Structure–Physicochemical Function Relationships of Soybean Glycinin at Subunit Levels Assessed by Using Mutant Lines

NOBUYUKI MARUYAMA,[†] KRISNA PRAK,[†] SHIORI MOTOYAMA,[†]
SEON-KANG CHOI,[†,‡] KAZUHIRO YAGASAKI,[§] MASAO ISHIMOTO,[||] AND
SHIGERU UTSUMI*,[†]

Laboratory of Food Quality Design and Development, Graduate School of Agriculture,
Kyoto University, Uji, Kyoto 611-0011, Japan, Marine Biotechnology Institute, Kamaishi, Iwate
026-0001, Japan, Nagano Chusin Agricultural Experiment Station, Shiojiri, Nagano 399-6461, Japan,
and National Agricultural Research Center for Hokkaido Region, Toyohira-ku, Sapporo, Hokkaido
062-8555, Japan

Glycinin is a hexameric protein composed of five kinds of subunits. The subunits are classified into two groups, group I (A1aB1b, A1bB2, and A2B1a) and group II (A3B4 and A5A4B3). We purified four mutant glycinins composed of only group I subunits (group I-glycinin), only group II subunits (group II-glycinin), only A3B4 (A3B4-glycinin), and only A5A4B3 (A5A4B3-glycinin) from mutant soybean lines. The physicochemical properties of these glycinin samples were compared with those of the normal glycinin (11S) composed of five kinds of subunits. The thermal stabilities (as measured by thermal denaturation midpoint temperatures) of 11S, group I-glycinin, and group II-glycinin were similar to each other, although that of A3B4-glycinin was significantly lower than those of the others. The orders of aromatic and aliphatic surface hydrophobicities were the same: A3B4-glycinin > group II-glycinin > A5A4B3-glycinin > 11S > group I-glycinin. The solubility of 11S as a function of pH at $\mu = 0.5$ was governed by that of group I-glycinin and followed this order at acidic pH: 11S = group I-glycinin > A3B4-glycinin > group II-glycinin = A5A4B3-glycinin. The order of emulsifying abilities was A5A4B3-glycinin > group II-glycinin > A3B4-glycinin > 11S > group I-glycinin. This order was consistent with that of the length of their hypervariable regions. Except for this relationship, there was no significant relationship among the other physicochemical properties of the mutant glycinins.

INTRODUCTION

Soybean proteins are known to lower cholesterol levels in human serum (*1*) and have a high nutritional value as well (*2, 3*). Therefore, expansion of the usage of soybean proteins is desired to address the current problems in the increase of heart disease and hypertension caused by high cholesterol levels prevalent in industrialized countries and the problem of food shortages in developing countries. Toward attaining these objectives, it is imperative to fully elucidate the structure– physicochemical function relationships of soybean proteins.

Soybean proteins are composed of two major components, β-conglycinin and glycinin. β-Conglycinin is a trimeric protein composed of three kinds of subunits, α ($\sim$67 kDa), α' ($\sim$71 kDa), and β ($\sim$50 kDa), and glycinin is a hexameric protein composed of five kinds of subunits, A1aB1b, A1bB2, A2B1a, A3B4, and A5A4B3 (*2, 3*). Structure–physicochemical function

relationships of β-conglycinin at the subunit level have been well-investigated by using recombinant β-conglycinins composed of only one kind of subunit (*4*) and mutant soybean lines containing β-conglycinin lacking the α or α' subunit (*5–7*). However, such investigations on glycinin subunits have so far been limited to gel forming ability (*8–11*) and none on other properties.

