# Molecular Design of Seed Storage Proteins for Enhanced Food Physicochemical Properties

Mary Rose G. Tandang-Silvas,[1]
Evelyn Mae Tecson-Mendoza,[2] Bunzo Mikami,[3]
Shigeru Utsumi,[1] and Nobuyuki Maruyama[1,*]

[1]Laboratory of Food Quality Design and Development, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan; email: marunobu@kais.kyoto-u.ac.jp

[2]Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, College 4031, Laguna, Philippines

[3]Laboratory of Basic and Applied Molecular Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan

Annu. Rev. Food Sci. Technol. 2011. 2:59–73

First published online as a Review in Advance on October 4, 2010

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi:
10.1146/annurev-food-022510-133718



1941-1413/11/0410-0059$20.00

*Corresponding author.

## Keywords

structure-function, protein engineering, emulsion, gelation

## Abstract

Seed storage proteins such as soybean globulins have been nutritionally and functionally valuable in the food industry. Protein structure-function studies are valuable in modifying proteins for enhanced functionality. Recombinant technology and protein engineering are two of the tools in biotechnology that have been used in producing soybean proteins with better gelling property, solubility, and emulsifying ability. This article reviews the molecular basis for the logical and precise protein designs that are important in obtaining the desired improved physicochemical properties.

This chapter is dedicated to the memory of Professor Shigeru Utsumi who passed away on 1 December 2008.

## INTRODUCTION

Seed storage proteins have been nutritionally and functionally valuable in the food industry. In 2002, the world's soybean production was reported to be 180 million metric tons (MTs) (FAO 2003). Although 85% of the production is processed into animal feed and vegetable oil, approximately 2% of the soybean meal is processed into flour and protein isolate for use as a food ingredient, and 6% is consumed directly as human food, mostly in Asia (Soyatech 2010). Sales of soyfood products such as tofu, soymilk, soycheese, energy bars, and meat alternatives reached close to $4 billion in 2004 (Soyatech & SPINS 2005). Most of these soy protein products, except those used in nutritional applications, are utilized for their functional properties, such as solubility, water absorption and binding, viscosity, gel formation, elasticity, emulsification, binding, cohesion-adhesion, fat absorption, flavor binding, foaming, and color control (Kinsella 1979).

Presently, the variety of soy products and their different characteristics are brought about by formulation and various processing treatments that may involve the use of solvents, enzymes, heat, fractionation, and pH adjustment, or any combination of these treatments. To develop more and better products, the main ingredient, the seed, could be modified using conventional breeding and genetic engineering, specifically, protein engineering. Through conventional breeding, soybean with increased protein levels, especially those of the 7S and 11S globulins (Kwanyuen et al. 1998), low levels of lipoxygenase to reduce the production of beany off-flavors (Nielsen et al. 1992), and higher level of β-conglycinin (Heller 2005) have been developed. Soybean with low levels of the flatulence factors raffinose oligosaccharides and lipoxygenase, and associated off-flavors, has been developed using genetic engineering (Johnson 1999).

Since the early 1990s, the research team of Professor Shigeru Utsumi of Kyoto University has pioneered the molecular design of storage proteins and protein engineering to enhance their physicochemical, functional, and nutritional qualities (Kim et al. 1990, Utsumi et al. 1993). These studies consist of isolation and cloning of the storage protein gene and expression in *Escherichia coli*, characterization of the recombinant protein, protein structure determination using X-ray crystallography, gene modification to enhance the protein's properties, analysis of the physicochemical properties of the modified storage proteins, and gene transformation and expression in selected crops, such as rice, soybean, and potato. These studies have covered storage proteins from several crops: soybean, adzuki bean, mungbean, rapeseed, and French bean. Ultimately, the goal is to develop crops with these modified genes that will produce the enhanced storage proteins.

This chapter focuses on the molecular design of storage proteins specifically for enhanced physicochemical and functional properties and covers (*a*) an overview of seed storage proteins, (*b*) their physicochemical properties, (*c*) their three-dimensional structure, and (*d*) protein engineering to improve their physicochemical properties.

## OVERVIEW OF SEED STORAGE PROTEINS

### Classification

The grouping of seed storage proteins based on their solubility (Osborne 1924) is not absolute but is still used for convenience. Seed storage proteins are classified into (*a*) globulins or salt soluble fraction, (*b*) albumins or water soluble fraction, (*c*) glutelins or dilute acid/alkali fraction, and (*d*) prolamins or alcohol-soluble fraction. The globulins are subdivided based on their sedimentation coefficients into 7S or vicilins and 11S or legumins. Although prolamins and

glutelins have different solubility, they are sometimes put in one group because of the relatedness in their amino acid sequence and content (Shewry et al. 1995). In addition, the subunits released by glutelins in a reduced condition are alcohol-soluble, as in the case of wheat glutenins (Weiser 2007). The prolamins were thus further classified into sulfur-poor, sulfur-rich, and high-molecular weight prolamins (Utsumi 1992). The 2S albumins are compact, globular, and cysteine-rich (Shewry et al. 1995, Moreno & Clemente 2008). The amount of these storage proteins in food crops varies. In general, legumes contain mostly globulins, whereas cereals contain mostly prolamins and glutelins (Utsumi 1992). Oats and rice are exceptions to most cereals because they have mostly 11S globulin-like storage proteins and only approximately 5% to 10% prolamins (Shewry et al. 1995).

