Improvement of nutritional value and functional properties of soybean glycinin by protein engineering

Chan-Shick Kim, Seigo Kamiya, Toshiro Sato, Shigeru Utsumi[1] and Makoto Kito

단백질공학에 의한 大豆 글리시닌의 營養價 및 機能特性의 向上

金贊植, 우쯔미*, 기또*

摘 要

글리시닌은 大豆의 주요 貯藏 단백질중의 하나이다. 글리시닌의 機能特性(겔화성과 乳化性)과 營養價를 改善하기 위하여 $A_{1a}B_{1b}$ proglycinin subunit를 여러 종류의 콩과식물 혹은 비콩과식물의 단백질 그리고 글리시닌의 構造와 機能特性과의 상관관계로부터 글리시닌 형태의 글로불린의 아미노산 배열의 비교로부터 제안된 유전적으로 可變領域의 domain을 기초로 하여 改變하였다. 그러므로 각 可變領域에 상당하는 핵산 염기배열을 $A_{1a}B_{1b}$ proglycinin을 code하고 있는 cDNA로부터 削除하거나, 4개의 연속된 methionine을 code하고 있는 合成 DNA를 각각의 可變領域의 domain에 상당하는 cDNA 領域에 挿入하였다. 改變된 cDNA의 발현 plasmid를 調製하고 대장균 JM 105에서 發現하였다. 改變된 단백질의 몇종류는 대장균체내에서 溶解性 단백질로 축적되었으며 self-assemble하였다. 改變된 단백질들은 자연에 있는 대두 글리시닌보다도 우수한 機能特性을 나타내었으며 이론적으로 高品質인 글리시닌을 創製하는 可能性을 확립하였다.

Introduction

Soybean (*Glycine max* L.) proteins are deficient in the essential sulfur-containing amino acids. Heat-induced gel forming and emulsifying abilities of soybean proteins are significant functional properties with respect to their utilization in food systems. Therefore, improvement of the nutritional value and functional properties of soybean proteins is a major objective in the food industry (Kinsella, 1979). Soybean proteins are composed of two major components, glycinin (11S globulin) and β-conglycinin (7S globulin) (Derbyshire *et al.*, 1976). Of these two proteins, glycinin contains more methionine, the limiting amino acid of soybean proteins, than β-conglycinin (Millerd, 1975). Therefore, glycinin is a suitable target for genetic manipulation to improve the nutritional and functional properties of soybean proteins. Protein engineering is a powerful approach to attain this goal. However, the effects of protein engineering on the stability and self-assembly of glycinin should be considered from the standpoint of its high cumulative level in the protein bodies of the beans. It is necessary to evaluate whether protein-engineered glycinins are able to form proper conformation and to exhibit expected functional properties before the modified genes are transferred to the soybean plant. To evaluate these points, we established a high-level expression system of an $A_{1a}B_{1b}$ proglycinin subunit cDNA in *Escherichia coli*, which can be employed for the evaluation of the self-assembly and the functional properties of protein-engineered glycinins (Kim *et al.*, 1990).

When attempts are made to improve the nutritional and functional properties of glycinin by protein engineering, the following two problems should be considered: (i) which regions of glycinin are susceptible to protein engineering? and (ii) what kinds of protein engineering are employed? Wright (1987, 1988) aligned the amino acid sequences to maximize the homology among the 11S globulins from various legumes and nonlegumes and suggested that they comprised a series of alternating conserved and variable domains. The existence of five variable domains, I−V, was suggested (Wright, 1988). Each variable domain exists in the hydrophilic region, which suggests that they are located on the surface of the protein. On the other hand, Argos *et al.* (1985) suggested the presence of two variable domains, domain I and a hypervariable region from the comparison of 7S and 11S globulins. Domain I and the hypervariable region correspond to the region from the variable domains I to II and the variable domain IV demonstrated by Wright (1988) respectively. The variable domains have little function in forming and maintaining the glycinin structure and may tolerate modification (Nielsen, 1985). The relationship between the structure and the functional properties of glycinin may answer the second question

relating to the kind of protein engineering that should be employed. Mori and coworkers (Nakamura *et al.*, 1984) studied the relationships between the structure at the subunit level and heat-induced gelation of glycinin and proposed that the heat instability of the constituent subunits was related to the heat-induced gel-forming ability. On the other hand, attachment of fatty acid to glycinin increases its emulsifying properties (Haque and Kito, 1982; Haque *et al.*, 1982). This indicates that hydrophobicity is an important factor in the emulsifying properties of glycinin. Kato and Yutani (1988) reported that the surface properties of a protein depend on the conformational stability: the more unstable, the higher the emulsifying properties. These facts suggest that removal of the variable domains and insertions of continuous plural methionines into the variable domains may be powerful methods of improving the food qualities of glycinin. Such modifications may induce a strengthening of the hydrophobicity and the destabilization of the glycinin molecule.