Constituent subunits of glycinin are classified into two groups: group I (A1aB1b, A1bB2, and A2B1a) and group II (A3B4 and A5A4B3). The sequence identity of each subunit is around 80 and 40% within the group and between the groups, respectively (*1, 2*). Many molecular species that have different subunit compositions with random combinations are present in soybean seeds (*2*). Therefore, from normal soybean cultivars, it is very difficult to isolate large quantities of glycinin molecular species of restricted subunit compositions, which are necessary for the investigation of structure–physicochemical function relationships of glycinin at the subunit level. Zhang et al. (*12*) succeeded in the isolation of glycinin molecular species composed of only group I subunits using a soybean cultivar lacking A5A4B3. Nakamura et al. (*9, 10*) prepared artificial glycinins with a homogeneous subunit composition by rena-

* To whom correspondence should be addressed. E-mail: sutsumi@ kais.kyoto-u.ac.jp.
† Kyoto University.
‡ Marine Biotechnology Institute.
§ Nagano Chusin Agricultural Experiment Station.
|| National Agricultural Research Center for Hokkaido Region.

8198 *J. Agric. Food Chem.,* Vol. 52, No. 26, 2004

Maruyama et al.

turation from isolated subunits (*10*) or polypeptides (*9*). However, these methods are tedious and laborious. Recently, mutant soybean lines containing glycinin composed of only group I, only group II, only A3B4, or only A5A4B3 were developed (*13, 14*). In this study, we purified such glycinins from these mutant soybean lines and compared their physico-chemical properties with those of glycinin containing all five kinds of subunits.

MATERIALS AND METHODS

Purification of Glycinins with Various Subunit Compositions. Glycinins composed of all subunits (11S), only group I subunits (group I-glycinin), only group II subunits (group II-glycinin), only A3B4 (A3B4-glycinin), and only A5A4B3 (A5A4B3-glycinin) were partially purified from corresponding normal and mutant soybean lines (*13, 14*) by the procedure of Nagano et al. (*15*). The partially purified fractions containing glycinins were subjected to ammonium sulfate fraction-ation: 11S, 50−65% saturation; group I-glycinin, 50−75%; group II-glycinin, 50−70%; A3B4-glycinin, 50−65%; and A5A4B3-glycinin, 50−70%. Their purities were assessed by sodium dodecyl sulfate (SDS)−polyacrylamide gel electrophoresis (PAGE) (*16*).

Protein Measurement. The protein concentration of the samples was determined using a Protein Assay Rapid Kit (Wako, Osaka, Japan) based on dye binding with bovine serum albumin as the standard.

Thermal Stability. The thermal stability of the glycinin samples in buffer A [35 mM sodium phosphate, pH 7.6, 0.4 M NaCl, 10 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, 0.1 mM (*p*-amidinophenyl) methanesulfonyl fluoride, and 0.02% NaN₃] was analyzed using a Microcal MC-2 ultrasensitive microcalorimeter (Micro Cal Inc., Northampton, MA) as described previously (*17*). All analyses were performed with a protein concentration of 0.5 mg/mL.

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

Solubility as a Function of pH. Solubilities of the glycinin samples were measured as described previously (*4*). The protein solutions (0.8 mg/mL) were kept at 4 °C for 18 h at various pH values at $\mu = 0.5$ after which the protein solutions were centrifuged, and the protein concentrations in the supernatant were determined as described in the section on protein measurement. The solubility was expressed as a percentage of the total protein content in the sample.

Emusifying Ability. The emulsifying abilities of the glycinin samples were measured as described previously (*4*). To prepare emulsions, 0.25 mL of soybean oil and 1.5 mL of the glycinin samples in buffer A (0.5 mg/mL) were homogenized for 30 s with a high-speed homogenizer (model NS-50, Nichion Irikakikai Ltd.) and further sonicated using an ultrasonic homogenizer (model US-150, Nihonseiki Kaisha Ltd.). The particle size distribution of the emulsions was measured using a laser diffraction instrument (model LA500, Horiba Seisakusho Ltd.). Each sample was measured several times, and a representative typical pattern was presented.

