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Comparison of Physicochemical Properties of 7S and 11S Globulins from Pea, Fava Bean, Cowpea, and French Bean with Those of Soybean—French Bean 7S Globulin Exhibits Excellent Properties

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Legume seeds contain 7S and/or 11S globulins as major storage proteins. The amino acid sequences of them from many legumes are similar to each other in the species but different from each other, meaning that some of these proteins from some crops exhibit excellent functional properties. To demonstrate this, we compared protein chemical and functional properties (thermal stability, surface hydrophobicity, solubility as a function of pH, and emulsifying properties) of these proteins from pea, fava bean, cowpea, and French bean with those of soybean as a control at the same conditions. The comparison clearly indicated that the 7S globulin of French bean exhibited excellent solubility (100%) at pH 4.2−7.0 even at a low ionic strength condition ($\mu = 0.08$) and excellent emulsion stability (a little phase separation after 3 days) at pH 7.6 and $\mu = 0.08$, although the emulsions from most of the other proteins separated in 1 h. These results indicate that our assumption is correct.

KEYWORDS: Physicochemical properties; 7S and 11S globulins; pea; fava bean; cowpea; French bean; soybean

INTRODUCTION

Legume seeds contain 7S and/or 11S globulins as storage proteins (*1, 2*). These globulins account for 70−80% of seed proteins (*1, 2*). Among legume seed proteins, soybean proteins are widely utilized as food materials probably because their properties have been extensively studied, which has led to expansion of their food usage. However, some crops must contain proteins with excellent physicochemical properties, since the amino acid sequences of 7S and 11S globulins from many legume and nonlegume seeds exhibit 30−70% difference from those of soybean globulins (*3*−*5*).

The 7S globulins are trimeric proteins. The soybean 7S globulin is composed of three kinds of subunits: α, α′, and β. The α- and α′-subunits consist of the extension region (α, 125 residues; α′, 141 residues) and the core region (418 residues) (*4*). The β-subunit consists of only the core region (416 residues). The core regions exhibit high sequence identities among them (90.4, 76.2, and 75.5% between α and α′, between α and β, and between α′ and β, respectively) (*4*). The extension regions of the α- and α′-subunits exhibit 57.3% sequence identity (*4*).

On the other hand, 11S globulins are hexameric proteins. The soybean 11S globulin consists of five kinds of subunits: A1aB1b, A1bB2, A2B1a, A3B4, and A5A4B3. These five subunits are classified into two groups based on homology in their sequences. Group I consists of A1aB1b, A1bB2, and A2B1a, and group II consists of A3B4 and A5A4B3 (*1, 2*). The sequence identities of each subunit are more than 84% within a group and 45−49% between groups (*2, 6*). Our group compared the physicochemical properties of each constituent subunit of 7S or 11S globulins with each other and with those of the native 7S or 11S globulins using cDNA expression system in *Escherichia coli* and mutant soybean lines with restricted subunit compositions (*4, 7*−*12*). The results showed that the α- and α′-subunit of 7S globulin exhibit much better solubility and emulsifying ability than does the β-subunit and that none of five subunits of 11S globulin exhibit any special excellent properties, although they have their own inherent properties.

The major seed storage proteins of pea and fava bean are 7S and 11S globulins, and those of French bean, cowpea, and mung bean are 7S globulins. Many studies on physicochemical properties of these proteins have been done by various groups (*13*−*22*), but most of them were done under different conditions, and comparisons of their physicochemical properties with those of soybean 7S and 11S globulins are therefore not possible or limited. Herein, we report the purification of the 7S and 11S globulins from the seeds of said crops and the

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examination of their physicochemical properties to know whether some of these globulins have excellent physicochemical properties. To say "excellent", we compared physicochemical properties of crop proteins with those of soybean globulins as a standard using the conditions that we used to determine the properties of soybean globulins (*4, 7*−*12*).

MATERIALS AND METHODS

Chemicals and Materials. All chemicals were obtained from Wako (Kyoto, Japan) or Nacalai Tesque (Kyoto, Japan). Seeds of pea (*Pisum sativum*), fava bean (*Vicia faba*), soybean (*Glycine max* L.), French bean (*Phaseolus vulgaris* L.) and black and red varieties of cowpea (*Vigna unguiculata* L.) were purchased from a local seed supplier, Mizuno Seeds (Kyoto, Japan).

