Structure−Physicochemical Function Relationships of Soybean β-Conglycinin Constituent Subunits

Nobuyuki Maruyama,† Ryohei Sato,† Yusuke Wada,† Yasuki Matsumura,† Hideyuki Goto,‡ Eiko Okuda,† Shuko Nakagawa,† and Shigeru Utsumi*,†

Research Institute for Food Science, Kyoto University, Uji, Kyoto 611-0011, Japan, and Department of Food Science, Ishikawa Agricultural College, 1-308 Suematsu, Nonoichi, Ishikawa 921-8836, Japan

β-Conglycinin, one of the dominant storage proteins of soybean, has a trimeric structure, being composed of three subunits α, α′, and β. The α and α′ subunits contain the extension regions in addition to the core regions common to all subunits, which are N-glycosylated. Physicochemical functions of recombinant nonglycosylated individual subunits and deletion mutants (α_c and α'_c) lacking the extension regions of the α and α′ subunits were examined at pH 7.6 and 3.7 at low ($\mu = 0.08$) and high ($\mu = 0.5$) ionic strengths. Although individual recombinant subunits exhibited different properties at all conditions, there were some consistencies. Surface hydrophobicities and thermal stabilities of the individual subunits were likely to be conferred by their core regions, and the carbohydrate moieties did not contribute to these properties at any conditions examined here. Solubility at $\mu = 0.08$, heat-induced association, and emulsifying ability remarkably depended on the extension regions and the carbohydrate moieties in addition to the structural features of the core regions. These findings indicate that various end products could be produced by the selection of soybean varieties containing β-conglycinin with different subunit compositions and suggest a direction for a principle of soybean breeding.

Keywords: *β-Conglycinin; structure−physicochemical function relationship; physicochemical functions; soybean; Glycine max L.*

INTRODUCTION

Soybean proteins have good nutritional and physicochemical functions among plant proteins. Thus, soybeans have been utilized for many kinds of traditional foods in East Asia. More than 80% of the annual production of soybean seeds is used for oil expression. The amount of the protein in the residues after oil expression is ∼35 million tonnes. Most of the residues are used as feed for domestic animals and fertilizer. It is known that soybean proteins have a physiological function that lowers cholesterol levels in human serum (Kito et al., 1993). Therefore, expansion of the usage of the residual proteins is desired to solve the current problems in the increase of heart disease and hypertension caused by high cholesterol levels and the future problem of food shortages. For this purpose we need to elucidate the structure−physicochemical function relationships of soybean proteins.

Soybean proteins are composed of two major components, β-conglycinin and glycinin, accounting for about 30 and 40% of the total seed proteins, respectively (Utsumi, 1992; Utsumi et al., 1997). Both are multisubunit proteins. Some structure−physicochemical function relationships of glycinin were investigated using various soybean cultivars containing glycinins with different subunit compositions (Nakamura et al., 1984) and by preparing artificial glycinins with a homogeneous subunit composition by renaturation from isolated subunits (Mori et al., 1982; Nakamura et al., 1985) at the subunit level and by means of protein engineering at the molecular level (Kim et al., 1990; Utsumi et al., 1993). However, such investigations have been scarcely carried out on β-conglycinin. One example is a study on the gelation of β-conglycinin suggesting that the contributions of individual constituent subunits were different (Nakamura et al., 1986). More detailed investigations are required for the purpose described above.

β-Conglycinin is a trimeric protein composed of three subunits α (∼67 kDa), α′ (∼71 kDa), and β (∼50 kDa). The amino acid sequences of these subunits were deduced from their nucleotide sequences (Doyle et al., 1986; Harada et al., 1989; Sebastiani et al., 1990; Maruyama et al., 1998). The α and α′ subunits are composed of the extension regions (α, 125 residues; α′, 141 residues) and the core regions (418 residues) (Maruyama et al., 1998). The β subunit consists of only the core region (416 residues). The core regions exhibit high absolute homologies among them (90.4, 76.2, and 75.5% between α and α′, between α and β, and between α′ and β, respectively) (Maruyama et al., 1998). The extension regions of the α and α′ subunits exhibit 57.3% sequence identity and a highly acidic property. Isoelectric points and hydrophobicities of the α and α′ subunits calculated from their amino acid sequences are lower than those of the β subunit (Katsube et al., 1998). Thermal stabilities of individual subunits are different (Maruyama et al., 1998). These findings suggest that individual subunits exhibit different physicochemical functions.

Although preparation of homogeneous subunits is necessary to investigate the structure−physicochemical

* Corresponding author (telephone 81-774-38-3760; fax 81-774-38-3761; e-mail utsumi@soya.food.kyoto-u.ac.jp).
† Kyoto University.
‡ Ishikawa Agricultural College.