RESULTS AND DISCUSSION

Isolation of 11S, and Groups I- and II- and A3B4- and A5A4B3-Glycinins. Glycinins composed of all five subunits (11S), only group I subunits (group I-glycinin), only group II (group II-glycinin), only A3B4 (A3B4-glycinin), and only A5A4B3 (A5A4B3-glycinin) were partially purified by the method of Nagano et al. (*15*) and further purified by ammonium sulfate fractionation. The isolated and purified glycinins had purities of around 90% as evaluated by SDS−PAGE (**Figure 1**) and were used for the following analyses.
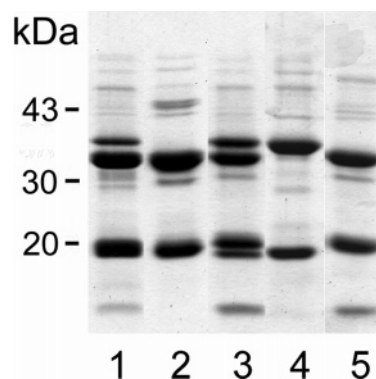
Figure 1. SDS−PAGE analysis of the purified glycinin samples. The purified glycinin samples were analyzed by means of SDS−PAGE using 11% gels. Lane 1, 11S; lane 2, group I-glycinin; lane 3, group II-glycinin; lane 4, A3B4-glycinin; and lane 5, A5A4B3-glycinin. The numbers on the left denote molecular masses.
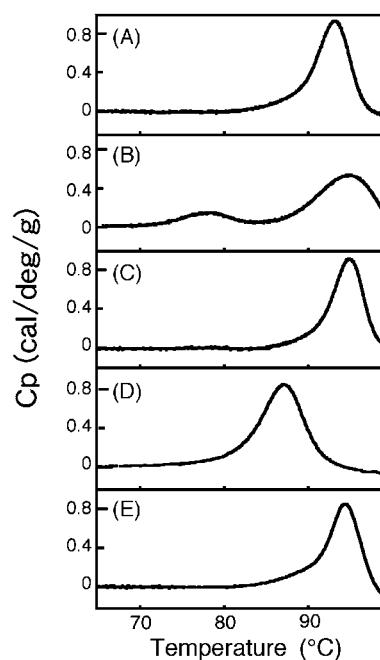
Figure 2. DSC scans of glycinin samples. (**A**) 11S, (**B**) group I-glycinin, (**C**) group II-glycinin, (**D**) A3B4-glycinin, and (**E**) A5A4B3-glycinin.

Thermal Stability. The thermal stability of proteins is an important factor for their heat-induced association and gelation. Partial denaturation of the native protein molecule is a prereq-uisite to the subsequent association of denatured molecules and the formation of the network structure (*3, 18*). Differential scanning calorimetry (DSC) profiles of the glycinin samples are shown in **Figure 2**.

The thermal denaturation midpoint temperature (T_m) of the peaks of the glycinin samples ranged from 77 to 95 °C. 11S containing all five subunits gave a sharp peak with a T_m of 92.9 °C. The T_m of A3B4 was 87.2 °C, which was the lowest among all glycinin species. Even 11S and group II-glycinin, which contain A3B4, exhibited higher T_m values than did A3B4-glycinin. Previously, we demonstrated that the order of T_m values of β-conglycinin subunits, α, α', and β, is $\alpha < \alpha' < \beta$, and T_m values of β-conglycinin composed of two or three kinds of subunits were basically conferred by the subunit that had the lowest T_m value (*5, 6*). Therefore, this is not the case with glycinin.

Stucture–Physicochemical Relationships of Soybean Glycinin

J. Agric. Food Chem., Vol. 52, No. 26, 2004 **8199**

Table 1. Elution Times of Glycinin Samples on Hydrophobic Chromatography

| columns	elution times (min)				
	group I-glycinin	11S	A5A4B3-glycinin	group II-glycinin	A3B4-glycinin
butyl sepharose	39.9[a]/0.2[b]	43.4/0.3	43.9/0.3	48.3/0.2	52.5/0.2
phenyl sepharose	57.7/0.3	60.9/0.3	61.2/0.2	66.6/0.2	71.7/0.3

[a] The value represents an average for three experiments. [b] Standard error.