In this paper, we describe the creation of novel glycinins designed to improve their nutritional and functional properties. The design strategy was based on the structural characteristics of glycinin and the relationships between the structure and the functional properties of glycinin.

Materials and methods

Bacterial strain, medium and plasmids

Escherichia coli strain JM105 was used as the host cell (Yanisch-Perron *et al.*, 1985). LB medium (pH 7.5) consisted of 1% bactotryptone (Difco), 0.5% yeast extract (Difco) and 1% NaCl. Plasmids employed here were pKK233-2 (Pharmacia), pUC19 (Yanisch-Perron *et al.*, 1985), pGST4-2-11-10 carrying $A_{1a}B_{1b}$ subunit precursor cDNA prepared according to Okayama and Berg (1982) (Utsumi *et al.*, 1987b), $pKGA_{1a}B_{1b}$-3, and $pKGA_{1a}B_{1b}$-11 (Utsumi *et al.*, 1988). In the latter two expression plasmids, the ATG codon under the control of *trc* promoter in pKK233-2 was joined to the forth and the twelfth codons in the cDNA encoding $A_{1a}B_{1b}$ proglycinin respectively (Utsumi *et al.*, 1988). Apart from these details, both plasmids have the same construction. The expressed proteins from $pKGA_{1a}B_{1b}$-3 and -11 were termed $A_{1a}B_{1b}$-3 and ΔI, respectively. The N-terminal methionine was retained in $A_{1a}B_{1b}$-3 and cleaved in ΔI (Utsumi *et al.*, 1988). Modified expression plasmids $pKGA_{1a}B_{1b}$ DI+4Met, $pKGA_{1a}B_{1b}$IV+4Met and $pKGA_{1a}B_{1b}$V+4Met, $pKGA_{1a}B_{1b}$-II, $pKGA_{1a}B_{1b}$-III, $pKGA_{1a}B_{1b}$-IV, $pKGA_{1a}B_{1b}$-V36 and $PKGA_{1a}B_{1b}$-V8 were constructed using $pKGA_{1a}B_{1b}$-3, pUC19, pKK233-2 and pGST4-2-11-10. The detailed procedures for the construction of these plasmids are described in Results.

Expression and detection of modified proteins from E.coli

Three hundred milliliters of LB medium containing 25 μg/μl ampicilin was inoculated with 3 ml of a full-grown culture of JM105 harboring individual expression plasmids and cultured at 37°C as described previously (Kim *et al.*, 1990). At A_{600} = 0.3 isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM. After incubation for 20 h at 37°C, the induced cells were harvested by centrifugation. The cells were disrupted by sonication as described previously (Kim *et al.*, 1990). The cell debris and the supernatant were fractionated by centrifugation. Aliquots of the total cells, the cell debris and the supernatant were dissolved in SDS-sample buffer (62.5 mM Tris−HCl, pH 6.8, 2% SDS, 20% glycerol and 0.2% 2-mercaptoethanol) and analyzed by SDS−PAGE to determine whether the expressed proteins were soluble.

SDS−PAGE was carried out according to the method of Laemmli (1970) using 11% polyacrylamide gels. Proteins were visualized with Coomassie Brilliant Blue R-250. The relative amount of the expressed protein in each sample was estimated from a densitometric scan using a Shimadzu dual-wavelength TLC scanner CS-910 densitometer.

Purification of expressed proteins from E.coli

Escherichia coli cells from 20−30 × 300 ml culture were disrupted by sonication and the cell debris and unbroken cells were removed by centrifugation as described previously (Kim *et al.*, 1990). From the resultant supernatant the expressed proteins were purified by ammonium sulfate fractionation, Q-Sepharose column chromatography and cryoprecipitation as described previously (Kim *et al.*, 1990).

Protein determination

Protein was determined by the method of Bradford (1976) with bovine serum albumin as a standard.

Analysis of self-assembly of expressed protein

The soluble extracts (~5 mg/0.4 ml) of JM105 cells harboring individual expression plasmids were dialyzed against 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl, 1.5 mM PMSF and 1 mM EDTA. After dialysis, assembly was assayed by sucrose density gradient centrifugation as described previously (Utsumi *et al.*, 1988). The 2S, 7S and 11S fractions purified from soybean according to Thanh and Shibasaki (1976) were run in parallel as size markers.

Computer analysis of secondary structure

The secondary structure was predicted according to the procedure of Chou and Fasman (1974a,b). Search distances of six and four residues were used for helical and sheet structures respectively. For the β-turn analysis, tetrapeptides with $Pt > 0.75 \times 10^{-4}$ were selected as probable turns.

Preparation of glycinin from soybean

The purified glycinin fraction was prepared from soybean (*Glycine max* L. var. Tsuru-no-ko) seeds as described previously (Mori *et al.*, 1979).