Purification of 7S and 11S Globulins from Soybean, Pea, Fava Bean, Cowpea, and French Bean. The 7S and 11S globulins of soybean were purified according to the method of Nagano et al. (*23*) as described previously (*7, 11*). Centrifugation was done at 9800*g* for 30 min at 4 °C. Briefly, proteins were extracted from defatted soybean meals with buffer A [30 mM Tris-HCl (pH 8.0), 10 mM β-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 1.2 μM leupeptin, 0.2 μM pepstatin A, and 0.02% (w/v) NaN_3]. The precipitate at pH 6.4 and 4 °C was collected by centrifugation and dissolved in buffer B [35 mM sodium phosphate, pH 7.6, 0.4 M NaCl, 10 mM β-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, 0.02% NaN_3, 1.2 μM leupeptin, 0.2 μM pepstatin A, and 0.02% (w/v) NaN_3], and then the precipitate at 45−65% ammonium sulfate saturation at room temperature was collected by centrifugation and dissolved in buffer B as the 11S fraction. Afterward, the precipitate at pH 4.8 and 4 °C was collected by centrifugation and dissolved in buffer B. Then, the precipitate at 55−87% ammonium sulfate saturation at 4 °C was collected by centrifugation and dissolved in buffer B as the 7S fraction.

Purification of 7S and 11S globulins from pea and fava bean and 7S globulins from French bean and red and black varieties of cowpea was done as follows: The protein extract from each defatted seed meal of pea and fava bean with buffer B was precipitated with ammonium sulfate of 50−65 and 35−65% saturation, respectively, as a fraction containing 11S globulin. After removal of 11S globulin fractions by centrifugation, the remaining 7S globulins from pea and fava bean were precipitated at 65−85 and 65−80% ammonium sulfate saturation, respectively. French bean and red and black cowpea 7S globulins were precipitated at 55−80% ammonium saturation. For further purification, the 7S and 11S globulin fractions were applied on a gel filtration column (Hi-Prep 26/60 Sephacryl S-300 HR) using buffer B as a mobile phase. After sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (*24*), the purity of 7S and 11S globulin fractions was estimated by analyzing the gel image with Image Master ID Elite, version 3.0 (Amersham-Pharmacia Biotech, Uppsala, Sweden).

Protein Measurement. The amounts of protein in the samples were determined using a Protein Assay Rapid Kit (Wako) with bovine serum albumin as the standard.

Detection of Carbohydrate Moiety. Carbohydrate detection was carried out by the method of Kijimoto-Ochiai et al. (*25*) as described previously (*26*). Briefly, protein samples were separated by electrophoresis on 11% SDS-polyacrylamide gels, and proteins on the gel were transferred to a nitrocellulose membrane at 40 mA for 2 h. Blotted proteins were treated with ConA-HRP (5 μg/mL in Tris-Tween-saline containing in 3% skim milk) for 1 h. After the membrane was washed with buffer C (15 mM phosphate buffer, pH 6.8), proteins were reacted with 3,3′-diaminobenzidine (1 mg/15 mL in buffer C containing 15 μL of 30% hydrogen peroxide) at room temperature.

Thermal Stability. The thermal denaturation of 7S and 11S globulins was studied by differential scanning calorimetry (DSC) experiments using a Microcal MC-2 ultrasensitive microcalorimeter (Micro Cal Inc.) at a rate of 1 °C/min as described previously (*4*). Protein concentrations were prepared to be 0.5 mg/mL with buffer B.

Surface Hydrophobicity. The surface hydrophobicity was measured by hydrophobic column chromatography using Phenyl Sepharose 6 Fast Flow and Butyl Sepharose 4 Fast Flow columns (Amersham Pharmacia Biotech) as described previously (*9*). All globulin samples were dialyzed against buffer B containing 2.3 M ammonium sulfate and applied to columns equilibrated with the same buffer. The samples were eluted with a linear gradient of 2.3−0 M ammonium sulfate over a period of 80 min at a flow rate of 0.25 mL/min.