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function relationships of β-conglycinin constituent subunits, it is very difficult to obtain a large amount of homogeneous trimers from soybean seeds because of molecular heterogeneity: the presence of many molecular species having different subunit compositions with almost random combinations (Thanh and Shibasaki, 1976, 1978). In a previous study, we constructed an *Escherichia coli* expression system for individual subunits and deletion mutants (α_c and α'_c) lacking the extension regions, which were found to adopt the correct conformation, and then investigated their structural features (Maruyama et al., 1998). Although β-conglycinin is a glycoprotein, recombinant proteins from an *E. coli* expression system are not glycosylated. Therefore, it was possible to elucidate roles of the extension regions and the carbohydrate moieties in structural features of β-conglycinin (Maruyama et al., 1998). In this study, we examined the physicochemical functions of recombinant normal and mutant subunits and compared them with those of the native β-conglycinin.

MATERIALS AND METHODS

Purification of Recombinant Normal and Mutant Subunits and Native β-Conglycinin. The recombinant normal and mutant subunits were synthesized in *E. coli* and purified from *E. coli* as described previously (Maruyama et al., 1998). The native β-conglycinin was purified from soybean seeds (*Glycine max* var. Shirotsurunoko and Suzuyutaka) according to the method of Nagano et al. (1992) [β homotrimers are absent because they have a tendency to form precipitates at pH 6.4 at low ionic strength, similar to glycinin, during the course of purification (Yamauchi et al., 1981), resulting in a coexistence in a glycinin-rich fraction].

Protein Measurement. Proteins in the samples were determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

Solubility as a Function of pH. The recombinant normal and mutant subunits and the native β-conglycinin were dissolved in a sodium phosphate buffer [10 mM sodium phosphate, pH 7.6, 0.5 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride, 0.02% NaN_3]. Ionic strength and pH were adjusted by adding 10−50 mM citrate buffer (for pH 3.2−6.5), 10−50 mM sodium phosphate buffer (for pH 6.2−7.8), or 10−50 mM ammonium buffer (for pH 7.2−8.9) containing 0−0.4 M NaCl. The protein solutions (0.8 mg/mL) were kept at 4 °C for 18 h. After centrifugation, protein concentrations in the supernatant were determined using the method of Bradford (1976). Solubility was expressed as a percentage of the total protein content in the sample.

Fluorescence Study. Fluorescence intensities arising from 8-anilino-1-naphthalenesulfonic acid (ANS) complexed with proteins were measured in a Hitachi F-3000 fluorescence spectrophotometer. Protein samples in the following were used for the measurement: (a) 35 mM sodium phosphate, pH 7.6, 0.4 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride, 0.02% NaN_3 for pH 7.6 and $\mu = 0.5$; (b) 10 mM sodium phosphate, pH 7.6, 0.05 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride, 0.02% NaN_3 for pH 7.6 and $\mu = 0.08$; (c) 0.2 mM NaH_2PO_4, 20 mM Na_2HPO_4, 22 mM citric acid, 0.36 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride, 0.02% NaN_3 for pH 3.7 and $\mu = 0.5$; and (d) 0.1 mM NaH_2PO_4, 2 mM Na_2HPO_4, 6 mM citric acid, 0.05 M NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM (*p*-amidinophenyl)-methanesulfonyl fluoride, 0.02% NaN_3 for pH 3.7 and $\mu = 0.08$. The excitation wavelength was 350 nm, and the emission spectra were recorded from 360 to 600 nm. The excitaion and emission spectral widths were 5.0 nm, and the scan rate was 120 nm/min. ANS solution was added to protein solutions 1 min before each measurement. All measurements were performed with a protein concentration of 2.7 μM and a molar ratio of ANS/protein of 66.

DSC Measurement. Protein samples (pH 3.7 or 7.6 and $\mu = 0.08$ or 0.5) prepared as described above were used for the differential scanning calorimetry (DSC) experiments, which were carried out on a Microcal MC-2 ultrasensitive microcalorimeter as described previously (Maruyama et al., 1998). All DSC experiments were performed with a protein concentration of 0.5 mg/mL. The DSC scan rate was 1 °C/min for all experiments.

Analysis of Heat-Induced Association. Protein solutions (1 mg/mL) (pH 7.6 and $\mu = 0.5$), prepared as described above except for the absence of 2-mercaptoethanol, were heated at 70, 80, and 90 °C for 5 min. After heating, the solutions were passed through a membrane filter (0.22 μm). Each sample (100 μL) was fractionated via gel filtration chromatography using Shodex KW803 and SB columns and subjected to the multi-angle laser light scattering experiments. Light scattering was measured on a Dawn DSP-F MALLS (Wyatt Technology, Santa Barbara, CA).

Protein solutions (1 mg/mL) ($\mu = 0.08$ and pH 3.7 or 7.6), prepared as described above, were heated at 70, 80, and 90 °C for 5 min and analyzed by polyacrylamide gel electrophoresis (PAGE) using 5−15% gradient acrylamide gels according to the method of Davis (1964).