Group I-glycinin gave two broad peaks (**Figure 2B**). Their T_m values were 77.0 and 95.0 °C. Our group developed transgenic tobacco (*19*) and rice (*20*) accumulating glycinin A1aB1b and demonstrated that the expressed A1aB1b can be processed and can assemble into hexamers. However, the assembly efficiency was less than 50% and most remained as trimers. Recently, our group further demonstrated using an *Escherichia coli* expression system that A1aB1b had a higher folding ability than A3B4 and assisted in the folding of A3B4 and its modified versions, which had a very low folding ability (*21*). Moreover, collaboration between Takaiwa's and Utsumi's groups revealed that A3B4 assists in the incorporation of A1aB1b into hexamers (this will be described elsewhere). These facts suggest that the peaks with T_m values of 95.0 and 77.0 °C originated from a hexamer and a trimer, respectively. This suggestion is consistent with the observation by Utsumi et al. (*22*) that a glycinin trimer (a half molecule of a hexamer) exhibits a much lower thermal stability than a glycinin hexamer. The fact that the 11S containing both groups I and II subunits exhibit one sharp peak (**Figure 2A**) supports the above suggestion. On the other hand, both peaks that group I-glycinin gave (**Figure 2B**) were broader than those of the other glycinin samples (**Figure 2A,C−E**), suggesting that group II subunits contribute to the structural rigidity of glycinin hexamers.

Tezuka et al. reported that the T_m values of group I-, A3B4-, and A5A4B3-glycinins are 95, 97.9, and 96 °C, respectively (*23*). These are not in agreement with our results probably because of the different measuring conditions used in the analysis of T_m values.

Surface Hydrophobicity. Surface hydrophobicity of proteins is related to some of their physicochemical properties such as emulsifying and forming abilities and solubility (*24, 25*). Kato and Nakai (*26*) described that *cis*-parinaric acid (CPA) is more suitable for the measurement of surface hydrophobicity than 1-anilino-8-naphthalenesulfonate (ANS). Hayakawa and Nakai (*27*) reported that hydrophobicity measured by phenyl sepharose column chromatography and ANS correlated well to the protein insolubility, whereas no significant correlation was observed between that by CPA and insolubility. They suggested that the aromatic hydrophobicity may play a more important role in protein solubility than the aliphatic hydrophobicity. Therefore, we employed two columns of phenyl and butyl sepharose (**Table 1**). With this analysis, the longer the elution time is, the higher the surface hydrophobicity will be of the sample.

Both columns gave similar trends, with A3B4-glycinin having the highest hydrophobicity, and the order was A3B4-glycinin > groupII-glycinin > A5A4B3-glycinin > 11S > group I-glycinin, indicating that there should not be any significant difference in aromatic and aliphatic hydrophobicities among glycinin samples. The proportions of groups I and II subunits in 11S and that of A3B4 and A5A4B3 in group II-glycinin were around 3:2 and 1:1, respectively. This indicates that the surface hydrophobicity of a heterohexamer is consistent with the arithmetic mean of those of its constituent subunits.
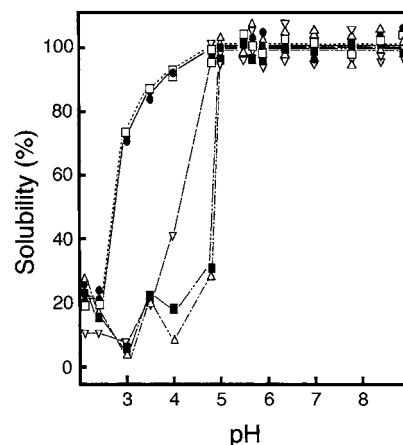

Figure 3. Dependency of the solubility of glycinin samples on pH at ionic strength 0.5. 11S, group I-glycinin, group II-glycinin, A3B4-glycinin, and A5A4B3-glycinin are shown by a solid line with closed circles, a dotted line with open squares, a dashed and double-dotted line with closed squares, a dashed line with inverted triangles, and a dashed and single-dotted line with open triangles, respectively.

Previously, we measured the surface hydrophobicities of the homotrimers of the constituent subunits α, α′, and β by the same procedure (*6*) and reported that the order of the surface hydrophobicity is α > α′ > β. From the comparison of the data obtained here and previously, the surface hydrophobicity of β is lower than that of group I-glycinin and that of α is intermediate between those of A3B4 and group II-glycinins. Recently, Tezuka et al. measured the surface hydrophobicities of group I-glycinin, A3B4-glycinin, A5A4B3-glycinin, and β-conglycinin using ANS and reported that A5A4B3-glycinin > A3B4-glycinin > group I-glycinin > β-conglycinin (*23*). The discrepancies in the results between Tezuka's and ours are probably due to the difference in the measuring method used by the two laboratories.