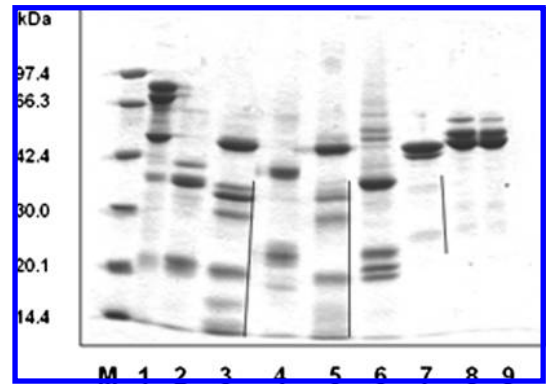

Figure 1. SDS-PAGE analysis of the purified 7S globulin samples. The purified 7S globulin samples were analyzed by SDS-PAGE using 11% gels. Lane 1, soybean 7S; lane 2, soybean 11S; lane 3, fava bean 7S; lane 4, fava bean 11S; lane 5, pea 7S; lane 6, pea 11S; lane 7, French bean 7S; lane 8, red cowpea 7S; and lane 9, black cowpea 7S. The numbers on the left denote molecular masses. The bands defined by a vertical line are mostly limited-proteolyzed subunits.

Solubility as a Function of pH. 7S and 11S globulins were dialyzed against buffer D (10 mM sodium phosphate, pH 7.6, 10 mM 2-mercaptoethanol, 0.5 M NaCl, 1 mM EDTA, 0.1 mM *p*-APMSF, and 0.02% NaN_3) or buffer E (10 mM sodium phosphate, pH 7.6, 0.05 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM *p*-APMSF, and 0.02% NaN_3). The protein solutions (0.8 mg/mL) were kept at 4 °C for 18 h at pH 2.2−9.5 and pH 3−9 at ionic strengths of 0.5 and 0.08, respectively, and then centrifuged at 4 °C (10000*g* × 20 min). The pH solubility profiles were obtained by measuring the protein concentration in the supernatant as the soluble fractions using a Protein Assay Rapid Kit (Wako). The percentage of protein solubility was calculated by the ratio of soluble protein to initial total protein.

Backbone Model of Soybean 7S Globulin α′ and French Bean 7S Globulin. A backbone model of soybean 7S globulin α′ and French bean 7S globulin was drawn using MOLSCRIPT (*27*) and Raster 3D (*28*) based on the three-dimensional data of soybean 7S globulin α′ (*29*) and French bean 7S globulin (*3*).

Emulsification. The emulsifying ability of 7S and 11S globulins was analyzed as described previously (*7*) using 1.5 mL of 0.5 or 1.0 mg/mL protein at pH 7.6 and ionic strengths of 0.5 and 0.08 and mixing with 0.25 mL of soybean oil. Each sample was measured at least three times, and a representative typical pattern was presented. The emulsions were kept at room temperature without agitation and visually observed after 20 h and 3, 5, and 15 days to assess stability.

RESULTS AND DISCUSSION

Purification of 7S and 11S Globulins. 7S and 11S globulins were purified from seeds of soybean, pea, and fava bean and 7S globulins from French bean and two cowpea varieties by ammonium sulfate fractionation and gel filtration. SDS-PAGE analysis of purified globulins (**Figure 1**) indicates that their purities were around 85−90% on the basis of band interisities and confirmed that the 7S globulins from fava bean, pea, and French bean seeds contain limited-proteolyzed subunits (*30*). Cowpea 7S globulin is likely not to be limited-proteolyzed (*20, 30*). The purified samples were used for the following analyses and were compared with soybean 7S and 11S globulins.

Detection of the Presence of Carbohydrates. The presence of carbohydrate moieties linked to 7S and 11S globulins from soybean, pea, fava bean, cowpea, and French bean was examined using Con A conjugated to peroxidase (**Figure 2**). We confirmed that French bean 7S globulin is glycosylated

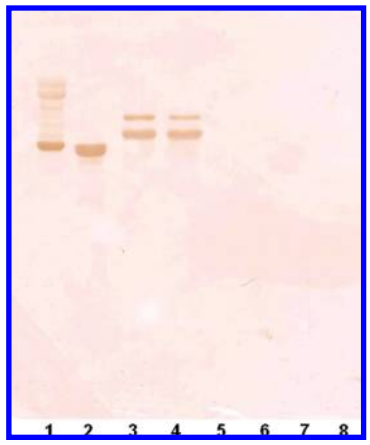

Figure 2. Detection of N-glycoproteins with Con A conjugated peroxidase. 7S globulins (5 μg each) on SDS-polyacrylamide gels were electrophoretically transferred to a nitrocellulose membrane and detected with ConA. Lane 1, soybean 7S; lane 2, French bean 7S; lane 3, red cowpea 7S; lane 4, black cowpea 7S; lane 5, pea 7S; lane 6, fava bean 7S; lane 7, pea 11S; and lane 8, fava bean 11S.

