates and a 3-4S soluble fraction, which are the dissociated subunits (Wolf and Tamura 1969). The dissociation of 11S protein into subunits depends on the ionic strength. At low-ionic strength, the dissociation begins at 70° C and is completed at 80° C. At higher-ionic strength, it starts at 90° C and is completed at 100° C (Hashizume et al 1975).

2-Mercaptoethanol (MCE). It is well known that 10 m*M* MCE prevents aggregation of 11 S protein by cleaving the intermolecular SS bonds (Briggs and Wolf 1957). However, a high concentration (0.2*M*) is required for extensive dissociation of 11S into subunits (Catsimpoolas et al 1970).

Urea and Guanidine-HC1. Urea (6*M*) causes complete and irreversible dissociation of 11S protein into subunits (Catsimpoolas et al 1969). Guanidine-HC1 (6*M*) exerts the same effects on 11S protein (Catsimpoolas et al 1971a). Dissociation of 11S protein by urea is reversible (Kelly and Pressey 1966, Kitamura et al 1977).

Storage Time. Storage of 11S protein produces dissociation ($\mu = 0.01$, pH 3–3.8), aggregation without precipitation ($\mu = 0.1$–0.2, pH 2.2–3), or aggregation with precipitation ($\mu = 0.1$, pH 2.2–3.8), depending on buffering conditions (Wolf et al 1958). An increase in the $\overline{7S}$ component was observed in 11S protein ($\mu = 0.5$, pH 7.6) with storage time (Yamauchi et al 1979). When stored, in addition to dissociating into monomer ($\overline{7S}$), the 11S protein is also associated into polymers, even though the dimer (11S) is thought to be the most stable form (Kitamura et al 1976,

Catsimpoolas 1969b).

Freeze-Drying. Freeze-drying the 11S protein results in the formation of monomer, dimer, and polymers (Catsimpoolas et al 1969). Also, when the 11S is freeze-dried and stored, it can partially break down into a $\overline{7S}$ component (Wolf et al 1962).

Chemical Modification. Soy 11S protein polymerizes when it is incubated with peroxidizing linoleic acid. The acidic subunits are lost faster than the basic subunits and form polymers. Succinylation and acetylation of 11S protein prevent polymerization, but succinylation is more effective than acetylation (Shimada and Matsushita 1978). However, highly acetylated 11S (>90%) undergoes drastic conformational changes, in that 75% of the modified protein polymerizes and the rest dissociates into 3S protein (Yamauchi et al 1979). During seed germination, amidolysis of 11S protein also results in the dissociation of 11S protein into subunits (Catsimpoolas et al 1968a, b).

Alcohol. Alcohol promotes the dissociation of 11S protein. Hydrophobicity of the alcohol is related to its ability to unfold protein, and increases with aliphatic chain length and decreases with branching of the aliphatic chain (Roberts and Briggs 1963, Wolf et al 1964, Fukushima 1968).

Detergent. Conformational changes in the 11S protein are promoted through increasing the intramolecular electrostatic repulsion forces of the negatively charged subunits of the molecule. The interaction of the 11S protein with an anionic detergent, such as sodium dodecyl sulfate and sodium n-octylbenzene sulfonate, on the alkaline side of the isoelectric point of the protein provides a means of increasing the negative charge on the molecule and brings about a complete conversion of the protein to the 3S form (Wolf and Briggs 1958).

EDTA. EDTA causes considerable dissociation of 11S protein into $\overline{7S}$ and 4S, with the result being the formation of a solution of mixed molecular species of 11S, $\overline{7S}$, and 4S in a ratio of 29%:45%:26% (Wolf and Briggs 1958, Appu Rao and Narasinga Rao 1975b). This dissociation can be reversed to some extent by adding metal ions. Addition of Mg^{2+} increases the 11S component and decreases the $\overline{7S}$ component, but the 4S component remains unchanged (Appu Rao and Narasinga Rao 1975b).

Thus, the different ultracentrifugal forms of soy 11S protein are: $2S \leftarrow \overline{7S} \rightleftharpoons 11S \rightleftharpoons 15S$, which correspond to the dissociated subunits, monomer (half-molecule), dimer, and polymers. The dissociation into subunits is irreversible, but the other steps are interconvertible (Wolf et al 1958).