## 7S and 11S Globulins

This review is primarily focused on 7S and 11S globulins, the most extensively studied seed storage proteins, perhaps because of their predominance in nature. The 7S globulins are trimer molecules with molecular weights of 150–200 kDa and have 40–70 kDa monomers. The 11S globulins, on the other hand, are hexamer molecules involving two trimers, have molecular weights of 300–400 kDa, and have 50–60 kDa monomers (Utsumi 1992). They are synthesized, sorted, processed and accumulated during seed development. Their monomers are translated into a single pre-propeptide in the rough endoplasmic reticulum (rER). After cotranslational cleavage of the signal peptide in the endoplasmic reticulum (ER), the propeptides accordingly assemble into trimers. The composition of the newly synthesized proteins in the ER was suggested to determine their transport pathway (Mori et al. 2004).

Unlike 11S globulins, 7S globulins are generally cotranslationally glycosylated at Asn residues of the consensus sequence Asn-X-Ser/Thr (Katsube et al. 1998). There are, however, rare reports of glycosylated 11S globulins such as lupin (Duranti et al. 1988) and cocosin (Garcia et al. 2005). Glycosylated (Derbyshire et al. 1976) and unglycosylated (Kimura et al. 2008) pea 7S globulins and unglycosylated coconut 7S globulins (Garcia et al. 2005) have been reported as well. The 7S globulins are usually lacking in cysteine residues, hence, they are devoid of disulfide bridges. The 11S globulins have two conserved disulfide bridges **(Figure 1)**. The cleavage of acidic and basic polypeptides by asparaginyl endopeptidase in the protein storage vacuoles (PSVs) was suggested to trigger hexamer formation to attain mature 11S globulins (Dickinson et al. 1989, Jung et al. 1998). The crystal structure of mature A3B4 revealed that the hydrophobic residues needed for the face to face association of the two trimers become exposed after the cleavage (Adachi et al. 2003a). Storage proteins are kept indefinitely in mature seeds in various organelles depending on the crop. Rice accumulates prolamins in protein bodies (Müntz 1998, Shewry & Halford 2002) whereas soybeans store 7S and 11S globulins in PSV (Mori et al. 2009). At the onset of germination, they are rapidly mobilized, used, and depleted.

The soybean 7S globulin called β-conglycinin has three types of subunits, namely, α (~67 kDa), α′ (~71 kDa), and β (~50 kDa) (Maruyama et al. 1998), as shown in **Figure 1**. All have a highly homologous core region but only α and α′ have a highly acidic extension region at the N-terminus. Both α and α′ have two N-glycans within their core regions, whereas β has only one (Maruyama et al. 2001). Soybean 11S globulin or glycinin has five types of subunits that are grouped into two based on their amino acid sequence. Group I consists of A1aB1b, A1bB2, and A2B1a, whereas group II consists of A3B4 and A5A4B3. A schematic representation of A1aB1b is shown in **Figure 1**. The sequence identity among the subunits is approximately 45%, whereas it is approximately 80% within the group (Maruyama et al. 2006). There are five variable regions among the 11S globulins.

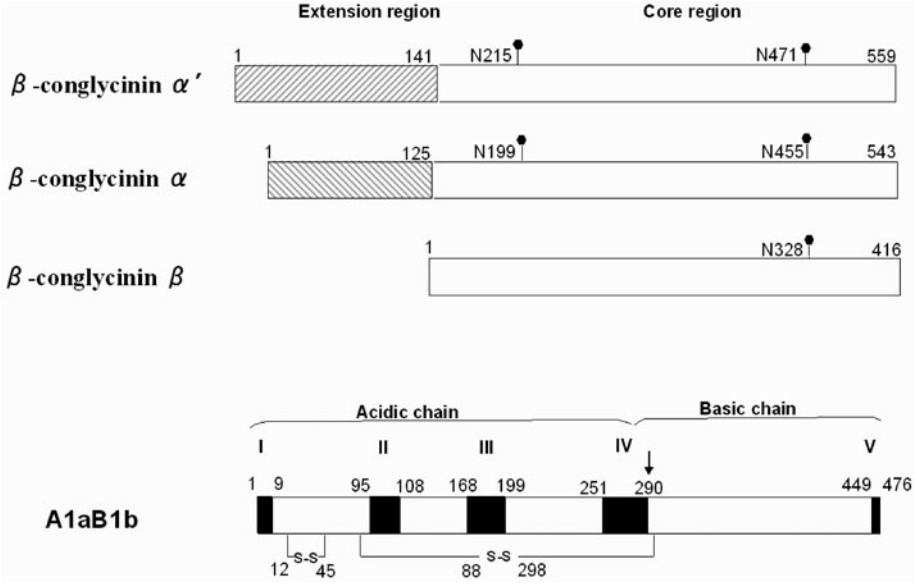



**Figure 1**

Schematic representation of β-conglycinin α′, α, β, and glycinin A1aB1b (*from top to bottom*). The small closed circles on β-conglycinin represent N-glycans. The five variable regions in 11S globulins are indicated by I, II, III, IV, and V, and the processing site is indicated by an arrow (Utsumi 1992, Maruyama et al. 1998).