similarly to soybean 7S globulin and that fava bean 7S globulin and pea and fava bean 11S globulins are not (*31*), suggesting that the presence and absence of carbohydrate moieties do not depend on the varieties of these crops. However, we could not detect a positive band in pea 7S globulin, although it was reported that a polypeptide with a molecular mass of 12500 Da is N-glycosylated (*30*). Because we applied sufficient amount of samples on SDS-PAGE, we believe that pea variety employed here does not contain N-glycosylated 7S globulin. On the other hand, we observed that the 7S globulins from both cowpea varieties were glycosylated, and such has not been reported before for cowpea 7S globulin. These differences maybe depend on the difference in their varieties.

Thermal Stability. Partial denaturation of the native protein molecule is a prerequisite to the subsequent association of denatured molecules and the formation of a network structure (*2, 32*). Therefore, the thermal stability of proteins is an important factor for their heat-induced association and gelation. DSC profiles of the 7S and 11S globulin samples are shown in **Figures 3** and **4**, respectively.

The thermal denaturation midpoint temperatures (T_m) of the peaks of the 7S globulins of black cowpea, red cowpea, French bean, fava bean, pea, and soybean were 86.1, 85.7, 88.3, 83.8, 82.7, and 78.5 °C, respectively, at $\mu = 0.5$ (**Figure 3**). It is to be noted that the 7S globulins from red cowpea, fava bean, and pea exhibited a broad peak, although those from black cowpea, French bean, and soybean exhibited a comparatively sharp peak. At $\mu = 0.08$, the T_m values of the 7S globulins from black cowpea, red cowpea, French bean, fava bean, pea, and soybean decreased to 76.8, 75.4, 80.8, 76.5, 71.7, and 65.7 °C, respectively. The extent of the decrease in T_m values from $\mu = 0.5$ to 0.08 was higher in the case of cowpea, pea, and soybean than in the others, indicating that subunit interactions in the 7S globulins from cowpea, pea, and soybean are sensitive to ionic strength. This suggests that forces, which are sensitive to ionic strength, for example, hydrophobic interactions, play a more important role in the maintenance of the structures of cowpea, pea, and soybean 7S globulins than those of French bean and fava bean 7S globulins.

The 7S globulins from French bean and soybean exhibited the highest and the lowest T_m values among those from the five crops. Therefore, French bean and soybean 7S globulins are suitable for the production of foods requiring high and low thermal stabilities, respectively.

The T_m values of the peaks of the 11S globulins of fava bean, pea, and soybeans were 95.4, 95.2, and 93.5 °C at $\mu = 0.5$ and 85.0, 80.8, and 81.2 °C at $\mu = 0.08$, respectively (**Figure 4**). The peak area of soybean 11S globulin was greater at $\mu = 0.5$ than at $\mu = 0.08$. These suggest that hydrophobic interaction is more important for the maintenance of the structure of soybean 11S globulin than those of fava bean and pea.

Surface Hydrophobicity. Surface hydrophobicity of proteins is related to some of their physicochemical properties such as emulsifying and foaming abilities and solubility (*33, 34*). Hayakawa and Nakai suggested that aromatic hydrophobicity may play a more important role in protein solubility than the aliphatic hydrophobicity (*35*). Therefore, we employed two columns of phenyl and butyl sepharose (**Table 1**). With this analysis, the longer the elution time, the higher the surface hydrophobicity of the sample.

Because the 7S globulin from fava bean eluted as a very broad peak on both columns (**Table 1**), we adopted the mid values in the case of fava bean 7S globulin. All 7S and 11S globulins gave similar trends on both columns to each globulin except red and black cowpea 7S globulins. The order of the surface hydrophobicity is as follows: for 7S globulins, cowpea ≥ soybean ≥ fava bean > French bean > pea, and for 11S globulins, fava bean ≥ soybean > pea. Interestingly, the red and black cowpea 7S globulins eluted from the butyl sepharose column with a time similar to those of soybean and fava bean 7S globulins but much more slowly on phenyl sepharose column as compared to the others. This means that the aromatic hydrophobicity of cowpea 7S globulins is quite high. On the other hand, both 7S and 11S globulins from pea exhibited the lowest surface hydrophobicity among crops. These indicate that pea globulins and cowpea 7S globulins are suitable for foods requiring low and high surface hydrophobicities, respectively.