Renaturation of 11S Protein. Renaturation of the 11S protein from denatured or reductively denatured state has been studied (Kitamura et al 1977). The yield of the renatured 11S protein from the denatured state (in urea, Gu HCl, and SDS) is high (70–100%). Reconstitution of 11S protein from the stoichiometric combination of the intermediary subunits (ie, IS1:IS2:IS3 = 1:1:1) previously fractionated in 8*M* urea (ie, all SS bonds are intact) yields 100% when renatured. In addition, 11S protein renatures more readily from 8*M* urea denaturation than from denaturation in 5*M* urea (Kamata et al 1979a). The purified renatured 11S protein is similar or identical to the native protein, as judged by various techniques (Kitamura et al 1977, Kamata et al 1979b).

In contrast, the yield of renaturation of 11S protein from the reductively denatured state is low (<50%). Renaturation is achieved by dialyzing 0.25% reductively denatured 11S protein against pH 7.6 phosphate buffer ($\mu = 0.5$) in the presence of 0.002–0.012*M* MCE at 25°C. The purified renatured 11S protein from the reductively denatured state is conformationally different from the native protein, and in the electrophoretic banding pattern of the renatured protein, the density of the A4 band is greatly reduced (Kitamura et al 1977). Therefore, the reduction of the interchain SS bonds has great influence on renaturation. Dissociation of 11S protein into intermediary subunits containing intact interchain SS bonds is reversible, but dissociation into constituent (acidic and basic) subunits, with the accompanying breakage of interchain SS bonds, is irreversible. The scheme for the association-dissociation of 11S protein is as follows:

$$\text{11S protein} \underset{\text{pH 7.6, } \mu = 0.5}{\overset{\text{urea}}{\rightleftharpoons}} \text{intermediary subunits}$$

$$\xrightarrow{\text{urea and MCE}} \text{acidic and basic subunits (irreversible in pH 7.6, } \mu = 0.5).$$

Mori et al (1979a) reported that reformation of the intermediary subunits was observed between AS1+2, AS2+3, AS4, and AS5 and BS, but not between AS6 and BS. The yields of the reconstituted intermediary subunits differed from one another.

Alkali-denatured 11S protein partially recovers the secondary and tertiary structures upon neutralization (Hachiya et al 1972). Renaturation of 11S globulin from the reductively denatured state is depressed significantly by the presence of other proteins (Mori et al 1979b).

Heat Denaturation and Thermal Aggregation. Heating of water-extracted proteins from soybean results in the formation of soluble aggregates. 11S, 15S, and a portion of 7S fraction disappear after 10 min at 80°C or higher temperatures (Watanabe and Nakayama 1962). Heating (100°C) of a crude 11S component solution (in a 0.5 ionic strength phosphate buffer, pH 7.6) causes two changes: Approximately one-half of the protein aggregates and, upon continued heating, precipitates, and the remaining protein sediments much more slowly (4S) than the 11S component. The precipitation reaction is accelerated when a reducing agent, such as 0.01~0.05*M* MCE, is added. Blocking the sulfhydryl groups with NEM (0.01*M*) prevents precipitation, but, in addition to the presence of a 3S fraction, an aggregate of approximately 67S is formed (Wolf 1956, Wolf and Tamura 1969). Lowering ionic strength from 0.5 to 0.1 or to 0.01 in 0.01*M* MCE accelerates the disappearance of 11S protein. Evidently, heating disrupts the quaternary structure of the 11S protein, resulting in the formation of two distinguishable fractions. One of these fractions is composed of stable and soluble 3-4S subunits. The other fraction has a tendency to interact to form a soluble aggregate and, upon continued heating, to form an insoluble aggregate (precipitate) in which SH groups are present (Wolf and Tamura 1969).

$$\text{Namely, 11S} \xrightarrow{a} \underset{\text{(3-4S)}}{\text{A-subunits}} + [\text{B-subunits}]$$

$$\downarrow b$$

$$\text{soluble aggregate}$$

$$\downarrow c$$

$$\text{insoluble aggregate}$$

$K_b \gg K_a$, b is not due to SS formation.

It is reasonable to speculate that step b involves hydrophobic interactions and that step c involves SS groups. A detailed study by Takagi et al (1979) on the chemical forces involved in the thermal aggregation concluded that this is the case.