Preparation and measurement of protein gels

The expressed proteins and the purified glycinin were thoroughly dialyzed against 3.5 mM potassium phosphate buffer (pH 7.6) (buffer A) just before use. After dialysis, the protein solutions were concentrated to 5−10% protein concentration by ultrafiltration and then diluted to the desired protein concentration with buffer A. The protein gels were heated for 30 min at 100°C according to the micro-method of Utsumi *et al.* (1982). Gel hardness was measured with a rheometer (Yamaden, RE-3305 Rheoner) using a plunger 8 mm in diameter. Force required to compress 0.4 mm into the gels was recorded as hardness according to the method described previously (Kim *et al.*, 1990).

Emulsifying activity

The emulsifying activity of the expressed proteins was measured by the method of Pearce and Kinsella (1978). To prepare the emulsion, 0.5 ml of soybean oil and 1.5 ml of 0.05% protein solutions in buffer A were sonicated at 25°C with a sonicator (Insonator Model 200M, Kubota, Japan) at 100 W for 30 s. Aliquots (30 μl) of the emulsions were taken from the bottom of the container immediately after sonication and diluted in 0.1% (w/v) SDS in water (20 ml). The turbidity of the diluted emulsions was then measured in a 1 cm pathlength cuvette at a wavelength of 500 nm. Absorbance of duplicate aliquots of each emulsion was measured in each case.

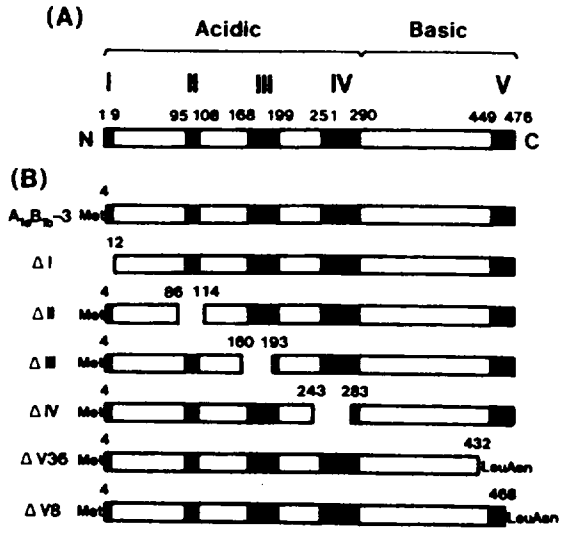

Fig. 1. (A) The variable and conserved domains of glycinin $A_{1a}B_{1b}$ subunit aligned by Wright (1988). The numbers of the residues from the N-terminus are described for the variable domains above the alignment. The five variable domains were labeled I–V. Shaded areas, variable domains; open areas, conserved domains. Acidic and Basic refer to the acidic and basic polypeptides respectively. (B) Construction of the deleted proteins and $A_{1a}B_{1b}$-3. $A_{1a}B_{1b}$-3 lacks the N-terminal three amino acids. ΔI N-terminal 11, ΔII from the 87th to the 113rd, ΔIII from the 161st to the 192nd, ΔIV from the 244th to the 282rd, ΔV38 from the 431st to the C-terminus and ΔV8 from the 469th to the C-terminus. The N-terminal methionine was retained in $A_{1a}B_{1b}$-3, ΔII, ΔIII, ΔIV, ΔV36 and ΔV8 and cleaved in ΔI. ΔV36 and ΔV8 have two extra amino acids Leu-Asn at their C-terminus.

Results and discussion

Construction of expression plasmids for modified proteins

Figure 1(A) shows the variable and conserved domains of the $A_{1a}B_{1b}$ proglycinin aligned by Wright (1988). To improve the functional properties of glycinin, we attempted to delete each variable domain of the $A_{1a}B_{1b}$ proglycinin as shown in Figure 1(B). We employed $pKGA_{1a}B_{1b}$-11 (see Materials and methods) as an expression plasmid for ΔI lacking the variable domain I. Here we constructed expression plasmids $pKGA_{1a}B_{1b}$-II for ΔII, $pKGA_{1a}B_{1b}$-III for ΔIII, $pKGA_{1a}B_{1b}$-IV for ΔIV, $pKGA_{1a}B_{1b}$-V36 for ΔV36 and $pKGA_{1a}B_{1b}$-V8 for ΔV8 using $pKGA_{1a}B_{1b}$-3 (see Materials and methods) by the procedures given in Figure 2. The nucleotide sequences in the vicinity of the translation initiation site of each individual expression plasmid constructed here are the same as that of $pKGA_{1a}B_{1b}$-3. Therefore, the expressed proteins lack the N-terminal three amino acids of the $A_{1a}B_{1b}$ proglycinin and retain the initiation methionine. ΔV36 and ΔV8 have two extra amino acids Leu-Asn at their C-terminus.