Solubility as a Function of pH. The solubilities of proteins are the most important factor for their physicochemical properties such as gelation, emulsification, and foaming (*35, 36*). The solubilities of 7S and 11S globulins as a function of pH are shown in **Figures 5** and **7**, respectively.

Although red and black cowpea and French bean 7S globulins were soluble at any pH examined at $\mu = 0.5$ similar with that of soybean 7S globulin, those from fava bean and pea exhibited lower solubility at a pH lower than 3. At $\mu = 0.08$, 7S globulins from cowpea, fava bean, pea, and soybean exhibited isoelectric precipitation, although the pH range where they were insoluble was variable. The insoluble pH range was wider with the 7S globulins from fava bean, pea, and red and black cowpea than from soybean. The 7S globulin of soybean is N-glycosylated and two (α and α′) of three (α, α′, and β) kinds of subunits contain extension regions that are quite rich in acidic amino acid residues. We reported that the carbohydrate moieties and the extension regions highly contribute to the solubility at neutral and weak alkaline pH at $\mu = 0.08$ (*8*). However, the 7S globulins from fava bean and pea do not contain both, and red and black cowpea 7S globulins do not contain the latter. This is probably the reason why the soybean 7S globulin exhibits a narrower insoluble pH range. Most importantly, the French bean 7S globulin was completely soluble at any pH examined even at $\mu = 0.08$. Although the French bean 7S globulin does not contain the extension region, it is N-glycosylated at one or two positions. On the other hand, soybean 7S globulin is also glycosylated at one (β) or two (α and α′) positions. Although one of the two positions is equivalent in French bean and soybean 7S globulins