Gel filtration of the soluble, heat-treated NEM-11S globulin yields two peaks: One consists primarily of the intermediary subunits (41-44S), and the other contains monomer of the acidic subunits and monomer of the IS (3-4S). Increasing heating time causes polymers to form via SS bonds in the first peak (Yamagishi et al 1981b).

Thermal denaturation of 11S protein is very sensitive to the ionic strength. The promotive effects of low-ionic strength (0.1 vs 0.4–2) and MCE on thermal aggregation of 11S globulin have also been noticed (Catsimpoolas et al 1970, Hermansson 1978, Iwabuchi and Shibasaki 1981). Increasing NaCl concentration from 0 to 1.0*M* increased the denaturation temperature of soy 11S globulin by 20°C, as determined by calorimetry (Hermansson 1978). When the ionic strength range is 0–4, 11S globulin becomes increasingly resistant to thermal denaturation (at 100°C for 5 min), with increasing salt concentrations (both KCl and potassium phosphate). There are three regions of salt stabilizing effects. At μ <0.7, potassium phosphate has no stabilizing effect and KCl has only a slight effect. At $\mu = 1$–1.5, the stabilizing effect increases (up

to 50% antigenicity retained), which represents a second transition to a different denatured state. This includes undissociated molecules. At μ = 2.5–3.5, up to 75–95% antigenicity is retained. In addition, pH affects 11S thermal aggregation. Maximal rate of aggregation occurs at pH 4–6 (Iwabuchi and Shibasaki 1981). Involvement of ionic bonds in the interaction is implied. Ca^{2+}, Mg^{2+}, and Al^{3+} exert little effect on thermal aggregation (Catsimpoolas et al 1970).

The protective effect of ionic strength can also be observed by comparing the maximum temperatures in which native 11S globulin remains soluble. At very low-ionic strength (0.001), the amount of 11S protein in the supernatant decreases drastically at 60°C and continues to decrease until, at 75–80°C, no 11S protein remains in the supernatant. At an ionic strength of 0.1, 11S protein remains in solution until 70°C, starts to become insoluble at 75°C, and is completely precipitated at 90°C. Thus, 11S protein dissociates into subunits at a temperature below 80°C (μ = 0.001) or 90°C (μ = 0.1) (Hashizume and Watanabe 1979). The conformation of these subunits continues to change at temperatures above 80 or 90°C. However, blocking of sulfhydryl residues brings about the retention of the hydrophobic region buried in the protein when heated; only one-half of the buried tyrosine residues and no buried tryptophan residues are exposed when soy 11S globulins are heated in the presence of N-ethylmaleimide (Yamagishi et al 1981a).

When heated at high-ionic strength, 11S protein turns turbid because of subunit aggregation. The aggregates are composed of basic subunits, and the soluble fraction contains acidic subunits (Hashizume and Watanabe 1979). The supernatants contain an acidic subunit monomer and at least three kinds of oligomers of acidic subunits, and the precipitates are composed of a basic subunit monomer, seven kinds of oligomers containing acidic and basic subunits in different proportions, and highly polymerized subunits. These oligomers are linked through SS bonds (Yamagishi et al 1980). However, Peng et al (1982b) showed that when the 11S protein was heated at lower temperatures (85–95°C) at 0.5 ionic strength, the supernatant contained traces of basic subunits, in addition to acidic subunits.

Protein concentration exerts an effect on the thermal association-dissociation behavior of soy 11S globulin. Heating 0.5 and 5% 11S protein solutions at 100°C for 1 min results in the formation of soluble aggregates with a molecular weight of 8×10^6. On subsequent heating, the soluble aggregates disappear and completely dissociate into acidic and basic subunits when protein concentration is low (0.5%), whereas at high protein concentration (5%), these soluble aggregates polymerize further and form gels after 5 min of heating. Therefore, the soluble aggregates appear to be transient intermediates in the course of gel formation for 11S globulin (Mori et al 1982).

It is apparent that thermal aggregates of 11S protein are aggregates of subunits, not aggregates of 11S molecules. In contrast, aggregates that form during acid precipitation and cryoprecipitation are formed from intact 11S protein molecules.