Solubility as a Function of pH. The solubility of proteins is the most important factor for their physicochemical properties (*27, 28*). Because we previously measured the solubilities of glycinin samples at $\mu = 0.08$ (*29*), we measured them at a higher μ of 0.5 (**Figure 3**). All glycinin samples exhibited a lower solubility at a lower pH with different extents. In particular, group II and A5A4B3-glycinins exhibited low solubilities below pH 5 and their profiles were very similar to each other. A3B4-glycinin was completely soluble at pH 4.8, but 40% soluble at pH 4.0, and less than 20% below pH 3.5. 11S and group I-glycinin became gradually insoluble with a lowering of pH, and their profiles were about the same. These results indicate that at $\mu = 0.5$, the solubilities of 11S and group II-glycinin are governed by those of group I subunits and A5A4B3, respectively. The acidic amino acid contents of group I subunits, A3B4, and A5A4B3 are 56 (average), 66, and 85%, respectively. Therefore, the higher the content of the acidic amino acids, the lower the solubility of the glycinin samples at low pH.

Hayakawa and Nakai (*27*) reported that the aromatic hydrophobicity of proteins correlates with their insolubility. However, our results indicate that there is no such relationship between them. One possible reason is that the conditions for measurement of solubility are different. They measured the solubility of proteins in 10 mM phosphate buffer (pH7.0). This condition is closer to our low ionic strength condition ($\mu = 0.08$) than our high ionic strength condition. Under $\mu = 0.08$ and pH 7.0, the order of solubility was reported to be A5A4B3-glycinin = group II-glycinin > A3B4-glycinin = 11S > group I-glycinin (*29*).

8200 *J. Agric. Food Chem.,* Vol. 52, No. 26, 2004

Maruyama et al.
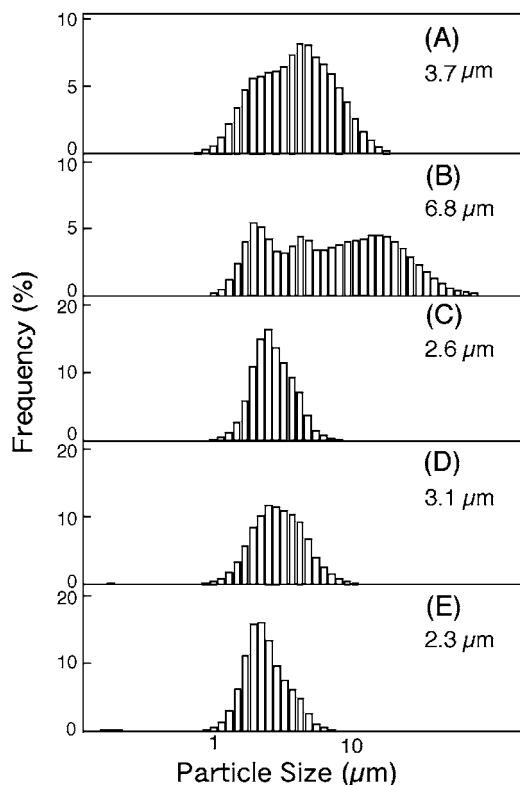
Figure 4. Particle size distributions of emulsions from glycinin samples: (**A**) 11S, (**B**) group I-glycinin, (**C**) group II-glycinin, (**D**) A3B4-glycinin, and (**E**) A5A4B3-glycinin.

There is no correlation between this order and the order of surface hydrophobicity that we obtained, indicating that the suggestion by Hayakawa and Nakai (*27*) is not applicable to glycinins.

All examined molecular species with different subunit compositions of β-conglycinin are completely soluble at pH 3−9 and μ = 0.5 (*4−6*), although all examined glycinin samples exhibited a lower solubility at pH < 5. Glycinin and β-conglycinin are derived from a common ancestor and share a common three-dimensional structure (*30−33*). Therefore, differences in the distribution of hydrophobic and charged residues on molecular surfaces may be related to the solubilities of these glycinins.