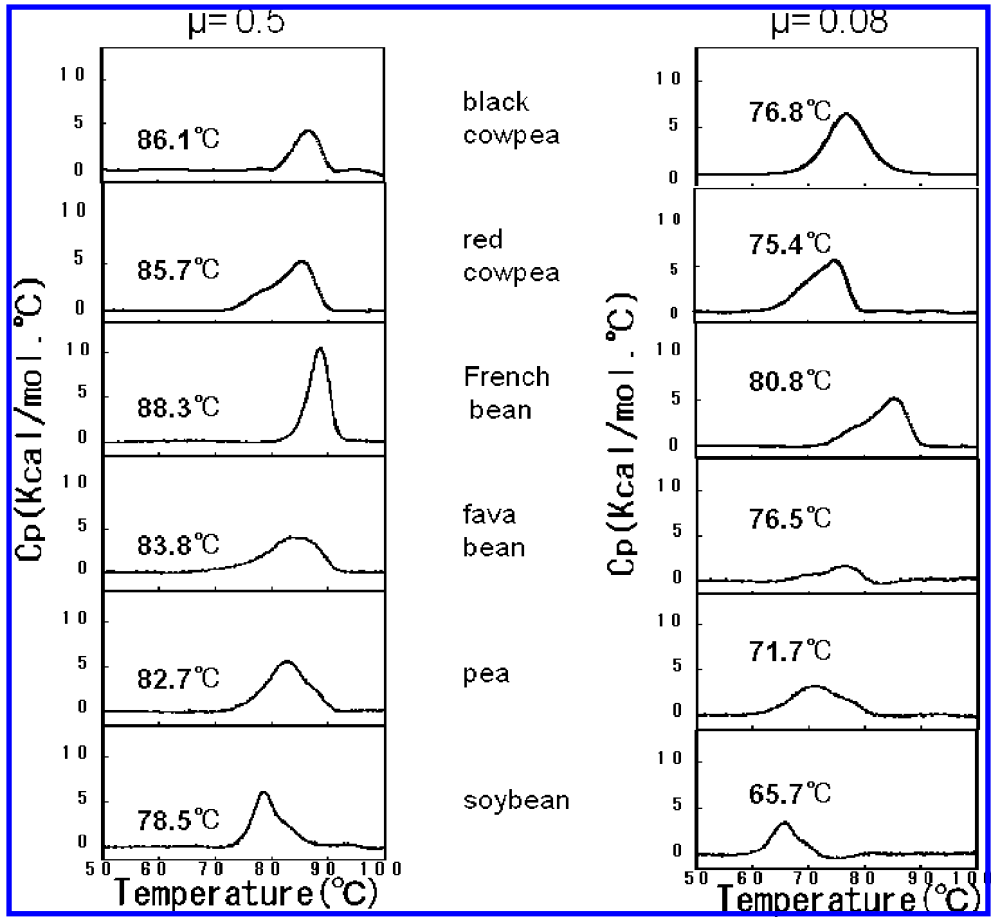

Figure 3. DSC scans of the purified 7S globulin samples at ionic strengths of 0.5 and 0.08.

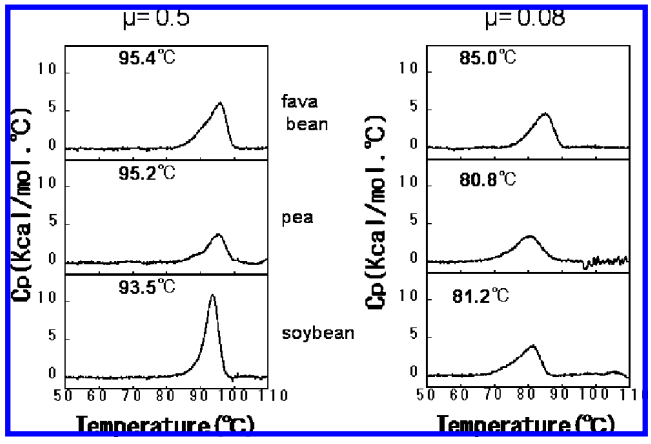

Figure 4. DSC scans of the purified 11S globulin samples at ionic strengths of 0.5 and 0.08.

(**Figure 6**, red), the other is completely different (**Figure 6**, green), resulting in the close positions of the two carbohydrate moieties in French bean 7S globulin. It is possible that this difference in the N-glycosylation site explains the higher solutibility of the French bean 7S globulin than the other 7S globulins. To elucidate this point, we will compare the solubility of the native French bean 7S globulin with that of its recombinant form, which is not N-glycosylated.

At $\mu = 0.5$, all 11S globulins exhibited a lower solubility at a lower pH to different extents (**Figure 7**). In particular, the 11S globulins from fava bean and pea exhibited low solubilities below pH 5 and pH 6, respectively, and again were fairly soluble near pH 2. These profiles were basically similar to each other. On the other hand, soybean 11S globulin became gradually insoluble with a lowering of pH. As shown previously (*11*), soybean 11S globulin composed of only group I subunits (group I-11S globulin) exhibits a solubility profile close to that of the normal 11S globulin composed of both groups I and II, and the solubility profile of 11S globulin composed of only group II subunits (group II-11S globulin) is similar to those of 11S globulins from fava bean and pea, especially fava bean, although soybean group II-11S globulin is not very soluble near pH 2 (*11*). Group I-11S globulin contains more acidic amino acids than group I-11S globulin. Therefore, the difference in the numbers of acidic amino acids is probably the reason why these 11S globulins exhibit different solubility profiles at $\mu = 0.5$.

At $\mu = 0.08$, all of the 11S globulins exhibited isoelectric precipitation at weak acidic pH. As compared with soybean 11S globulin, the profiles of 11S globulins from pea and fava bean shifted a little bit to basic and acidic pH values, respectively. The profiles of group I- and group II-11S globulins reside at basic and acidic sides of that of 11S globulin containing both group subunits, respectively (*37*). Therefore, the solubility profile of pea 11S globulin was similar to that of group I-11S globulin. Thus, the similarity of pea 11S globulins to that of soybean 11S molecular species was different between $\mu = 0.08$ and 0.5.

These results suggest that the distribution of hydrophilic and hydrophobic amino acid residues on the molecular surface as well as amino acid compositions are important determinants of solubility pH dependence. This will be further elucidated at the determination of their three-dimensional structures.