Cryoprecipitation. Cryoprecipitation occurs when a concentrated water extract of soybean proteins is stored in the cold (2–4°C) for 3–5 hr. CIF contains primarily 11S protein. Factors influencing CIF are: extraction temperature, pH, NaCl, sucrose, $CaCl_2$, protein concentration of the water extract, aging of the soybean meal, and variety of the soybeans (Wolf and Sly 1967).

Extracting proteins from defatted soy flour at 40°C (meal to water = 1:5, w/v) before cooling increases the yield of CIF 1.86-fold. Extraction at pH 5.2 yields twice as much CIF than extraction at pH 6.3. Complete precipitation of the 2S and 7S components are also observed. If sodium chloride (0.3*M* or greater) or sucrose (0.6*M* or greater) are added to the water extract before cooling, cryoprecipitation does not occur. Addition of 0.1*N* $CaCl_2$ to the water extract causes nearly quantitative (100%) precipitation of the 11S and 15S proteins and part of 2S and 7S proteins. Cryoprecipitation occurs only when the total protein concentration of the water extract is greater than 1% or when the 11S protein concentration in the extract exceeds 4 mg/ml. Aging of the soybean meal (32 months) decreases the 11S extractability, resulting in a CIF containing only 69–74% 11S protein.

Digestibility. The state of denaturation of the 11S protein relates to the digestibility. The native 11S protein molecules are quite compact and are not easily hydrolyzed by proteases (Fukushima 1968). The digestibility of 11S protein with trypsin increases with higher concentration of denaturants and heating temperature. Further addition of the denaturants and 120°C heating causes decreased digestibility. Evidently, the refolding of the protein plays an important role in decreasing the digestibility. Ionic strength also affects the trypsin digestion of 11S protein (Kamata et al 1979a). At low-ionic strength (no NaCl), both the native (no urea) and urea-denatured (2–8*M* urea) proteins are well digested; at high-ionic strength (0.5*M* NaCl), the extent of digestion depends on the state of denaturation. At high-ionic strength (0.5*M* NaCl), the native protein (no urea) resists tryptic digestion, but 5*M* urea-denatured 11S protein is most extensively degraded (more extensive than the 8 *M* urea-denatured 11S protein). Gel filtration and electrophoretic analyses on the trypsin-digestion products in the renaturation study showed the presence of glycinin-T. Because glycinin-T is the digestion intermediate from the trypsin-treated native glycinin (Kamata et al 1979a), the existence of an almost completely renatured protein species after the urea is removed from the renatured 11S proteins is suggested by gel filtration and electrophoretic data. Therefore, the decreased digestibility of the 8 *M* urea-denatured 11S protein (compared to the 5*M* urea-denatured protein) may be due to its increased ability to renature from a completely denatured state (Kamata et al 1979b).

Immunological Reactions. Approximately 1 mol of 11S protein combines with 2 mol of rabbit antibody at the maximum point in the quantitative precipitation of the 11S protein with anti-11S protein antiserum. Heating affects the immunological properties of the 11S protein. The antigenic properties of 11S protein are retained when heated for 30 min at up to 65°C, but are lost rapidly if heated at 70–90°C (Catsimpoolas and Meyer 1968b, Catsimpoolas et al 1971c). Loss of antigenicity is associated with destruction of the quaternary structure and possibly the alternation of the secondary and tertiary structures of the individual subunits. At higher temperatures, the protein becomes increasingly insoluble. After being treated at 110°C for 1 hr, the protein cannot be detected immunologically (Catsimpoolas and Meyer 1968b). The reason for this may be that the protein lacks diffusion due to the loss of solubility or the modification of the antigenic sites by excessive heat. Thus, although some antigenic determinants are located on the intermediary subunits, the major determinants are mostly conformation-dependent (Iwabuchi and Shibasaki 1981).