To improve the nutritional and functional properties simultaneously, we inserted synthetic DNAs encoding four continuous methionines to the cDNA regions corresponding to domain I (DI) indicated by Argos *et al.* (1985) and the variable domains IV and V indicated by Wright (1988), and constructed $pKGA_{1a}B_{1b}$DI+4Met, $pKGA_{1a}B_{1b}$IV+4Met and $pKGA_{1a}B_{1b}$ V+4Met using $pKGA_{1a}B_{1b}$-3 as shown in Figure 3. DI+4Met expressed from $pKGA_{1a}B_{1b}$DI+4Met has Arg-Met-Met-Met-Met between Arg61 and Arg62. As a result of the insertion, the secondary structure in the vicinity of the insertion site predicted according to the procedure of Chou and Fasman (1974a,b) varies from random to α-helix. IV+4Met expressed from $pKGA_{1a}B_{1b}$ IV+4Met has Arg-Met-Met-Met-Met-Gly between Pro281 and Arg282 which results in the alteration from β-turn to α-helix. V+4Met expressed from $pKGA_{1a}B_{1b}$V+4Met has Glu-Met-Met-Met-Met-His between Pro467 and Gln468 resulting in the alteration from random to α-helix.

Abilities of modified proteins to form the correct conformation

To improve the glycinin qualities by means of protein engineering, the modified proteins should be able to assume the correct conformation. We demonstrated previously that *E.coli* strain JM105 accumulated expressed proteins from $pKGA_{1a}B_{1b}$-3 at the level of 20% of the total bacterial proteins, and that the expressed protein was soluble and self-assembled into trimers with the secondary structure similar to that of soybean glycinin (Kim *et al.*, 1990). Moreover, we suggested that the folding of the expressed protein with the signal peptide could be disturbed on account of the strong hydrophobicity of the signal peptide rendering the protein susceptible to proteinase digestion (Utsumi *et al.*, 1987a, 1988). Accordingly, the following criteria were employed for the formation of the correct conformation: (i) high-level expression, (ii) solubility and (iii) self-assembly into trimers.

Production of modified proteins in JM105 cells harboring individual expression plasmids was analyzed by SDS–PAGE (Figure 4). ΔI, ΔV8 and IV+4Met were accumulated in the cells at a high level, and ΔIV, ΔV36 and V+4Met at a moderately high level. However, ΔII, ΔIII and DI+4Met were not detected by Coomassie Brilliant Blue staining, although they were detected by immunoblotting. After the disruption of the cells harboring individual expression plasmids by sonication, the debris and the soluble fractions were applied to SDS–PAGE and the expressed proteins were detected by Coomassie Brilliant Blue staining or