Emulsifying Ability. The emulsifying ability of proteins is one of the most important physicochemical properties for food processing. We assessed the emulsifying abilities of glycinin samples by measuring the sizes of their emulsions (**Figure 4**). 11S and group I-glycinin gave broad peaks with average particle sizes of 3.7 and 6.8 μm, respectively. Group II-, A3B4-, and A5A4B3-glycinins gave sharp peaks with average particle sizes of 2.6, 3.1, and 2.3 μm, respectively. The emulsifying ability of group II-glycinin was better than that of group I-glycinin, and that of 11S was intermediate between those of groups I- and II-glycinins. Similarly, the emulsifying ability of A5A4B3-glycinin was better than that of A3B4-glycinin, and that of group II-glycinin was intermediate between those of A5A4B3- and A3B4-glycinins. These results suggest that the emulsifying ability of a heterohexamer corresponds to the arithmetic mean of the emulsifying ability values of its constituent subunits.

Group I-glycinin composed of three different subunits gave very broad peaks. This suggests that the three subunits exhibit a different emulsifying ability to each other. This is consistent with the observation that 11S-containing group I subunits also gave a broad peak. However, the broadness of the 11S peak

was much milder than that of the group I-glycinin peak. In analogy with the case of thermal stability, group II subunits are likely to contribute to the sharpness of 11S peak.

Suitable conformational change of proteins at the interface between oil and water and their balance of hydrophilicity and hydrophobicity are important factors for emulsifying ability (*34*). Similarly, correlations of surface properties including emulsifying activity with conformational stabilities (*35*) and surface hydrophobicities of proteins (*26*) are reported. Previously, we demonstrated that the structural stability as well as the extension region are important to the emulsifying abilities of β-conglycinin homotrimers (*4*). However, the results obtained from the analyses of glycinin samples indicate that there is no relationship between emulsifying ability and structural stability assessed by DSC (**Figure 2**) and surface hydrophobicity (**Table 1**) as measured by hydrophobic chromatography. This discrepancy may be due to the difference in the methods employed for the assessment, or such relationships may not be the same for glycinin.

Each subunit of glycinin has several disordered regions, which are not visible by X-ray crystallography (*32, 33*). The longest one of them is called a hypervariable region (*36*), which is rich in acidic amino acid residues. The lengths of the hypervariable regions are different among subunits, thus A5A4B3 (103) > A3B4 (70) > group I (A1aB1b, 42; A1bB2, 29; and A2B1a, 35). This order is consistent with that of emulsifying ability. Therefore, it seems that there is a relationship between emulsifying ability and the length of the hypervariable region of each subunit. This relationship is similar to the important role that the extension region plays in the emulsifying property of β-conglycinin (*4*).

Comparison of some physicochemical properties of glycinin samples that have restricted subunit compositions from mutant soybean lines with those of normal glycinin indicated that individual subunits have intrinsic properties and that glycinin composed of only group I subunits is suitable for foods requiring high solubility at acidic pH and high ionic strength and glycinin composed of only group II subunits for food requiring emulsifying ability and/or high solubility at neutral pH and low ionic strength.

LITERATURE CITED

(1) Kito, M.; Moriyama, T.; Kimura, Y.; Kambara, H. Changes in plasma lipid levels in young healthy volunteers by adding an extruder cooked soy protein to conventional meals. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 354−355.

(2) Utsumi, S. Plant food protein engineering. In *Advances in Food and Nutrition Research*; Kinsella, J. E., Ed.; Academic Press: SanDiego, CA, 1992; pp 89−208.

(3) Utsumi, S.; Matsumura, Y.; Mori, T. Structure−function relationships of soy proteins. In *Food Proteins and Their Applications*; Damodaran, S., Paraf, A., Eds.; Dekker: New York, 1997; pp 257−291.