Analysis of Emulsifying Ability. To prepare emulsions, 0.25 mL of soybean oil and 1.5 mL of protein solutions prepared as described above (0.5 mg/mL) (pH 3.7 or 7.6 and $\mu = 0.08$ or 0.5) were homogenized for 30 s with a high-speed homogenizer (Nichion Irikakikai Ltd.) operating at 2.2×10^4 rpm, and further emulsions were sonicated using an ultrasonic homogenizer (Nihonseiki Kaisha Ltd.) for 1 min. The particle size distribution of the emulsions was measured using a laser light scattering instrument (Model LA 500, Horiba Seisakusho Ltd.). Each sample was analyzed five times, and the data were presented as averages.

RESULTS AND DISCUSSION

Solubility. β-Conglycinin is globulin, which is soluble in salt solution but not in water (Osborne, 1924). It is known that the isoelectric point of β-conglycinin is pH 4.8−4.9 (Koshiyama, 1968). Thanh and Shibasaki (1979) reported a detailed study of the effect of pH and ionic strength on the solubility of β-conglycinin. However, such a study on individual constituent subunits has not yet been reported.

Solubilities as a function of pH of the recombinant normal and mutant subunits and the native β-conglycinin (unless otherwise stated, the native β-conglycinin from var. Shirotsurunoko was used) were measured at $\mu = 0.5$ and 0.08. All recombinant proteins were soluble at all pH values examined here at $\mu = 0.5$, in analogy with the native β-conglycinin, coinciding with the findings reported by Thanh and Shibasaki (1979) and Maruyama et al. (1998). This is because electrostatic interactions between polypeptides of the recombinant normal and mutant subunits and the native β-conglycinin are suppressed by the presence of 0.5 M salt. However, the β subunit and the deletion mutants α_c and α'_c were insoluble at pH >4.8 and $\mu = 0.08$, although the α and α′ subunits exhibited isoelectric precipitation at pH 4−5.5 and 4−6, respectively (Figure 1). Thus, the core regions of the α and α′ subunits exhibited solubility profiles as a function of pH different from those of the α and α′ subunits but similar to that of the β subunit. This indicates that the extension regions play an important role in the solubility of the α and α′ subunits above pH 5.5 and pH 6.0, respectively, due to their high hydrophilicity. The pH range causing insolubility of the native β-conglycinin at $\mu = 0.08$ was narrower compared

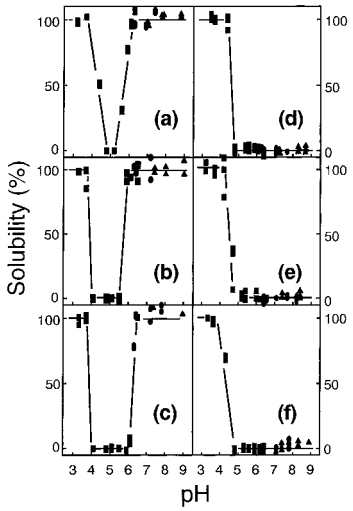

Figure 1. pH dependence of the solubility of the recombinant normal and mutant subunits and the native β-conglycinin at $\mu = 0.08$. The pH values of the samples were adjusted by adding a citrate buffer (square), a phosphate buffer (circle), or an ammonium buffer (triangle). Samples: (a) the native β-conglycinin; (b) α; (c) α'; (d) β; (e) α_c; (f) α'_c.

with those of the α and α' subunits. The carbohydrate moieties of the native β-conglycinin probably contributed to this difference.

Surface Hydrophobicity. Surface hydrophobicity of a protein is an important factor for physicochemical functions such as solubility, emulsification, and foaming (Nakai and Li-Chan, 1988). The surface hydrophobicities of the recombinant normal and mutant subunits and the native β-conglycinin were assessed by measuring fluorescence derived from the binding of ANS at pH 7.6 and 3.7 at $\mu = 0.5$ and 0.08 (Figure 2). Because the β subunit and the deletion mutants were insoluble at $\mu = 0.08$ and pH 7.6, they could not be subjected to the measurement of surface hydrophobicity under such a condition. The fluorescence intensities were very variable: higher at pH 3.7 than at pH 7.6 and higher at $\mu = 0.08$ than at $\mu = 0.5$ at pH 3.7. These are probably due to protonation of the sample at pH 3.7 and exposure of hydrophobic regions by partial loss of the conformational integrity at $\mu = 0.08$ and pH 3.7. The order of the intensities was always $\alpha' > \alpha > \beta$ under all conditions, and the intensities of the deletion mutants α_c and α'_c were almost identical with those of the α and α' subunits under all conditions, respectively, indicating that the surface hydrophobicities of the α and α' subunits are conferred by their core regions. This is consistent with the fact that the extension regions are very poor in hydrophobic amino acids. The observed intensities of the native β-conglycinin were similar to those calculated from the values of individual subunits on the basis of their proportion (α:α':β = 2:1.7:1) under all conditions, although the native β-conglycinin contains both homo- and heterotrimers. This suggests that the interactions for trimerization between the different subunits do not cause any differences in the surface hydrophobicity.