Emulsifying Ability and Stability. The emulsification of protein is one of the most important physicochemical properties for food processing. We assessed the emulsifying abilities of protein samples by measuring the sizes of their emulsions

Table 1. Elution Time of 7S and 11S Globulins on a Hydrophobic Column

	elution time (min)								
	7S						11S		
hydrophobic column	soybean	pea	fava bean	French bean	red cowpea	black cowpea	soybean	pea	fava bean
butyl sepharose	42.3	33.1	37.4−44.7	36.5	41.9	42.0	43.4	37.2	44.0
phenyl sepharose	63.5	50.8	56.6−61.0	52.0	71.2	69.8	60.9	56.0	64.0

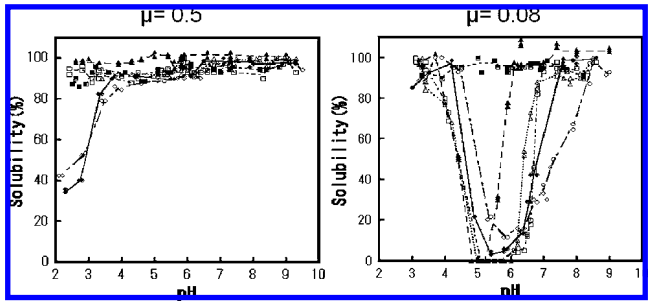

Figure 5. Dependency of the solubility of 7S globulins on pH at ionic strengths of 0.5 and 0.08. Symbols: Δ, red cowpea; □, black cowpea; ■, French bean; ○, fava bean; ●, pea; and ▼, soybean.

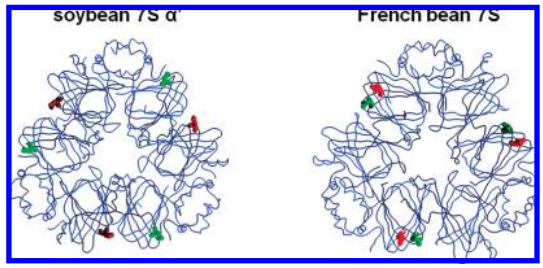

Figure 6. Comparison of the N-glycosylation sites of soybean 7S globulin α′ and those of French bean 7S globulin. This figure was prepared by MOLSCRIPT and Raster3D. Carbohydrate moieties are colored by red and green.

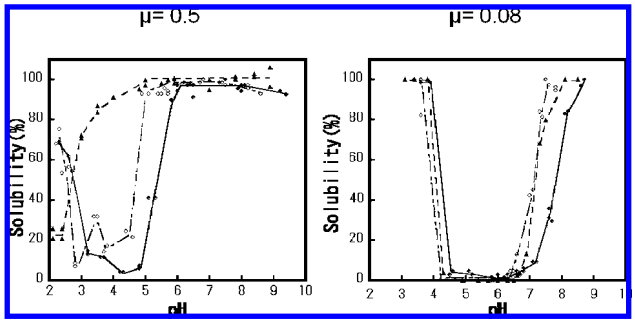

Figure 7. Dependency of the solubility of 11S globulins on pH at ionic strengths 0.5 and 0.08. Symbols: ○, fava bean; ●, pea; and ▼, soybean.

(**Table 2**); the smaller the sizes are, the better the emulsifying abilities. We prepared emulsions at two ionic strengths (μ = 0.08 and 0.5) and two protein concentrations (0.5 and 1.0 mg/mL).

In the case of the 7S globulins, those from soybean and French bean gave smaller average particle sizes than those from pea, fava bean, and red and black cowpea (**Table 2**). It is noteworthy that the French bean 7S globulin gave very small average particle sizes at μ = 0.08. The 7S globulins from soybean, French bean, and red and black cowpea are N-glycosylated, but those from pea and fava bean are not. Previously, we demonstrated that the extension regions of the α- and α′-subunits contribute very much to emulsifying ability,

Table 2. Emulsifying Activity of the Protein Samples at High (μ = 0.5) and Low (μ = 0.08) Ionic Strengths Conditions As Indicated by Mean Droplet Diameter

		mean droplet diameter (μm)			
		7S		11S	
sample	mg/mL	μ = 0.5	μ = 0.08	μ = 0.5	μ = 0.08
soybean	0.5	2.68/0.5[a]	2.32/0.09	3.97/0.2	4.54/0.3
	1.0	2.67/1.2	2.56/0.02	4.78/0.06	12.32/0.07
pea	0.5	6.74/0.8	15.21/0.04	4.24/0.2	4.70/0.6
	1.0	15.25/0.7	23.56/0.1	3.57/0.2	5.39/2.0
fava bean	0.5	9.21/0.6	26.28/1.0	2.40/0.1	2.41/0.3
	1.0	9.89/1.0	20.41/0.9	4.89/0.4	2.80/0.03
French bean	0.5	2.65/0.02	1.85/0.02		
	1.0	2.59/0.05	1.55/0.05		
red cowpea	0.5	7.65/0.06	20.5/1.6		
	1.0	[b]	14.5/0.2		
black cowpea	0.5	7.1/2.0	20.6/0.7		
	1.0	[b]	15.0/0.9		