### Recombinant Technology

Recombinant technology has been used to rapidly obtain highly pure protein in an appreciable quantity. This technique takes advantage of the ability of hosts such as bacteria and yeasts to produce non-endogenous proteins with the aid of expression plasmids. One of the limitations of the usual expression host, *E. coli*, is its inability to carry out posttranslational modifications. Hence, the recombinant 7S globulins are unglycosylated, and the recombinant 11S globulins are uncleaved and unprocessed into mature forms. Both were, however, able to fold correctly and assemble into trimers (Kim et al. 1990, Maruyama et al. 1998, Lauer et al. 2004, Garcia et al. 2006). There is a good number of reports on purification and characterization of food plant proteins over the last three decades as a result of this technology. Soybean is one of the crops that has been intensively investigated (Utsumi et al. 1997, Maruyama et al. 2007). Most initial studies on plant proteins were primarily focused towards improving their nutritional properties, followed by functional and pharmacological studies. Recently, studies were prompted by the search for the molecular basis of their allergenicity and cross reactivity (Albillos et al. 2008; Jin et al. 2009a,b).

## FOOD PHYSICOCHEMICAL PROPERTIES OF SEED STORAGE PROTEINS

### Overview of Food Physicochemical Properties

Storage proteins are the predominant source of amino acids and peptides in seeds. Therefore, they mostly determine the seed's nutraceutical and functional values. Physicochemical properties such as thermal stability, surface hydrophobicity, solubility, and emulsifying and gelling ability are important in their application in the food industry. Protein structure affects physicochemical

properties, and it imparts characteristic texture, hardness, viscosity, and water and fat absorption, among other properties, in foods such as sausages, mayonnaise, salad dressing, gravies, bread, cake, and beverages.

Solubility is perhaps an initial intrinsic property that should be considered in determining the potential use of food proteins because other properties depend highly on this. Fukuda et al. (2005) correlated the emulsifying ability of 7S and 11S globulins in wild-type and cultivated soybeans to solubility. The emulsion formation was found to be most favored by the variety with the most soluble globulins. Consumer acceptability of food products is highly influenced by appearance, which is also dictated by solubility. Furthermore, surface hydrophobicity is correlated with solubility, thermal stability, and foaming and emulsifying abilities. Gelling property is highly influenced by thermal stability. These results established that these functional properties do not work independently of each other. Also, numerous studies have shown that factors like ionic strength and pH greatly affect these properties.



## Food Physicochemical Properties of Soybean Seed Storage Proteins

Soybean has long been used in various food products like tofu gel. Protein gel is a three-dimensional random network of partially denatured proteins formed by heating and subsequent cooling. It can be composed of either random aggregates or polymers of strings of beads (Doi 1993). Both glycinin (Nakamura et al. 1984b) and β-conglycinin (Mohamed Salleh et al. 2004) gels are composed of strings of beads as verified by transmission and scanning electron microscopy. Glycinin is known to have better gelling property than β-conglycinin (Nakamura et al. 1986b, Kohyama et al. 1995). β-conglycinin α, α′, and β were earlier reported to participate uniformly in 7S soy protein gel (Utsumi & Kinsella 1985). A more recent study (Mohamed Salleh et al. 2004) clarified that although no secondary structural differences were observed in the three subunits, they formed gels with different density and strand thickness. The hardest gel was formed from an α′-lacking sample, followed by normal 7S and α-lacking sample. The different wild soybeans surveyed by Fukuda and coworkers (2005) did not have uniform distribution of globulins, and microheterogeneity in the subunits was also reported. Slight variations, such as the natural deletion of four amino acids in the hypervariable region of wild soybean glycinin, affected thermal stability that could in turn affect gelation. Some soybean cultivars were demonstrated to have better gelling property than the others (Nakamura et al. 1984a). Glycinin from the cultivars with the A5A4B3 subunit formed gels twice as fast as those without it, whereas the cultivars with higher A3B4 subunit formed harder gels. Apparently, the five glycinin subunits impart different gelling properties.