Metal Binding. Soy 11S protein binds Ca^{2+} and Mg^{2+}. At pH 5.5 (Appu Rao and Narasinga Rao 1975a) and pH 6 and 7 in 0.02*M* Tris-HCl buffer containing 0.18*M* KCl (Sakakibara and Noguchi 1977), no binding of Ca^{2+} occurs. At pH 8.8 and 8, the Ca^{2+} binding of 11S protein was found to be 17 mol $Ca^{2+}/10^5$g protein and 4–11 mol $Ca^{2+}/10^5$g protein, depending on the $CaCl_2$ concentration. Calcium binding is decreased by 0.5*M* NaCl and prior treatment with ethylenediamine tetraacetic acid (EDTA). The probable binding sites on the protein molecule are the imidazole groups. Adding Ca^{2+} causes an increase in 11S protein solution volume, which reaches a maximum of 0.04*M* $CaCl_2$ and drops off at 0.05*M* $CaCl_2$ but does not cause association or dissociation of the protein. $CaCl_2$ (0.05*M*) does not cause any change in the secondary structure of the 11S protein. Heat coagulation (80–97°C) is increased by the addition of Ca^{2+}, and the protein is almost quantitatively precipitated at 0.01*M* $CaCl_2$. This precipitation is decreased when NaCl is added. Thus, Ca^{2+} may serve as a salt bridge in the precipitation.

Magnesium binding is 16 mol $Mg^{2+}/10^5$g protein in a 0.1*M* borate buffer, pH 7.8; and 0.5*M* NaCl suppresses Mg^{2+} binding. Addition of urea or prior treatment of the 11S protein with EDTA also reduces Mg^{2+} binding. EDTA causes dissociation of the protein, which is partially reversed by the addition of Mg^{2+}. Upon Mg^{2+} binding, no conformational change occurs. The 11S protein, before and after EDTA treatment, is quantitatively precipitated by 0.01*M* Mg^{2+}. Again, this precipitation is suppressed by 0.5*M*

NaCl. Binding sites on the 11S protein molecule are thought to be the imidazole group of the histidine residues. Adding the Mg^{2+} ions to the water extract of defatted soybean meal yields a precipitate that consists almost entirely of the 11S protein (Appu Rao and Narasinga Rao 1975b).

Physical Properties of Soy 11S Protein

The physical properties of soy 11S protein are shown in Table II, and are based on the work of Koshiyama (1972b) and Badley et al (1975).

Functional Properties of Soy 11S Protein

Solubility. Soy 11S protein has an isoelectric point of 4.64 (Koshiyama 1972b) and a minimal solubility at this pH. However, the solubility in sodium acetate-sodium chloride buffer at pH 4.64 varies according to the ionic strength and temperature (Eldridge and Wolf 1967). At pH 4.6, 0.25 ionic strength, and 0–2° C, the 11S component is nearly insoluble, whereas at 0.8 ionic strength, this protein has a high solubility (8 mg/ml).

The solubility of 11S protein in a pH 7.6 phosphate buffer also varies with ionic strength. It decreases to a minimum in 0.1*M* NaCl and increases again in 1.0*M* NaCl in the pH range of 6–8 (Wolf and Briggs 1956). The solubility (extractability) of 11S protein by water extraction of defatted soy meal and subsequent cold precipitation varies, depending on the extraction temperature, age of the defatted soy meal, variety of soybeans, pH of the water extract, and presence of $CaCl_2$, sucrose, and NaCl (Wolf and Sly 1967). The solubility of 11S globulin is sensitive to the lowering of pH and decreases more rapidly than 7S globulin when the pH is lower than 6.5 (Thanh and Shibasaki 1976a, Wolf and Sly 1967). This may partially account for the decreased solubility of soy protein extractability after the beans have been stored (Saio and Arisaka 1978, Saio et al 1980).

Soy 11S protein forms disulfide polymers of various sizes, ranging from large soluble aggregates of 14–22S to insoluble polymers. The addition of 10 m*M* MCE, a high concentration (0.73N) of H_2O_2, 0.01*M* cysteine, Na_2S, Na_2SO_3, or NaCN increases the solubility, as shown by the increased 11S peak in the ultracentrifugal pattern (Briggs and Wolf 1957).

The redispersibility of soy 11S and 7S globulins after storage depends on the storage conditions. When stored at 50° C, the redispersibility of the globulins decreases in a short time at 96% relative humidity (R.H.), but it does not decrease for 45 days at 11% R.H. This decrease in redispersibility is due to the formation of polymers primarily through disulfide bonds (Hoshi et al 1982).

Freeze-drying, frozen storage and thawing, and storing 11S protein in acid-precipitated form also decrease the solubility of protein in a 0.5 ionic strength, pH 7.6 buffer, due to the formation of polymers. Adding 10 m*M* MCE improves the solubility. The presence of alcohols decreases solubility of the 11S protein because their denaturing effects result in nonspecific aggregation of the protein (Roberts and Briggs 1963, Wolf et al 1963, 1964).