[a] Standard error. [b] These samples were not measured, as they separated to two phases just after the emulsification.

whereas the carbohydrate moieties of the α- and α′-subunits do not contribute to emulsifying ability at all, but that of the β-subunit having no extension region is likely to do so (*6*). These suggest that a carbohydrate moiety contributes to emulsifying ability in the case of 7S globulin without an extension region. However, this is not the case for red and black cowpea 7S globulins and also not for mung bean 7S globulin (*20*). Thus, the effect of the carbohydrate moiety is not so simple.

In the case of 11S globulins, there was not a big difference in the average particle sizes among 11S globulin samples, although fava bean 11S globulin gave slightly smaller sizes of emulsions. However, the emulsion sizes of fava bean 11S globulin were larger than those of French bean 7S globulin emulsions. These suggest that none of these 11S globulins have a specific structural feature suitable for emulsification.

Furthermore, we examined the emulsion stability of the 7S and 11S globulins. We sealed and kept the test tubes containing the emulsion without agitation at room temperature, and the stability of the emulsions was observed visually. The emulsions of all samples (pea 11S shown in **Figure 8A**) completely separated into water and cream phases after 20 h except for French bean 7S globulin. Most of them significantly separated even after only 1 h (data not shown). However, the emulsion of French bean 7S globulin was much more stable; at μ = 0.08, a little phase separation was observed after 3 days, and its water phase was still turbid even after 15 days, and at μ = 0.5, phase separation was observed after 20 h, but its water phase was still fairly turbid after 20 days (**Figure 8B**). These results indicate that French bean 7S globulin has an excellent ability to form stable emulsion.

Even the molecular species composed of only the α- or α′-subunit of soybean 7S globulin could not form the emulsion with good stability (data not shown), although they have an extension region and two glycans. This indicates that the extension region and carbohydrate moieties in the α- and α′-

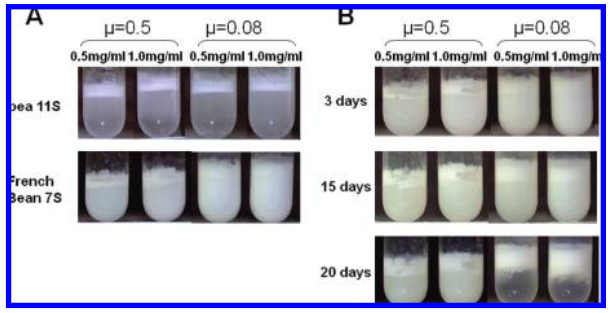

Figure 8. Emulsion stability. (**A**) Emulsions formed using pea 11S globulin and French bean 7S globulin as an emulsifier after 20 h. (**B**) Time dependence of emulsion stability of French bean 7S globulin at room temperature.

subunits do not contribute to emulsion stability. Interestingly, replacement of the extension region of the α′-subunit from its N terminus to the C terminus resulted in improvement of the emulsion stability (*38*). This suggests that the position of the extension region (hydrophilic cluster) on the molecular surface is an important factor for emulsifying stability. As described in the previous section, the positions of the carbohydrate moieties are different between French bean and soybean 7S globulins. There is a possibility that this difference in the positions of the carbohydrate moieties is one of the reasons why French bean 7S globulin can form much more stable emulsions than does soybean 7S globulin. This will be further confirmed with studies on recombinant French bean 7S globulin without the carbohydrate moieties.

Conclusion. On the basis of the assumption that diversity of the amino acid sequences of 7S and 11S globulins from many legume and nonlegume seeds suggests the presence of some globulins with excellent physicochemical properties, we compared such properties of 7S and 11S globulins from some crops. We found that French bean 7S globulin exhibits excellent solubility and emulsion stability at an ionic strength of 0.08. This indicates that our assumption is correct, and there is a possibility that other globulins exhibit better properties than French bean 7S globulin.

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