β-conglycinin and glycinin gels have different properties (Nakamura et al. 1986a). The gelation mechanism between the two are not the same because sulfhydryl (SH) and disulfide (SS) exchange reactions in β-conglycinin are unlikely. Gel formation of glycinin was described in two steps by Mori et al. (1981). The first step involves the formation of soluble aggregates. Further heating leads to the second step in which the soluble macroaggregates form gel. If protein concentration is low, the dissociation of acidic and basic polypeptides only results in soluble aggregates and no gelation proceeds. The forces involved in gel formation include hydrophobic interactions, hydrogen bonds, ionic interactions, and disulfide bonding (Catsimpoolas & Meyer 1970, Nakamura et al. 1984a, Mori et al. 1986). Two proposed mechanisms for the formation of crosslinked aggregates of proteins during heating are (*a*) inaccessible cysteine residues and disulfide bonds becoming available and reactive to form intermolecular crosslinks and (*b*) aggregation lowering the unfavorable exposure of hydrophobic patches (Visschers & de Jong 2005). Some factors that affect gel formation and quality include pH, ionic strength, protein concentration, heating time, and temperature (Doi 1993). A prerequisite for heat-induced gelation is partial

protein denaturation such that the heating temperature should be above the thermal denaturation midpoint value ($T_m$) of the protein. Sufficient heating time for denaturation to occur is also important. Higher protein concentration forms harder gels.

Other physicochemical properties of α, α′, and β have been studied individually (Maruyama et al. 1998, 1999). Versions of α and α′ subunits without an extension region, $\alpha_c$ and $\alpha'_c$, were also constructed and studied. Their $T_m$ values were as follows: 78.6°C for α, 82.7°C for α′, 90.8°C for β, 77.3°C for $\alpha_c$, and 83.3°C for $\alpha'_c$. Thermal stability, therefore, was dictated mostly by the core region, whereas the extension region showed very little influence. The $T_m$ value of β-conglycinin heterotrimer was determined by the subunit having the lowest $T_m$ value (Maruyama et al. 2002b), but glycinin heterotrimer did not follow this trend (Maruyama et al. 2004a). Surface hydrophobicity was determined by the core regions. Solubility of α and α′ was similar to native proteins, but $\alpha_c$ and $\alpha'_c$ have lower solubility, as with β at $\mu = 0.08$. This showed that the highly acidic extension regions contribute to solubility. Glycan and extension regions prevented heat-induced association and improved the emulsifying ability of β-conglycinin subunits (Maruyama et al. 1999, 2002a).

Other physicochemical properties of glycinin were also studied individually, and their solubility profiles were found to be unique from each other at both $\mu = 0.08$ and $\mu = 0.5$. Prak et al. (2005) reported the following patterns: (*a*) for emulsifying ability, A1bB2 < A2B1a < A5A4B3 < A3B4 ≤ A1aB1b; (*b*) for surface hydrophobicity, A5A4B3 < A1aB1b ≤ A3B4 < A1bB2 < A2B1a; and (*c*) for thermal stability, A1bB2 < A2B1a ≤ A5A4B3 < A3B4 ≤ A1aB1b.

## Food Physicochemical Properties of Seed Storage Proteins of Other Food Crops

Some recombinant 7S globulins and their native forms evaluated for physicochemical and functional properties were from adzuki bean (Fukuda et al. 2007, 2008), mungbean (Garcia et al. 2006), and French bean (Kimura et al. 2008, 2010). The native French bean 7S globulin, phaseolin, has $T_m$ values of 88.3°C and 80.8°C at $\mu = 0.5$ and $\mu = 0.08$, respectively, which are relatively higher than the other 7S globulins from cowpea, fava bean, pea, and soybean. Soybean 7S globulin has the least $T_m$ values of 78.5°C and 65.7°C at $\mu = 0.5$ and $\mu = 0.08$, respectively. The solubility of recombinant phaseolin was better than the recombinant β-conglycinin β, suggesting that phaseolin was inherently more soluble. Like in β-conglycinin, glycan improved the solubility of mungbean 8S globulin or 8Sα, and it had no effect on $T_m$ values of adzuki 7S and mungbean 8Sα. Native adzuki 7S globulins have better emulsifying ability than β homotrimers even if both have no extension region. Glycan has minimal contribution to emulsifying ability of mungbean 8Sα, unlike in β-conglycinin. The remarkable emulsifying ability of native phaseolin was attributed to the glycans. Comparison of native and recombinant phaseolin revealed that N-glycans lower the molecule's surface hydrophobicity by possibly covering a hydrophobic patch on the surface, and they prevented protein aggregation at low ionic strength.

Pea prolegumin, rapeseed procruciferin, and pumpkin pro11S were among the 11S globulins studied (Tandang et al. 2004, Tandang-Silvas et al. 2010). Comparison between cruciferin and glycinin revealed that (*a*) glycinin is more thermally stable, (*b*) cruciferin has higher surface hydrophobicity, (*c*) cruciferin can form harder gels, (*d*) cruciferin has better solubility at $\mu = 0.08$ but was less soluble at $\mu = 0.5$, and (*e*) soybean proteins have better emulsifying ability (Mohamed Salleh et al. 2002). Like procruciferin, pumpkin pro11S has high surface hydrophobicity (Tandang-Silvas et al. 2010). The 11S globulin from sunflower meal, helianthinin, has a high $T_m$ value of 105°C at pH 7.5 and $\mu = 0.54$ (Molina et al. 2004). Hence, it is not a very good candidate in producing gel. Furthermore, it has very low water solubility.