Thermal Stability. In a previous study we examined the thermal stability of the recombinant normal and mutant subunits and the native β-conglycinin at pH 7.6 and $\mu = 0.5$. We demonstrated the following: (1) The order of thermal stability was β (90.8 °C) > α' (82.7 °C) > α (78.6 °C). (2) The core regions (α_c, 77.3 °C; α'_c, 83.3 °C) prescribed the thermal stabilities of the α and α' subunits. (3) The thermal stability of heterotrimers was conferred by the subunit having the lowest denaturation temperature among the constituent subunits (Maruyama et al., 1998). Here we measured DSC profiles of the samples at pH 7.6 and $\mu = 0.08$ and at pH 3.7 at $\mu = 0.5$ and 0.08 (Figure 3). The β subunit and the deletion mutants could not be subjected to measurement at pH 7.6 and $\mu = 0.08$ because of their insolubility. All samples exhibited higher denaturation temperatures at $\mu = 0.5$ than at $\mu = 0.08$ at pH 3.7 (Figure 3B,C). Comparison of the results at pH 7.6 and $\mu = 0.08$ (Figure 3A) with those at pH 7.6 and $\mu = 0.5$ in the previous paper (Maruyama et al., 1998) exhibited a tendency similar to that at pH 3.7. The effect of ionic strength on the thermal stability probably results from strengthen-

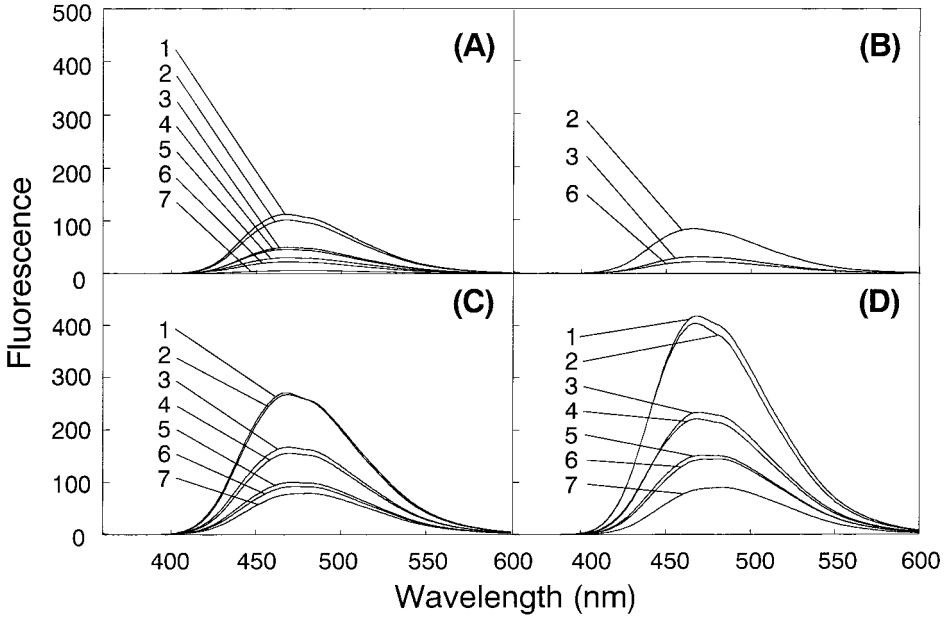

Figure 2. Emission spectra of the recombinant normal and mutant subunits and the native β-conglycinin at pH 7.6 and $\mu = 0.5$ (A), at pH 7.6 and $\mu = 0.08$ (B), at pH 3.7 and $\mu = 0.5$ (C), and at pH 3.7 and $\mu = 0.08$ (D): curve 1, α'_c; curve 2, α'; curve 3, the native β-conglycinin; curve 4, the spectrum calculated from those of individual α, α', and β; curve 5, α_c; curve 6, α; curve 7, β.