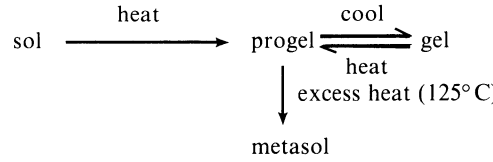
Gelation. Early work on the gelation of soy protein was done with soy protein isolates or acid-precipitated proteins, which are a mixture of proteins that consist primarily of the 11S and 7S components with minor quantities of the 2S and 15S components.

Table II
Physical Properties of Soy 11S Protein[a]

Sedimentation constant, $S^\circ_{20,w}$	12.3 ± 0.1S
Partial specific volume, V, at 20° C	0.730 ± 0.001 ml/g
Diffusion constant, $D^\circ_{20,w}$	3.44 ± 0.1 10 cm^2/sec
Frictional ratio	1.40
Stokes' radius, r	58.5 Å
Radius of gyration, R_g	44 Å
Hydration, δ	0.36 g/g
Intrinsic viscosity, η	0.0485 dl/g
$E^{0.1\%}_{280\ nm}$	8.04
Isoelectric point, pH	4.64

[a] Compiled from Biochim. Biophys. Acta 412:214, 1975, and Int. J. Peptide Protein Res. 4:167, 1972.

Catsimpoolas and Meyer (1970) proposed a scheme for the gelation phenomena of soy proteins:



When heated, the soy protein dispersion (sol) increases in viscosity and undergoes an irreversible change to the progel state. Progel becomes a gel when it is cooled, and viscosity increases again. This step is reversible. However, excessive heating irreversibly converts the gel or progel into metasol, which does not gel at all, due to thermal degradation of the proteins.

Information concerning gelation of the purified 11S and 7S protein has accumulated in the past 10 years. Basically, there are four types of gels: heat-induced gels, heat- and calcium-induced coagulum (tofu) gels, alkali-induced gels, and alkali-alcohol-induced gels.

Heat-induced (≥ 80° C) 11S PRF gels have higher tensile strain, tensile stress, and shear strength than do the 7S PRF gels. The 11S PRF gels also retain more water than do 7S PRF gels (Saio and Watanabe 1978, Saio et al 1973a). When heated without NaCl, the 11S gels obtain maximal hardness at 80° C, whereas at high-ionic strength, the maximal gel hardness occurs at 90° C (Hashizume et al 1975). 11S protein is sensitive to lower-ionic strengths and dissociates to subunits, which partially aggregate. At low-ionic strength, the dissociation occurs at 70° C and is complete at 80° C. At high-ionic strength, the dissociation starts at 90° C and is complete at 100° C. Thus, a close relationship exists between gel hardness and conformational change of protein and its dissociation into subunits and aggregates. Soy 11S protein requires higher protein concentration (2% higher) than does 7S protein for gelation, upon heating at 80° C for 30 min. Adding salts to 11S protein suppresses the formation of gels even with high protein concentration, but when they are added to 7S protein, gel formation is promoted. Higher pH (pH 9) requires lower protein concentration for gelation than does lower pH (pH 7.5). Soy 11S seems to be able to form more hydrogen bonds and ionic bonds and less hydrophobic interactions than 7S (Shimada and Matsushita 1980).

The lowest protein concentration for 11S globulin to form gel is 2.5% when heated at 100° C. The gel becomes harder with increasing concentrations of protein and heating time. Gel formed at 20% protein concentration has a very low turbidity and is transparent (Utsumi et al 1982).

Heat- and calcium-induced coagulum (tofu gels), made from the 11S fraction, are harder than that from the 7S fraction (Saio et al 1969). This difference is attributable to more SS bonds in the 11S tofu gels than in the 7S tofu gels (Saio et al 1971) because changes in the number of SH groups due to heating or treatment with urea are more rapid in the 11S protein than in the 7S protein. Furthermore, the physical properties of tofu from 11S protein are more significantly affected by the change in the number of SH groups in protein by NEM, MCE, and dithiothreitol (DTT). The hardness of the calcium gels from the 11S PRF is much greater than those from 7S PRF. The cohesivensss is also higher in 11S PRF gels (Saio et al 1973b).