## THREE DIMENSIONAL STRUCTURES OF SEED STORAGE PROTEINS

### Overview

**Figure 2** shows a schematic representation of the secondary structures present in mature glycinin A3B4 subunits and the superimposed structures of 7S and 11S globulins. The acidic N-terminal and the basic C-terminal domains are very similar and can be divided by a pseudo-dyad axis. Each terminal consists of a conservative beta barrel core domain and a divergent alpha helix domain (Itoh et al. 2006, Jin et al. 2009b, Tandang-Silvas et al. 2010). The variable regions among the 11S globulins roughly coincide with the disordered regions in their crystal structures. The 7S and 11S globulins, which both belong to the cupin superfamily, have similar tertiary structures such that their peptide backbones are highly superimposable with each other. Representative cartoon models showing the biological assembly of the 7S, pro11S, and mature 11S globulins are shown in **Figure 3**.

A summary of the seed storage proteins with known crystal structures is presented in **Supplemental Table 1** (follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**). The effects of some structural features on their physicochemical and functional properties have been reported, but some correlations are not clear and still cannot be explained. The deposited structures in protein data bank can be useful in understanding their currently known properties and in conceptualizing their future food applications.

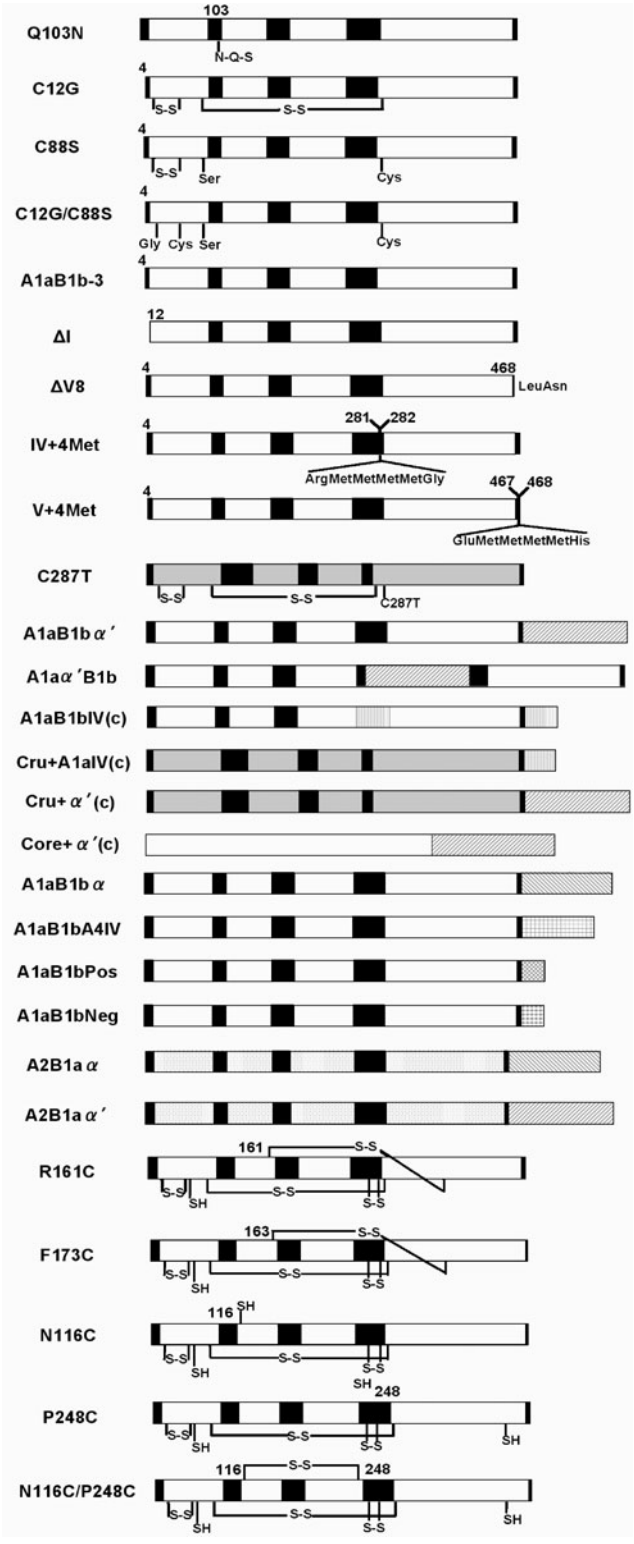
Supplemental Material

### Hydrophilic Domains

The hydrophilic domains in 7S globulins are extension regions, whereas in 11S globulins, they are the variable regions. Glycans of 7S globulins also affect a hydrophilicity. The presence or absence of a glycan had no influence on the crystal structures of recombinant and native β-conglycinin β homotrimers. The root mean square deviation (rmsd) for 343 common $C_\alpha$ atoms within 2.0 Å of the two structures was 0.43–0.51 Å (Maruyama et al. 2001). The extension region, on the other hand, apparently hampers the crystal formation of α′ and α because none of their crystal structures have been determined until after it was removed in α′ (Maruyama et al. 2004b). The crystal structures of 11S globulins generally do not include the five variable regions because they are often disordered. The modified proteins in which variable regions I–V were separately deleted from A1aB1b were still able to assemble into trimers (Kim et al. 1990). These regions are at the molecular surface and were suggested to be suitable targets for modification by protein engineering. Studies have shown that although the variable and extension regions are not important in the structure formation and maintenance of the seed globulins, they can greatly alter their physiochemical and functional properties.