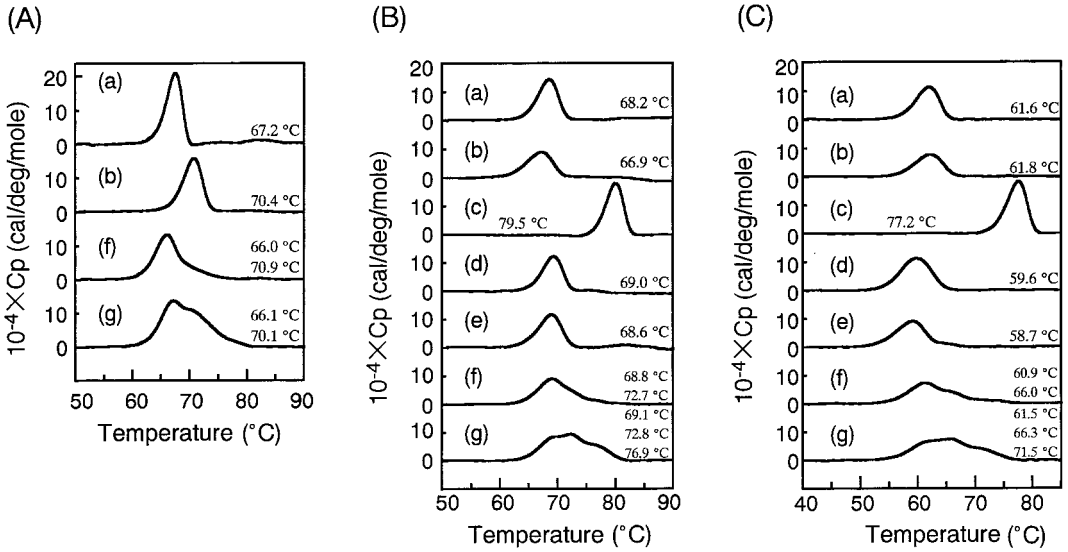

Figure 3. DSC scans of the recombinant normal and mutant subunits and the native β-conglycinin at pH 7.6 and $\mu = 0.08$ (A), at pH 3.7 and $\mu = 0.5$ (B), and at pH 3.7 and $\mu = 0.08$ (C). Scan rates were 1 °C/min. Samples: (a) α; (b) α′; (c) β; (d) α_c; (e) α'_c; (f) the native β-conglycinin (α:α′:β = 2.0:1.7:1.0); (g) the native β-conglycinin (α:α′:β = 2.0:1.3:1.7).

ing of the hydrophobic interactions (Boye et al., 1997). However, comparison of Figure 3A with Figure 3B and of Figure 3B with the results at pH 7.6 and $\mu = 0.5$ in the previous paper (Maruyama et al., 1998) indicates that at both ionic strengths all samples examined exhibit a higher denaturation temperature at pH 7.6 than at pH 3.7. It was shown that in the presence of 2.5% NaCl the t_m values decreased with decreasing pH below 6 (Nagano and Nishinari, 1994). Together with this, the findings obtained here indicate that the protonation of carboxyl groups is a driving force for destabilization at pH 3.7.

The t_m values of the deletion mutants α_c and α'_c were very close to those of the α and α′ subunits under all conditions, respectively. Thus, thermal stabilities of the α and α′ subunits are conferred by the core regions regardless of pH and ionic strength.

The native β-conglycinin containing α, α′, and β subunits in the proportion 2.0:1.7:1.0 exhibited one peak (66.0 °C) with a shoulder (70.9 °C) at pH 7.6 and $\mu = 0.08$ (Figure 3Af), which was close to those of the α (67.2 °C) and α′ (70.4 °C) subunits, respectively. At pH 3.7, the native β-conglycinin exhibited two peaks at $\mu = 0.5$ (68.8 and 72.7 °C) and 0.08 (60.9 and 66.0 °C) (Figure 3Bf,Cf). The t_m values of the α and α′ subunits were similar to each other at pH 3.7 (α, 68.2 °C, and α′, 66.9 °C, at $\mu = 0.5$; α, 61.6 °C, and α′, 61.8 °C, at $\mu = 0.08$). These values were similar to those of the first peaks of the native β-conglycinins at $\mu = 0.5$ (68.8 °C) and 0.08 (60.9 °C) at pH 3.7, respectively. The t_m values of the second peaks of the native β-conglycinin were between those of the β homotrimer (79.5 and 77.2 °C at $\mu = 0.5$ and 0.08, respectively) and those of the α and α′ homotrimers. The native β-conglycinin prepared here did not contain the β homotrimer as described under Materials and Methods. These findings suggest that the second peaks of the native β-conglycinin probably are derived from the heterotrimers containing the β subunit. To examine this assumption, we measured DSC profiles of the native β-conglycinin purified from var. Suzuyutaka, which contained α, α′, and β subunits in the proportion 2.0:1.3:1.7. The native β-conglycinin from var. Suzuyutaka exhibited two peaks (66.1 and 70.1 °C) at pH 7.6 and $\mu = 0.08$ (Figure 3Ag), which were similar to those (66.0 and 70.9 °C) of the β-conglycinin from var. Shirotsurunoko (Figure 3Af), and exhibited three peaks (69.1, 72.8, and 76.9 °C at $\mu = 0.5$ and 61.5, 66.3, and 71.5 °C at $\mu = 0.08$) at pH 3.7 (Figure 3B,Cg). The two lower t_m values were similar to those of the native β-conglycinin from var. Shirotsurunoko at both ionic strengths. The proportion of the β subunit in the native β-conglycinin prepared from var. Suzuyutaka was much higher than that in one from var. Shirotsurunoko. Thus, these findings support the assumption described above and suggest that the second and third peaks of the β-conglycinin from var. Suzuyutaka are derived from heterotrimers containing one and two β subunits, respectively. Furthermore, comparison of the area of the peak having the lowest t_m value with the areas of the other two peaks indicates that the proportions of the homo- and heterotrimers composed of α and/or α′ in the β-conglycinins from vars. Shirotsurunoko and Suzuyutaka are about 70 and 50%, respectively.