Alkali-induced gels are made when alkaline dope solutions of the acid-precipitated protein (7S or 11S protein prepared at pH above 11) are dialyzed against phosphate buffer, pH 7.6, 0.3 ionic strength (Ishino and Okamoto 1975, Ishino and Kudo 1979). The relationship between changes in viscosity and the aggregation phenomena of neutralized dope solutions has been investigated by viscosity measurements, disk electrophoresis, and gel filtration (Ishino and Kudo 1979). The gels consist of macromolecular aggregates. To form the aggregates suitable for gelation, the following conditions must be met: unfolding and dissociation into subunits (pH above 11); high-ionic strength in the media ($\mu = 0.3$); formation of hydrogen, hydrophobic, and disulfide bonds; and

high protein concentration (>8%). Maximum viscosity is obtained at pH 12.9. No gelation occurs for 11S protein alkaline dope solution dialyzed against low-ionic strength buffer or buffer containing MCE (ie, low-ionic strength depresses gelation). Viscosity increases with ionic strength and plateaus at $\mu = 0.3$. The degree of aggregation of the alkali-denatured 11S protein depends on protein concentration. At low protein concentrations, a decreased intrinsic viscosity and increased proportions of protein aggregates which enter the polyacrylamide gels during electrophoresis (Ishino and Kudo 1980b) indicate that the protein aggregates formed at lower protein concentrations are smaller in size.

Transparent alkali-alcohol-induced gels containing 2% protein can be obtained by mixing alkaline dope solution of 11S or 7S protein with alcohol. The 11S component forms weaker gels than the 7S component. This gelation depends on pH and alcohol concentration. In 66% ethanol, the viscosity of 11S protein reaches a maximum at pH 11.2. The viscosity decreases at pH values above 11.2, when further unfolding and dissociation into subunits of the protein molecules occurs. The effectiveness of alcohols to induce gelation varies inversely with the hydrophobicity (ie, the ability to unfold protein) of the alcohols. Methanol is the most effective, and 1-butanol is the least effective of the alcohols tried; ethylene glycol is completely ineffective. Adding NaCl or MCE to ethanol-mixed alkaline dope solution increases the viscosity. Increasing ethanol concentration results in greater formation of the ordered structure from unfolded random structure. Under conditions in which an alkaline dope solution shows a remarkable increase of viscosity by the addition of ethanol, the α-helical transition is more predominant than others. The β-structure depends less on the alcohol concentration (Ishino and Kudo 1977, 1980a). Evidently, a three-dimensional structure of gel obtained by alkali-alcohol treatment is constructed by orienting protein molecules having ordered structures, especially α-helix.

Emulsifying Properties. The 11S PRF has lower values of emulsifying capacity and stability than 7S PRF between pH 2 and pH 10. Partially HCl-hydrolyzed 7S PRF also shows better emulsifying capacity and emulsion stability than the partially HCl-hydrolyzed 11S PRF. The emulsion stability of 11S PRF is lowest around pH 7, indicating that this property does not correlate to the solubility curve (pH = 4.64) for 11S protein. The breaking stress of the emulsion formed with 11S PRF increases in accordance with the oil phase volume and with increasing protein concentration. Heat treatment of the 11S PRF at 95°C for 5 min before emulsification increases the breaking stress two to four times higher than that of an emulsion without heat treatment (Aoki et al 1980). Heat treatment apparently exposes the previously buried hydrophobic regions, resulting in better interaction with the oil phase.

Phosphatidyl choline can be nonspecifically bound to either 7S or 11S protein by sonification. The protein-phosphatidyl choline complexes that sediment to the bottom by sucrose density gradient centrifugation increase with the ultrasonic power. Oil associates with the protein-phosphatidyl choline complexes to form ternary complexes (Kanamoto et al 1977).

Research has been done to create hydrophobic patches on the surface of glycinin molecules for interacting with hydrocarbon or amphiphile molecules without significantly altering the solubility. Glycinin can be lipophilized by incorporating the N-hydroxysuccinimide esters of fatty acids. The rate of incorporation depends on the melting point of the active esters and the length of the hydrophobic ligands (Haque and Kito 1982).