(4) Maruyama, N.; Sato, R.; Wada, Y.; Matsumura, Y.; Goto, H.; Okuda, E.; Nakagawa, S.; Utsumi, S. Structure-physicochemical function relationships of soybean β-conglycinin constituent subunits. *J. Agric. Food Chem.* **1999**, *47*, 5278−5284.

(5) Maruyama, N.; Mohamad Ramlan, M. S.; Takahashi, K.; Yagasaki, K.; Goto, H.; Hontani, N.; Nakagawa, S.; Utsumi, S. The effect of the N-linked glycans on structural features and physicochemical functions of soybean β-conglycinin homotrimers. *J. Am. Oil Chem. Soc.* **2002**, *79*, 139−144.

Stucture–Physicochemical Relationships of Soybean Glycinin

J. Agric. Food Chem., Vol. 52, No. 26, 2004 **8201**

(6) Maruyama, N.; Mohamad Ramlan, M. S.; Takahashi, K.; Yagasaki, K.; Goto, H.; Hontani, N.; Nakagawa, S.; Utsumi, S. Structure-physicochemical function relationships of soybean β-conglycinin heterotrimers. *J. Agric. Food Chem.* 2002, *50*, 4323−4326.

(7) Mohamad Ramlan, M. S.; Maruyama, N.; Takahashi, K.; Yagasaki, K.; Higasa, T.; Matsumura, Y.; Utsumi, S. *Biosci., Biotechnol., Biochem.* 2004, *68*, 1091−1096.

(8) Mori, T.; Utsumi, S.; Nakamura, T. Formation of pseudoglycinins and their gel hardness. *J. Agric. Food Chem.* 1982, *30*, 828−831.

(9) Nakamura, T.; Utsumi, S.; Kitamura, K.; Harada, K.; Mori, T. Cultivar differences in gelling characteristics of soybean (*Glycine max*) glycinin. *J. Agric. Food Chem.* 1984, *32*, 647−651.

(10) Nakamura, T.; Utsumi, S.; Mori, T. Formation of pseudoglycinins from intermediary subunits of glycinin and their gel properties and network structure. *Agric. Biol. Chem.* 1985, *49*, 2733−2740.

(11) Lee, D.-S.; Matsumoto, S.; Hayashi, Y.; Matsumura, Y.; Mori, T. Differences in physical and structural properties of heat-induced gels from glycinins among soybean cultivars. *Food Sci. Technol. Res.* 2002, *8*, 360−366.

(12) Zhang, G.-Y.; Hayashi, Y.; Matsumoto, S.; Matsumura, Y.; Mori, T. Molecular species of glycinin in some soybean cultivars. *Phytochemistry* 2002, *60*, 675−681.

(13) Teraishi, M.; Takahashi, M.; Hajika, M.; Matsunaga, R.; Uematsu, Y.; Ishimoto, M. Suppression of soybean β-conglycinin genes by dominant gene, Scg-1. *Theor. Appl. Genet.* 2001, *103*, 1266−1272.

(14) Takahashi, M.; Uematsu, Y.; Kashiwaba, K.; Yagasaki, K.; Hajika, M.; Matsunaga, R.; Komatsu, K.; Ishimoto, M. Accumulation of high levels of free amino acids in soybean seeds through integration of mutations conferring seed protein deficiency. *Planta* 2003, *217*, 577−586.

(15) Nagano, T.; Hirotsuka, M.; Mori, H.; Kohyama, K.; Nishinari, K. Dynamic viscoelastic study on the gelation of 7S globulin from soybean. *J. Agric. Food Chem.* 1992, *40*, 941−944.

(16) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, *227*, 680−685.

(17) Maruyama, N.; Katsube, T.; Wada, Y.; Oh, M. H.; Barba de la Rosa, A. P.; Okuda, E.; Nakagawa, S.; Utsumi, S. The roles of the *N*-linked glycans and extension regions of soybean β-conglycinin in folding, assembly and structural features. *Eur. J. Biochem.* 1998, *258*, 854−862.

(18) Clark, A. H.; Lea-Tuffnell, C. D. Gelation of globular proteins. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Ledword, D. A., Eds.; Elsevier Applied Science: London, 1986; pp 203−272.