Heat-Induced Association. Heat-induced association of proteins is an important physicochemical function and is related to thermal stability. Protein solutions heated at pH 7.6 and $\mu = 0.5$ were subjected to multiangle light scattering detection to determine the molecular masses of aggregates (Figure 4). The α (panel A, peak 2) and α′ (panel B, peak 2) subunits formed soluble aggregates having molecular masses of 2−3 million daltons at heating temperatures >80 °C with a concomitant decrease of the intact species (panels A and B, peak 1). The efficiency of the conversion of the intact species of the α subunit to soluble aggregates at 80 °C was higher than that of the α′ subunit. This was due to the difference in their t_m values (α, 78.6 °C; α′, 82.7 °C). In contrast, the β subunit (panel C) and the deletion mutants α_c (panel D) and α'_c (panel E) formed insoluble aggregates depending on their t_m values. These findings indicate that the abilities of the α and α′ subunits to form soluble aggregates are conferred by the extension regions, which implies that the α and α′ subunits form transparent gels and the β subunit and the deletion mutants α_c and α'_c form turbid gels or cannot form gels.

The native β-conglycinin (panel F) did not form an insoluble aggregate despite the presence of the heterotrimers containing the β subunit and showed a pattern

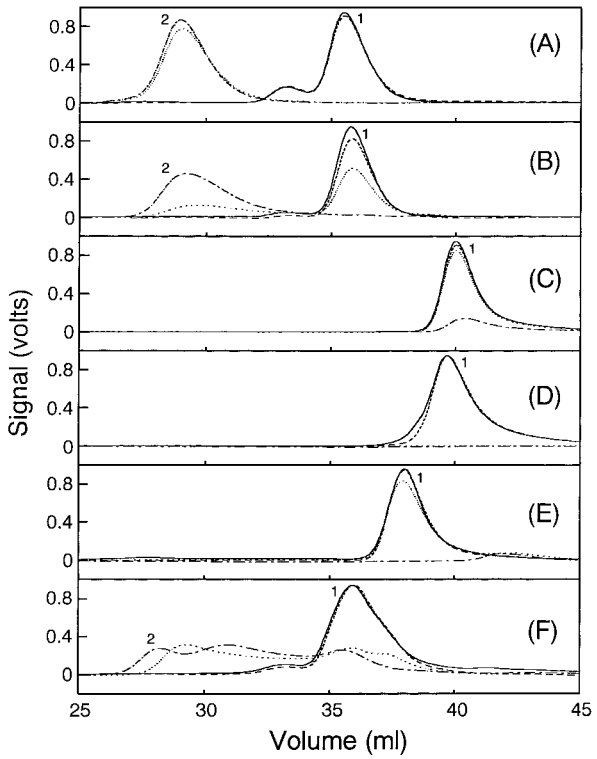

Figure 4. Elution patterns of the recombinant normal and mutant subunits and the native β-conglycinin heated at pH 7.6 and $\mu = 0.5$. Samples: (A) α; (B) α'; (C) β; (D) α_c; (E) α'_c; (F) the native β-conglycinin; solid line, nonheated; dashed line, 70 °C; dotted line, 80 °C; dashed and single-dotted line, 90 °C. Numbers indicate the peaks.

similar to those of the α and α' subunits. The native β-conglycinin heated at 90 °C showed larger soluble aggregates (panel F, peak 2) than the α and α' subunits heated at 90 °C, suggesting that the carbohydrate moieties of the native β-conglycinin also play an important role for the formation of the soluble aggregates.

Because the samples could not be analyzed at $\mu = 0.08$ at pH 3.7 and 7.6 by gel filtration because of their adsorption onto the column, the heat-induced aggregates were investigated using PAGE. The unheated α and α' subunits exhibited the tendency to form hexamers and to dissociate to monomers at $\mu = 0.08$ at both pH values, respectively (Figure 5A,B, lanes 1 and 5), indicating that the effects of the environmental condition on the dissociation and association behaviors of the α and α' subunits were different from each other. At both pH values the unheated native β-conglycinin showed broad bands, compared with trimers of the α and α' subunits, probably due to the molecular heterogeneity of the native β-conglycinin (Thanh and Shibasaki, 1976), and exhibited neither association nor dissociation, although it should have contained both the α and α' homotrimers (Figure 5C, lanes 1 and 5). This suggests that the carbohydrate moieties play an important role in prevention of their association and dissociation. After heating, no samples formed insoluble aggregates. In the cases of the α and α' subunits and the native β-conglycinin, aggregates that could not enter the 5% acrylamide gel appeared with concomitant disappearance of the bands corresponding to those of the unheated samples with heating depending on their t_m values (Figure 5, lanes 3, 4, 7, and 8). However, the β subunit and the deletion mutants α_c and α'_c could not be analyzed using PAGE because of insolubility under the conditions of PAGE, although they did not form insoluble aggregates. Thus, we analyzed the heated samples of the β subunit and the deletion mutants α_c and α'_c using dynamic light scattering measurements and observed the appearance of the soluble aggregates with heating (data not shown).