Thermal Expansion. High-temperature treatment of food proteins has become a common practice in such areas as texturization and fried tofu. The following is a description of the expansion properties of Ca^{2+}-CIF gels at 100–170°C: 1) Calcium coagulated CIF gels have a higher expansion ratio than do acid coagulated CIF gels. 2) A high concentration of $CaCl_2$ (0.04M) for coagulation produces gels with higher expansion ratio than does a lower $CaCl_2$ concentration. 3) A higher expansion ratio is obtained for gels in buffers with higher NaCl concentration (0.1M vs 0M NaCl). 4) A maximum expansion ratio is observed at 130°C in the temperature range of 100–170°C. 5) Maximum expansion occurs at pH 2.5 and pH 8.5. 6) High Na_2SO_3 in the buffer inhibits expansion. 7) 11S PRF produces gels with a higher expansion ratio than does 7S PRF (Saio et al 1974).

In the 11S gels before or after heating at 132°C, disulfide cross-linkages contribute the most to the gel structure. The formation of disulfide cross-linkages after weakening of hydrogen or electrostatic bonds occurs during expansion of gels. In acid-CIF gels, the solubility of the high-temperature-treated gels (100–170°C) in a phosphate buffer containing 0.05M SDS and 0.025M MCE increases with greater ionic strength and at higher pH (pH 8.5 vs pH 7.6). The solubility decreases to a minimum at 110°C, increases rapidly above 110°C, and plateaus at 150°C. On the molecular level, the subunits in the heat-treated gels remained unchanged up to 120–130°C and degraded to lower-molecular weight materials at 150°C. A higher expansion ratio correlates with weaker cross-linkages in the heated insoluble gels (Saio et al 1975a). The content of basic amino acid residues (Lys, His, and Arg) does not change significantly (Saio et al 1975b), but the number of amide groups in CIF-gels begins to decrease at 105°C and decreases rapidly as the temperatures of heating increased. Liberated NH_3 increases at 105–110°C. The changes in the characteristics of 11S protein at high temperature heating are shown in Table III.

Surface Film Formation. Surface films form when water evaporates from the surface of soybean milk. Yuba is made by repeatedly removing the previously formed film from the surface. The tensile strength of films prepared from 5% protein solutions by surface evaporation on continuous heating is pH-dependent, which indicates the involvement of ionic interaction. Glycinin films always have higher tensile strength than 7S films. The 11S films have elastic characteristics, but 7S films are hard and fragile (Okamoto 1973, Shirai et al 1974). This may also indicate differences in the ability of these two proteins to hold water.

Interaction with Myosin. Interaction between soy 11S globulin and myosin, the major muscle protein, has been reported to occur at temperatures above 80–85°C (Yamamoto et al 1973, Peng et al 1982a, b). The interaction is not between native soy 11S protein and myosin, but rather between partially dissociated soy 11S (IS') or fully dissociated soy 11S (basic subunits) and myosin heavy chains. Hydrophobic interactions seem to be involved in the interaction (Peng et al 1982a, b).

SUMMARY

Soy 11S protein is the prominent soybean protein in terms of quantity and functionality. The molecular aggregation reactions

Table III
Changes in the Characteristics of 11S Protein During High-Temperature Heating[a]

	Temperature of Heating (°C)								
	100	105	110	120	130	140	150	160	170
Gross structure of subunits	intact				little degraded			degraded	
Solubility	rapid decrease			slow decrease				rapid decrease	
Binding force (degree of aggregation)	rapid increase			slow decrease				rapid decrease	
Expansion property	increase						rapid decrease		
Texture	hard fragile		soft elastic				sol-like		

[a] Reprinted from J. Food Sci. 40:541, 1975. Copyrighted by Institute of Food Technologists.

dictate the solubility properties of 11S protein. The association-dissociation characteristics of this protein have a direct influence on the gelation phenomena caused by heat, calcium ions, alcohol, or alkaline treatment. The solubility of soy 11S protein in a given solvent is influenced by the history of molecular aggregation reactions. The emulsification properties of soy 11S protein are improved by modifying the protein structure with mild hydrolysis or mild heat treatment. The higher thermal expansion properties of soy 11S protein have been correlated with weaker intermolecular bonding. The tensile strength of surface films is related to the ionic interactions among the molecules. The interaction of soy 11S protein with myosin is preceded by a dissociation of both native proteins. The physicochemical characteristics of 11S protein directly relate to the functional properties of this protein.

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