### Disulfide Bridges

Disulfide bridges are known to aid in correct folding and in maintaining the tertiary structure of proteins. The highly conserved intra- and interdisulfide bridges among 11S globulins were, however, found to be unimportant on these aspects. Previous mutations on proA1aB1b to disrupt the intra- (Cys12-Cys45) and interdisulfide (Cys88-Cys298) bridges **(Figure 1**) by creating C12G, C88S, and C12G/C88S (**Figure 4)** mutants did not hamper correct assembly (Utsumi et al. 1993). The crystal structures of C12G and C88S had $C_\alpha$ rmsd values of 0.207 Å and 0.325 Å against the unmodified A1aB1b (Adachi et al. 2003b).



## Structural Factors for Thermal Stability

Structural basis for the higher thermal stability of β ($T_m$ = 90.8°C) compared with α′$_c$ ($T_m$ = 83.3°C) (Maruyama et al. 1998) was addressed by looking at their crystal structures. Structural features of α′$_c$ that could have caused this difference are its larger total cavity volume, its lack of the intermonomer salt bridge present in β, its smaller cluster of charged residues at intermonomer interface, its more hydrophobic solvent accessible surface, its lower percent of proline residues, and its longer loops (Maruyama et al. 2004b). Mungbean 8Sα has relatively lower $T_m$ value (77.5°C) compared with other 7S globulins, mostly owing to its larger cavity size (Itoh et al. 2006). Fukuda et al. (2008) have emphasized that the $T_m$ values of 7S globulins in soybean, mungbean, and adzuki are inversely correlated to cavity size. Adzuki 7S1 and 7S3 have $T_m$ values of 92.4°C and 92.5°C because of their smaller cavity size.

Mature 11S globulins have been reported to be more thermally stable than their proform counterparts (Maruyama et al. 2004a, Tandang et al. 2004, Kimura et al. 2008). Structural analysis of proA3B4 and mature A3B4 revealed that the mature form has smaller cavity size, more number of H bonds, increased hydrophobic interactions, and shorter loops (Tandang-Silvas et al. 2010). Structural features of pro11S globulins from soybean, pea, pumpkin, and rapeseed that may influence thermal stability were also looked at, but no specific feature was singled out as being responsible (Tandang-Silvas et al. 2010). Collective factors such as smaller cavity size, shorter loop, higher aliphatic hydrophobic residues on molecular surface, and more proline residues were mentioned.



# IMPROVEMENT OF PHYSICOCHEMICAL PROPERTIES OF SEED STORAGE PROTEINS BY PROTEIN ENGINEERING

## Protein Engineering

Protein engineering is a systematic approach to alter the primary structure of a protein with the hope of attaining a desired specific effect. The cDNA that encodes the desired protein and the polymerase chain reaction (PCR) primers that will introduce precise nucleotide modifications are the basic requirements in protein engineering. Prior knowledge of the physicochemical and functional properties, and structural information, especially the three dimensional structure, are important tools in developing rational molecular designs for improved food functionality. An expression system for the cDNA and a suitable host are needed to produce the desired proteins. Not all designed modifications lead to successful protein expressions. Very low expression level, insolubility, and instability of the expressed proteins were some of the problems encountered (Utsumi et al. 2002, Tandang et al. 2005).

Safety evaluation and expression of the modified storage globulin genes in transgenic plants to obtain proteins with enhanced functional properties are the final aims of plant food protein engineering. Expression, accumulation, and safety assessments on the introduction of normal or modified soybean genes in crops such as rice and potato had been done to address these goals

**Figure 4**

Schematic representation of the modified proteins. Q103N (Katsube et al. 1998); Aa1B1b-3, C12G, C88S, and C12G-C88S (Utsumi et al. 1993); ΔI, ΔV8, IV+4Met, and V+4Met (Kim et al. 1990); C287T (Tandang et al. 2004); A1aB1bα′, A1aα′B1b, A1aB1bIV(c), Cru+A1aIV(c), Cru+α′(c), and Core+α′(c) (Tandang et al. 2005); A1aB1bα, A1aB1bA4IV, A1aB1bPos, A1aB1bNeg, A2B1aα, and A2B1aα′ (Prak et al. 2007); R161C, F163C, N116C, P248C, and N116C/P248C (Adachi et al. 2004).

(Utsumi et al. 1994, Hashimoto et al. 1999, Momma et al. 2000, Taikawa et al. 2008, Motoyama et al. 2010).