In contrast, at $\mu = 0.5$ and pH 3.7 all of the samples including the native β-conglycinin formed insoluble aggregates with heating (data not shown), indicating that the ionic strength had a great effect on the solubility of the heat-induced aggregates at pH 3.7.

Emulsifying Ability. Surface hydrophobicity and hydrophilicity and molecular stability are related to the emulsifying ability of proteins. These properties of the β-conglycinin constituent subunits are different from each other, suggesting that emulsifying abilities of individual subunits are different. Therefore, we assessed emulsifying abilities of the recombinant proteins and the native β-conglycinin by measuring the sizes of particles formed by homogenization and sonication of the samples with soy oil at pH 7.6 and 3.7 at $\mu = 0.08$ and 0.5 (Figure 6). The β subunit and the deletion mutants could not be subjected to measurement at pH

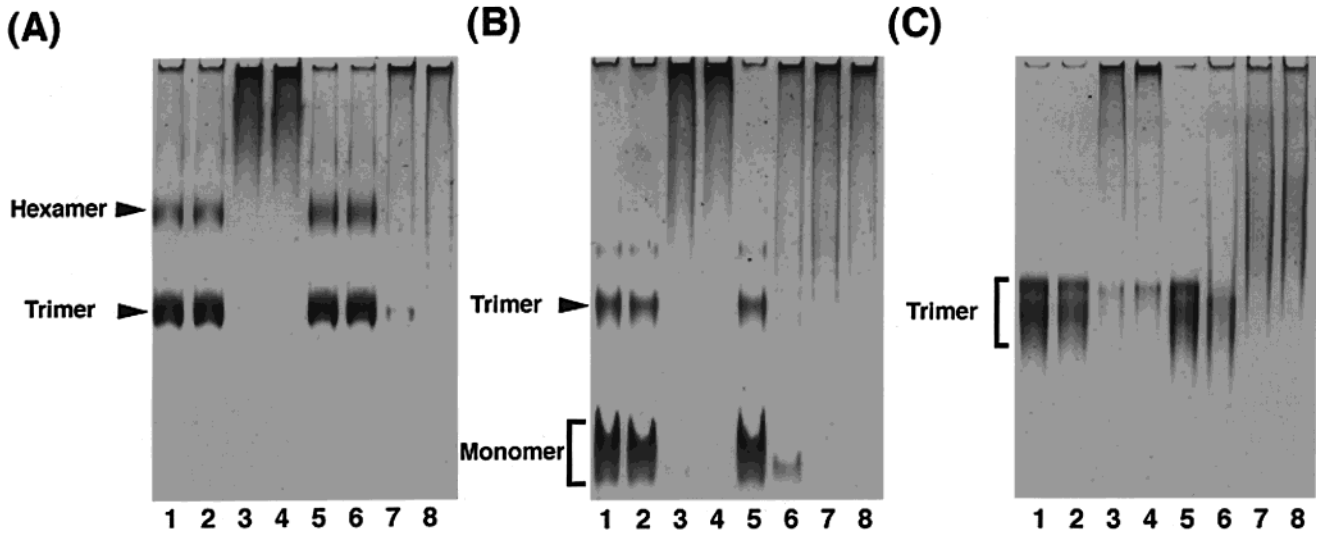

Figure 5. PAGE analysis of the α (A) and α' (B) subunits and the native β-conglycinin (C) heated at $\mu = 0.08$ at pH 7.6 (lanes 1−4) and 3.7 (lanes 5−8): lanes 1 and 5, unheated; lanes 2 and 6, 60 °C; lanes 3 and 7, 70 °C; lanes 4 and 8, 80 °C.

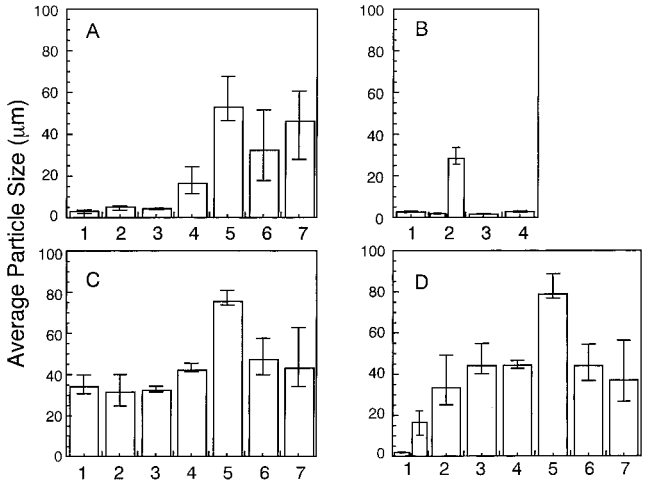

Figure 6. Average particle sizes of emulsions from the recombinant normal and mutant subunits and the native β-conglycinin at pH 7.6 and $\mu = 0.5$ (A), at pH 7.6 and $\mu = 0.08$ (B), at pH 3.7 and $\mu = 0.5$ (C), and at pH 3.7 and $\mu = 0.08$ (D): bar 1, BSA; bar 2, the native β-conglycinin; bar 3, α; bar 4, α′; bar 5, β; bar 6, α_c; bar 7, α'_c.