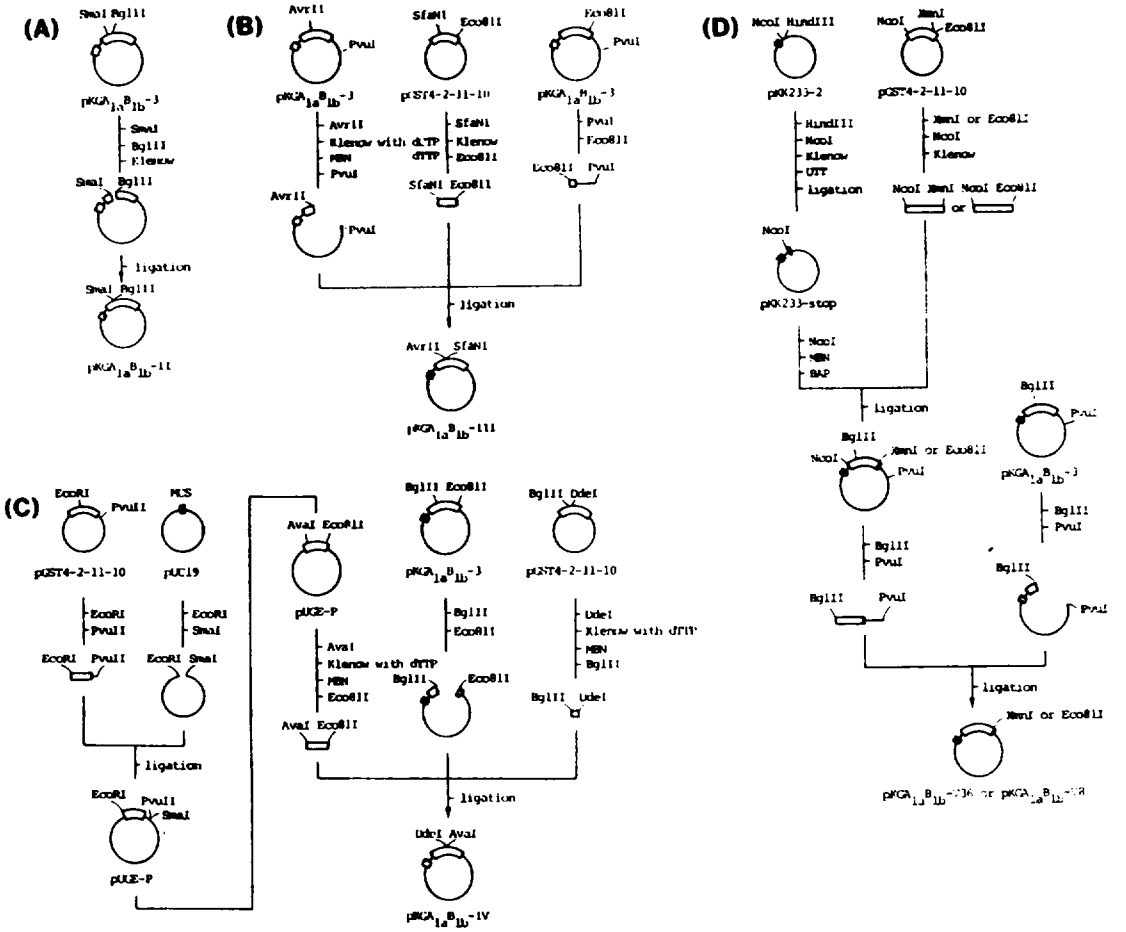

Fig. 2. Scheme for construction of expression plasmids pKGA$_{1a}$B$_{1b}$-II (A), pKGA$_{1a}$B$_{1b}$-III (B), pKGA$_{1a}$B$_{1b}$-IV (C), and pKGA$_{1a}$B$_{1b}$-V36 and pKGA$_{1a}$B$_{1b}$-V8 (D). Stippled, closed and open boxes represent *trc* promoter, UTT and A$_{1a}$B$_{1b}$ cDNA respectively. MBN, mung bean nuclease; BAP, bacterial alkaline phosphatase; MCS, multi cloning site; UTT, universal translation terminator [5'-d(GCTAAATTAATTAAGC)-3', Pharmacia].