Over the past two decades, proA1aB1b with improved gelling property (Kim et al. 1990, Utsumi et al. 1993, Adachi et al. 2004) and several pro11S globulins with improved solubility and emulsifying property (Kim et al. 1990, Tandang et al. 2005, Prak et al. 2007) were designed successfully at a molecular level through the use of protein engineering. Schematic representations of the modified proteins are shown in **Figure 4,** and their acquired physicochemical and functional attributes discussed above are summarized in **Supplemental Table 2**. Introduction of these specific modified genes in transgenic crops, however, has not yet been done.

Supplemental Material

## Modifications for Improved Emulsifying Ability

Balance between the molecule's hydrophobicity and hydrophilicity defines its surface behavior, which is manifested in functional properties like emulsifying activity. Amphipathic proteins that have polarized hydrophilic and hydrophobic regions can exhibit emulsifying ability (Utsumi 1992).

An earlier approach to improve the emulsifying ability of proA1aB1b was to increase its hydrophobicity by removing the hydrophilic variable regions. The modified proteins A1aB1b-3, ΔI, ΔV8, IV+4Met, and V+4Met exhibited better emulsifying ability than the native glycinin (Kim et al. 1990). The extent of improvement in ΔV8 and V+4Met was more than twice that of the native glycinin. A more recent and more pronounced improvement in solubility and emulsifying ability was achieved by attaching a hydrophilic domain. A1aB1bα′ exhibited a remarkable improvement in both emulsifying ability and emulsion stability (Tandang et al. 2005). A1aα′B1b, however, did not exhibit any improvement on emulsifying ability. These results indicated that the insertion of an α′ extension region was more effective at the C terminus than within variable region IV. One of the rationales behind this, as revealed by the proA1aB1b crystal structure (Adachi et al. 2001), is the higher molecular accessibility of the C terminus because it is situated at the periphery of the molecule. The C-terminal region of 11S globulins seems to be an ideal site of modification to improve emulsifying ability through increasing either its hydrophobicity or hydrophilicity.

Aside from the site where the hydrophilic region will be introduced, the nature of the insert was also found to be critical. For instance, A1aB1bIV(c) and cru+A1aIV(c) did not result in better emulsifying ability (Tandang et al. 2005). Variable region IV from proA1aB1b was not an effective insert for improving emulsion. Prak et al. (2007) correlated the hydrophilicity of the last 20–30 amino acids added to the C terminus with emulsifying ability. A1aB1bα exhibited the best emulsifying ability followed by A1aB1bα′ and A1aB1bA4IV. The α extension region was the most hydrophilic insert followed by the α′ extension region and A4IV polypeptide. A1aB1bPos and A1aB1bNeg exhibited less superior emulsion ability than A1aB1bα even if positive and negative peptides are more hydrophilic than the α extension region. Emulsions from A2B1aα′, cru+α′(c), and core+α′(c) were better than those from their original proteins. These results corroborated the beneficial effect of adding a hydrophilic domain at the C terminus on the emulsifying properties of 11S globulins.

## Modifications for Improved Gelling Properties

Modified proA1aB1b with disrupted disulfide bonds, C12G and C88S, exhibited an improved gelling property (Utsumi et al. 1993). In contrast, the introduction of new disulfide bonds to the same molecule resulted in increased gel hardness (Adachi et al. 2004). Molecular designs to increase the sulfhydryl and disulfide bonds on proA1aB1b included N116C, P248C, R161C, F163C, and N116C · 248C. All of the mutants formed harder gels than proA1aB1b, particularly N116C · 248C.

Remarkable 3.7°C and 4.8°C increases in the $T_m$ values of F163C and N116C · 248C, respectively, were observed. The introduced cysteine residues were designed so that they are far from any inherent cysteine residues. Based on this study, gel hardness increased with the number of cysteine residues. Heat-induced gelation was suggested to be favored by the topology and number of sulfhydryl groups.

In procruciferin, there is a free Cys287 that is very close to Cys283 that forms an interchain disulfide link with Cys83. Modified procruciferin C287T has similar thermal stability with the original protein but heating for 5 min at 80°C and 90°C resulted in more soluble aggregates in C287T (Tandang et al. 2004). The SH/SS exchange reaction with Cys287 was suggested to cause unfavorable association.

The effect of glycosylation in proA1aB1b was investigated by introducing glycosylation consensus sequence Asn-X-Ser/Thr at its variable regions II, III, IV, and V (Katsube et al. 1998). Signal peptide processing, trimer assembly, and targeting of proA1aB1b in the yeast vacuoles were not disturbed by the introduced glycans. Q103N is a glycosylated proA1aB1b at residue 103 due to mutation of Gln103 to Asn, thereby creating Asn-Gln-Ser glycosylation site. Q103N did not acquire thermal stability, but beyond the onset of its $T_m$ value, less coagulation was observed such that it has higher percent of soluble fraction than normal proA1aB1b. The glycan was suggested to inhibit protein-protein interaction. Glycosylated proteins should therefore be avoided in food products that involve thermal gelation. Instead, proteins that easily destabilize and readily coagulate are desirable for food gel products.