(19) Takaiwa, F.; Katsube, T.; Kitagawa, S.; Higasa, T.; Kito, M.; Utsumi, S. High level accumulation of soybean glycinin in vacuole-derived protein bodies in the endosperm tissue of transgenic tobacco seed. *Plant Sci.* 1995, *111*, 39−49.

(20) Katsube, T.; Kurisaka, N.; Ogawa, M.; Maruyama, N.; Ohtsuka, R.; Utsumi, S.; Takaiwa, F. Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiol.* 1999, *120*, 1063−1073.

(21) Choi, S. K.; Adachi, M.; Yoshikawa, M.; Maruyama, N.; Utsumi, S. Soybean glycinin A1aB1b subunit has a molecular chaperone-like function to assist the folding of the other subunit having low folding ability. *Biosci., Biotechnol., Biochem.* 2004, *68*, 1991−1994.

(22) Utsumi, S.; Nakamura, T.; Harada, H.; Mori, T. Occurrence of dissociable and undissociabel soybean glycinin. *Agric. Biol. Chem.* 1987, *51*, 2139−2144.

(23) Tezuka, M.; Yagasaki, K.; Ono, T. Changes in charactors of soybean glycinin groups I, IIa, and IIb caused by heating. *J. Agric. Food Chem.* 2004, *52*, 1693−1699.

(24) Nakai, S. Structure−function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. *J. Agric. Food Chem.* 1983, *31*, 676−683.

(25) Nakai, S.; Li-Chan, E. Hydrophobicity-functionality relationship of food proteins. *Hydrophobic Interactions in Food Systems*; CRC Press: Boca Raton, FL, 1988; pp 23−41.

(26) Kato, A.; Nakai, S. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta* 1980, *624*, 13−20.

(27) Hayakawa, S.; Nakai, S. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Food Sci.* 1985, *50*, 486-491.

(28) Kinsella, J. E. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* 1979, *56*, 242−258.

(29) Mori, T.; Maruyama, N.; Nishizawa, K.; Higasa, T.; Yagasaki, K.; Ishimoto, M.; Utsumi, S. The composition of newly synthesized proteins in the endoplamic reticulum determines the transport pathways of soybean seed storage proteins. *Plant J.* 2004, *40*, 238−249.

(30) Maruyama, N.; Adachi, M.; Takahashi, K.; Yagasaki, K.; Kohno, M.; Takenaka, Y.; Okuda, E.; Nakagawa, S.; Mikami, B.; Utsumi, S. Crystal structures of recombinant and native soybean β-conglycinin β homotrimers. *Eur. J. Biochem.* 2001, *268*, 3595−3604.

(31) Maruyama, Y.; Maruyama, N.; Mikami, B.; Utsumi, S. Structure of the core region of the soybean β-conglycinin α′ subunit. *Acta Crystallogr.* 2004, *D60*, 289−297.

(32) Adachi, M.; Takenaka, Y.; Gidamis, A. B.; Mikami, B.; Utsumi, S. Crystal structure of soybean proglycinin A1aB1b homotrimer. *J. Mol. Biol.* 2001, *305*, 291−305.

(33) Adachi, M.; Kanamori, J.; Masuda, T.; Yagasaki, K.; Kitamura, K.; Mikami, B.; Utsumi, S. Crystal structure of soybean 11S globulin: Glycinin A3B4 homohexamer. *Proc. Natl. Acad. Sci. U.S.A.* 2003, *100*, 7395−7400.

(34) Damodaran, S. Protein-stabilized forms and emulsions. In *Food Proteins and Their Applications*; Damodaran, S., Paraf, A., Eds.; Marcel Dekker: New York, 1997; pp 57−110.

(35) Kato, A.; Yutani, K. Correlation of surface properties with conformational stabilities of wild-type and six mutant tryptophan synthase α-subunits substituted at the same position. *Protein Eng.* 1988, *2*, 153−156.

(36) Argos, P.; Narayana, S. V. L.; Nielsen, N. C. Structural similarity between legumin and vicilin storage proteins from legumes. *EMBO J.* 1985, *4*, 1111−1117.