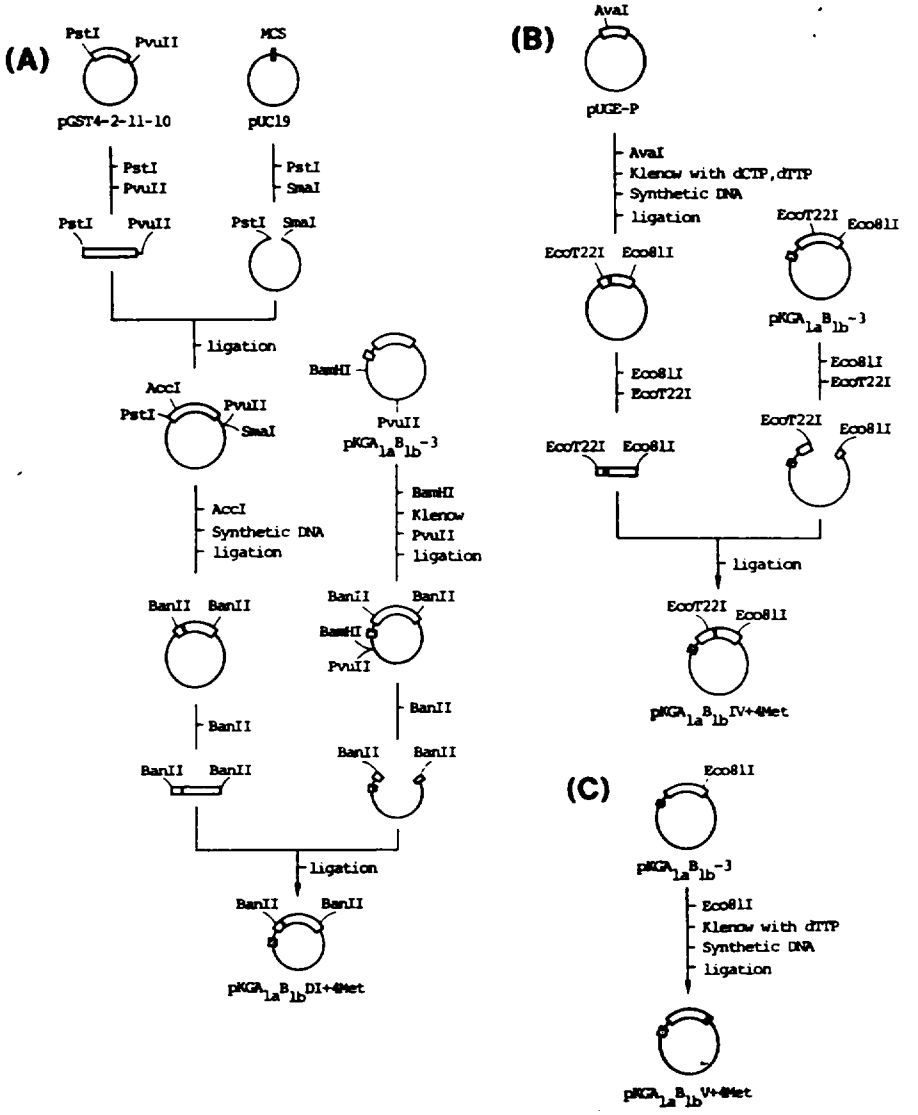

Fig. 3. Scheme for construction of expression plasmids $pKGA_{1a}B_{1b}DI+4Met$ (A), $pKGA_{1a}B_{1b}IV+4Met$ (B) and $pKGA_{1a}B_{1b}V+4Met$ (C). Synthetic DNAs used for $pKGA_{1a}B_{1b}DI+4Met$, $pKGA_{1a}B_{1b}IV+4Met$ and $pKGA_{1a}B_{1b}V+4Met$ were 5'-AGAATGATGATGATG-3' 3'-TTACTACTACTAC-5', 5'-GAATGATGATGATGGG-3' 3'-TACTACTACTACCCGG-5', and 5'-CAGATGATGATGATGCA-3' 3'-CTACTACTACTACGTAG-5', respectively.

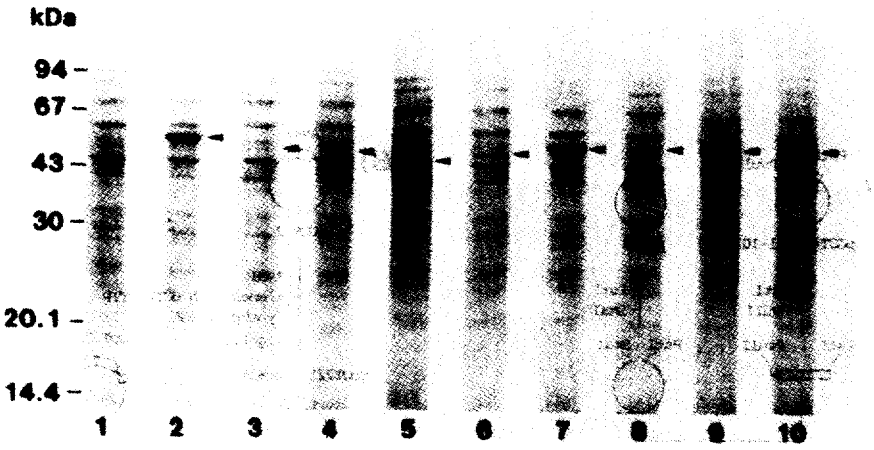

Fig. 4. SDS-PAGE analysis of expression of individual expression plasmids. The *E.coli* strain JM105 cells harboring individual expression plasmids were analyzed on SDS-PAGE in 11% gels. Lane 1, pKK233-2; lane 2, pKGA$_{1a}$B$_{1b}$-II; lane 3, pKGA$_{1a}$B$_{1b}$-II; lane 4, pKGA$_{1a}$B$_{1b}$-III; lane 5, pKGA$_{1a}$B$_{1b}$-IV; lane 6, pKGA$_{1a}$B$_{1b}$-V36, lane 7, pKGA$_{1a}$B$_{1b}$-V8; lane 8, pKGA$_{1a}$B$_{1b}$DI+4Met; lane 9, pKGA$_{1a}$B$_{1b}$IV+4Met; lane 10, pKGA$_{1a}$B$_{1b}$V+4Met. The numbers on the left denote mol. wts. The arrowheads indicate the position of the expressed proteins.

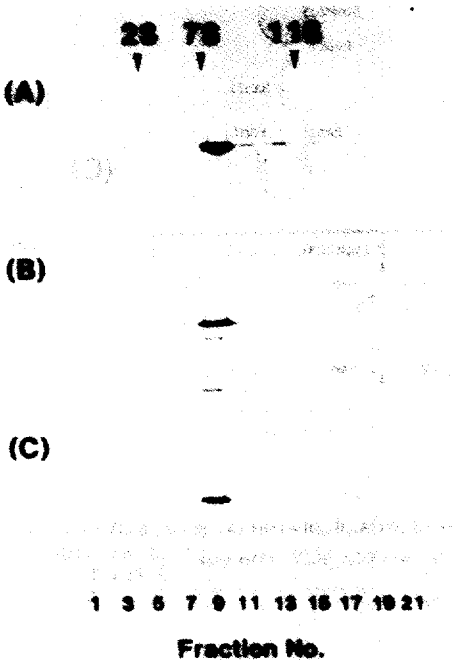

Fig. 5. Self-assembly of the modified proteins. Assembly was assayed by sucrose density gradient centrifugation and the proteins in each fraction were detected by immunoblotting as described previously (Utsumi *et al.*, 1987a). Panel A, ΔV8; Panel B, IV + 4Met; Panel C, V + 4Met. Sedimentation is from right to left. Sedimentation standards are given.