7.6 with $\mu = 0.08$ because of their insolubility. Proteins with a good emulsifying ability exhibit a small average particle size. At pH 7.6 and $\mu = 0.5$ BSA, one such protein, exhibited an average particle size of 3.0 μm (Figure 6A, bar 1). The α subunit exhibited the best value (4.2 μm) among the three subunits, close to that of BSA, and then α′ (16.4 μm) and β (52.9 μm). This order corresponds to that of the thermal stabilities of the three subunits (Maruyama et al., 1998). In contrast, the deletion mutants α_c and α'_c exhibited poorer values (33.3 and 46.2 μm, respectively) than the α and α′ subunits but better values than the β subunit. The order of α_c, α'_c, and β was similar to that of their t_m values, but not to that of their surface hydrophobicities. These findings indicate that the extension regions and molecular stabilities of the core regions play important roles in the emulsifying abilities of the β-conglycinin subunits. However, the native β-conglycinin exhibited an average size of 5.1 μm, which was similar to that of the α subunit, despite the presence of the α′ and β subunits. This may be because the carbohydrate moieties had a great influence on the particle size of the heterotrimer of the native β-conglycinin.

The average particle sizes of emulsions of the α and α′ subunits (1.6 and 2.8 μm, respectively) at pH 7.6 and $\mu = 0.08$ were much smaller than those at pH 7.6 and $\mu = 0.5$ (Figure 6B). This is probably due to the differences in intensities of mutual repulsions at $\mu = 0.5$ and 0.08. The native β-conglycinin gave two major peaks (1.9 and 28.3 μm) (Figures 6B and 7A). The average particle size of the1.9 μm peak was similar to those of the α (1.6 μm) and α′ (2.8 μm) subunits under the condition of pH 7.6 and $\mu = 0.08$ (Figure 7). It is likely that the 1.9 μm peak was derived from the homo- and heterotrimers composed of α and/or α′. The proportion of the 1.9 μm peak in emulsions was ~30%. This value was smaller than the proportion of the homo- and heterotrimers composed of α and/or α′ (~70%) calculated from the DSC findings (Figures 3Bf,Cf). This may be because the heterotrimers containing the β subunit in the native β-conglycinin had a great influence on the particle size at pH 7.6 and $\mu = 0.08$.

At pH 3.7 at both ionic strengths all samples exhibited poorer emulsifying abilities than at pH 7.6 (Figure 6).

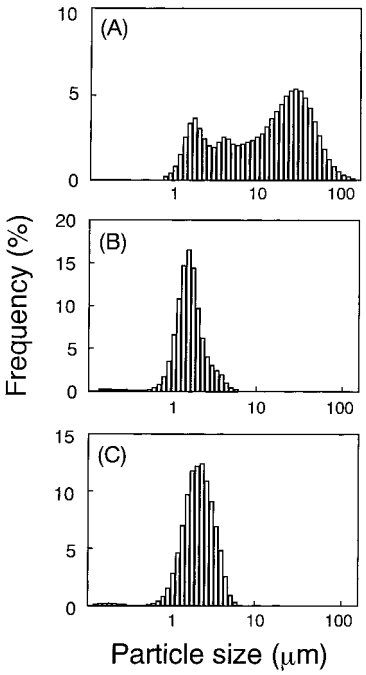

Figure 7. Typical particle size distributions of the native β-conglycinin (A) and the recombinant α (B) and α′ (C) subunits at pH 7.6 and $\mu = 0.08$.

The particle sizes of emulsions of the α and α′ subunits were similar to those of α_c and α'_c, and that of the β subunit was larger than those of the other samples (Figure 6C,D). This tendency of the particle sizes was similar to that of thermal stabilities. Thus, at pH 3.7 the extension regions of the α and α′ subunits had little effect on the particle sizes of the emulsion, and the structural stabilities of the core regions played an important role in the emulsifying ability regardless of ionic strength. The carboxyl groups were protonated at pH 3.7 and then the electrostatic repulsive forces were reduced, resulting in the large average particle size at pH 3.7.

Conclusion. Surface hydrophobicities, thermal stabilities, solubilities, heat-induced association, and emulsifying abilities of the β-conglycinin constituent subunits are different from each other and are variable among environmental conditions such as pH and ionic strength. However, there are some consistencies: surface hydrophobicities and thermal stabilities are conferred by their core regions, and solubilities, heat-induced association, and emulsifying abilities are remarkably dependent on the extension regions and the carbohydrate moieties as well as the core regions. These findings indicate that various end products can be produced by the selection of soybean varieties containing β-conglycinin with different subunit compositions and suggest a direction for a principle of soybean breeding.

ABBREVIATIONS USED

ANS, 8-anilino-1-naphthalenesulfonic acid; BSA, bovine serum albumin; DSC, differential scanning calorimetry; PAGE, polyacrylamide gel electrophoresis.

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