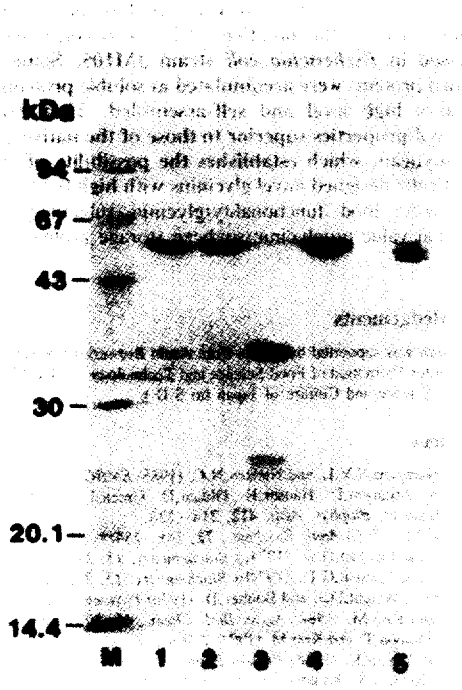

Fig. 6. SDS-PAGE analysis of the purified modified proteins. SDS-PAGE was carried out in the presence (lanes M, 1-4) and in the absence (lane 5) of 2-mercaptoethanol. Lane M, mol. wt markers; lane 1, ΔI; lane 2, ΔV8; lane 3, IV+4Met; lane 4, V+4Met; lane 5, IV+4Met. The numbers on the left denote mol. wts.

Table I. Emulsifying activity of the modified glycinins

Samples	Emulsifying activity[a] (%)
Soybean glycinin	100
$A_{1a}B_{1b}$-3	130
ΔI	132
ΔV8	215
IV+4Met	134
V+4Met	211

[a]Emulsifying activity was expressed as relative value (%) compared with the soybean glycinin.

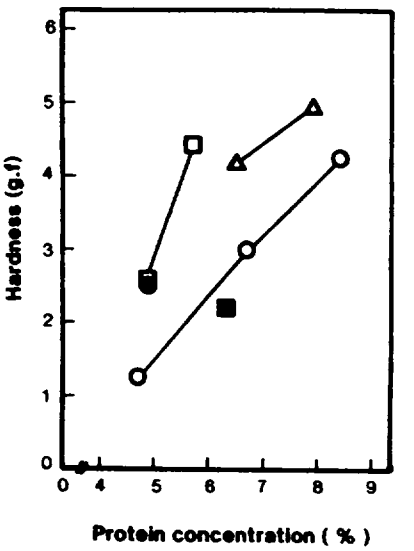

Fig. 7. Hardness of the gels from the modified proteins. ○, the native glycinin; □, ΔI; △, IV+4Met; ●, V+4Met; ■, ΔV8.

immunoblotting (data not shown). ΔI, ΔV8, DI+4Met, IV+4Met and V+4Met were predominantly detected in the soluble fraction and the others, ΔII, ΔIII, ΔIV and ΔV36, were in the debris fraction.

To determine whether ΔI, ΔV8, IV+4Met and V+4Met which were accumulated as soluble proteins at a higher level in the cells are able to self-assemble into trimers like the native proglycinin, the soluble fractions of the cells were subjected to sucrose density gradient centrifugation. After fractionation, proteins in each fraction were analyzed using SDS-PAGE and immunoblotting. As Figure 5 shows, ΔV8, IV+4Met and V+4Met predominantly sedimented in fraction 9 which corresponds to a size of 7-9S (trimer), which was similar to those observed with $A_{1a}B_{1b}$-3 and ΔI (Utsumi *et al.*, 1988). Therefore, we concluded that ΔI, ΔV8, IV+4Met and V+4Met are able to self-assemble into a conformation similar to that of the native glycinin, which indicates that the variable domains I, IV and V could tolerate modifications. However, in the case of IV+4Met, bands with molecular weights smaller than the expected were also observed (Figure 5B). These bands may be derived from limited proteolysis of the full-size product, which suggests that the conformational integrity of IV+4Met is partly lost.

Since the constructs of individual expression plasmids, except pKG$A_{1a}B_{1b}$-11, in the vicinity of the translation initiation site and the promoter are identical, the efficiency of translation and transcription may be identical. However, the amounts of ΔII, ΔIII and DI+4Met accumulated in the cells were much smaller than those of the other modified proteins (Figure 4). Therefore, the modifications introduced into ΔII, ΔIII and DI+4Met may disturb the correct folding, and make them susceptible to proteinase digestion: This suggests that the domain I specified by Argos *et al.* (1985) may not tolerate modification. In fact, when Arg-Gly-Met-Met-Met instead of Arg-Met-Met-Met-Met was inserted between Arg61 and Arg62 in domain I, the secondary structure in the vicinity of the modification site predicted by the procedure of Chou and Fasman (1974a,b) is the same as that of the native $A_{1a}B_{1b}$ subunit, whereas the modified protein did not accumulate in the cells (data not shown). Moreover, a modified $A_{1a}B_{1b}$ protein which lacks 31 of the N-terminus amino acids did not accumulate in the cells (data not shown). However, it is difficult to conclude that the variable domains II and III could not be targets for modification, because ΔII and ΔIII lack part of the conserved regions. On the other hand, ΔIV and ΔV36 accumulated in the cells in much larger amounts than ΔII and ΔIII, although all of ΔII, ΔIII, ΔIV and ΔV36 were insoluble. This together with the fact that IV+4Met is soluble suggests that the deletion of the hydrophilic variable domains IV and V did not disturb the folding of ΔIV and ΔV36, but made them insoluble after folding.

Purification and functional properties of modified proteins

ΔI, ΔV8, IV+4Met and V+4Met expressed in *E.coli* strain JM105 were purified according to the procedure employed for the purification of $A_{1a}B_{1b}$-3 (Kim *et al.*, 1990). Every modified protein exhibited the same behavior during the course of purification as that of $A_{1a}B_{1b}$-3. ΔI, ΔV8 and V+4Met gave predominantly the band with a mol. wt of ~55 kd as shown in Figure 6. However, IV+4Met gave two larger bands with mol. wts of ~35 kd and two smaller bands with mol. wts of ~21–25 kd. IV+4Met was intact in size in the cells (Figure 4). However, a small portion of IV+4Met was cleaved after dialysis against 35 mM potassium phosphate buffer containing 0.4 M NaCl (Figure 5), and the same pattern was observed as that shown in Figure 6 after dialysis against 35 mM potassium phosphate buffer containing 0.15 M NaCl for Q-Sepharose column chromatography (data not shown). The mol. wts of ~35 and 21–25 kd of the cleaved products were similar to those of the acidic and the basic polypeptides of the mature glycinin subunits, and they were disulfide linked as in the mature glycinin subunits (Badley *et al.*, 1975; Kitamura *et al.*, 1976; Mori *et al.*, 1979; Staswick *et al.*, 1981, 1984) (Figure 6). Therefore, the 55 kd product was affected by limited-proteolysis to give two species of protein with mol. wts of ~35 and 21–25 kd. This is analogous to the phenomenon whereby fragmentation of the proglycinin subunit into 35 kd acidic and 20 kd basic polypeptides occurs in the protein bodies of soybean. The proteinase participating in this cleavage prefers low ionic strength.

The functional properties (gelation and emulsification) of the modified proteins (ΔI, ΔV8, IV+4Met and V+4Met) were compared with those of native glycinin. Table 1 shows the emulsifying activity of the modified proteins and the native glycinin. Every modified protein, especially ΔV8 and V+4Met, exhibited higher values than native glycinin. This suggests that the hydrophobicity of the C-terminal region may be closely related to the emulsifying properties of glycinin.

All the modified proteins examined here could form gels by heating at 100°C. Figure 7 shows the hardness of the gels from the modified proteins and native glycinin. The gels from ΔI, IV+4Met and V+4Met exhibited significantly higher hardness than the native glycinin gel, while that from ΔV8 was slightly softer. However, it is difficult to explain the difference in hardness among ΔI, IV+4Met, V+4Met and ΔV8.

SUMMARY

Glycinin is one of the predominant storage proteins of soybean. To improve its functional properties (heat-induced gelation and emulsification) and/or nutritional value, the $A_{1a}B_{1b}$ proglycinin subunit was modified on the basis of genetically variable domains suggested from the comparison of amino acid sequences of glycinin-type globulins from various legumes and nonlegumes and the relationships between the structure and the functional properties of glycinin. Thus, nucleotide sequences corresponding to each of the variable domains were deleted from the cDNA encoding the $A_{1a}B_{1b}$ proglycinin, and a synthetic DNA encoding four continuous methionines was inserted into the cDNA region corresponding to each of the variable domains. Expression plasmids carrying the modified cDNAs were constructed and expressed in *Escherichia coli* strain JM105. Some of the modified proteins were accumulated as soluble proteins in the cells at a high level and self-assembled. They exhibited functional properties superior to those of the native glycinin from soybean, which establishes the possibility of creating theoretically designed novel glycinins with high food qualities.

Key words: food functionality/glycinin subunit precursor/nutritional value/proglycinin/soybean storage protein

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