## CONCLUSION

Genetic crop improvement of seed storage proteins in legumes, cereals, and other edible plants is important in addressing food quality- and processing-related issues. Recombinant DNA technology and protein engineering have led to expansive and direct studies on the individual subunits of seed storage proteins. Knowledge derived from this research can provide baseline data for crop breeders to produce superior or enhanced genetic lines of industrially important crops. Public awareness on the studies on quality evaluation and safety assessment of genetically modified foods are important to make this powerful approach more acceptable.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

We apologize to all colleagues whose relevant work we couldn't cite because of space limitations. Research in our laboratories was supported in part by grants from Ministry of Education, Culture, Sports, Science, and Technology (S.U. and N.M.), from the Program for Promotion of Basic Research Activities for Innovative Biosciences (S.U. and B.M.) and from the Development of Fundamental Technologies for Production of High-Value Materials Using Transgenic Plants project from the Ministry of Economy, Trade, and Industry (S.U. and N.M.).

## LITERATURE CITED

Adachi M, Chunying H, Utsumi S. 2004. Effects of designed sulfhydryl groups and disulfide bonds into soybean proglycinin on its structural stability and heat-induced gelation. *J. Agric. Food Chem.* 52:5717–23

Adachi M, Kanamori J, Masuda T, Yagasaki K, Kitamura K, et al. 2003a. Crystal structure of soybean 11S globulin: glycinin A3B4 homohexamer. *Proc. Natl. Acad. Sci. USA* 100:7395–400

Adachi M, Okuda M, Kaneda Y, Hashimoto A, Shutov AD, et al. 2003b. Crystal structures and structural stabilities of the disulfide bond–deficient soybean proglycinin mutants C12G and C88S. *J. Agric. Food Chem.* 51:4633–39

Adachi M, Takenaka Y, Gidamis AB, Mikami B, Utsumi S. 2001. Crystal structure of soybean proglycinin A1aB1b homotrimer. *J. Mol. Biol.* 305:291–305

Albillos SM, Jin T, Howard A, Zhang Y, Kothary MH, Fu TJ. 2008. Purification, crystallization and preliminary X-ray characterization of prunin-1, a major component of the almond (*Prunus dulcis*) allergen amandin. *J. Agric. Food Chem.* 56:5352–58

Catsimpoolas N, Meyer EW. 1970. Gelation phenomena of soybean globulins. I. Protein-protein interactions. *Cereal Chem.* 47:559–70

Derbyshire E, Wright DJ, Boulter D. 1976. Legumin and vicilin, storage proteins of legume seeds. *Phytochem.* 15:3–24

Dickinson CD, Hussen EHA, Nielsen NC. 1989. Role of posttranslational cleavage in glycinin assembly. *Plant Cell* 1:459–69

Doi E. 1993. Gels and gelling of globular proteins. *Trends Food Sci. Technol.* 4:1–5

Duranti M, Guerrieri N, Takahashi T, Cerletti P. 1988. The legumin-like storage protein of *Lupinus albus* seeds. *Phytochem.* 27:15–23

FAO (Food and Agriculture Organization of the United Nations). 2003. *Selected indicators of food and agriculture development in Asia-Pacific region 1992–2002*. **http://www.fao.org/docrep/004/ad452e/ad452e1i.htm**

Fukuda T, Maruyama N, Kanazawa A, Abe J, Shimamoto Y, et al. 2005. Molecular analysis and physicochemical properties of electrophoretic variants of wild soybean *Glycine soja* storage proteins. *J. Agric. Food Chem.* 53:3658–65

Fukuda T, Maruyama N, Mohamed Salleh MR, Mikami B, Utsumi S. 2008. Characterization and crystallography of recombinant 7S globulins of adzuki bean and structure-function relationships with 7S globulins of various crops. *J. Agric. Food Chem.* 56:4145–53

Fukuda T, Prak K, Fujioka M, Maruyama N, Utsumi S. 2007. Physicochemical properties of native adzuki bean (*Vigna angularis*) 7S globulin and the molecular cloning of its cDNA isoforms. *J. Agric. Food Chem.* 55:3667–74

Garcia RN, Adachi M, Tecson-Mendoza EM, Bernardo AEN, Utsumi S. 2006. Physicochemical properties of native and recombinant mungbean (*Vigna radiata* L. Wilczek) 8S globulins and the effects of the N-linked glycans. *J. Agric. Food Chem.* 54:6005–10

Garcia RN, Arocena RV, Laurena AC, Tecson-Mendoza EM. 2005. 11S and 7S globulins of coconut (*Cocos nucifera* L.): purification and characterization. *J. Agric. Food Chem.* 53:1734–39

Hashimoto W, Momma K, Katsube T, Ohkawa Y, Ishige T, et al. 1999. Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analyses of the potato tubers and digestibility of the newly expressed protein in transgenic potatoes. *J. Sci.Food Agric.* 79:1607–12

Heller L. 2005. *Monsanto, Solae to create new soy protein line*. **http://www.foodnavigator-usa.com/content/view/print/172486**

Itoh T, Garcia RN, Adachi M, Maruyama Y, Tecson-Mendoza EM, et al. 2006. Structure of 8S$\alpha$ globulin, the major seed storage protein of mung bean. *Acta Cryst.* D62:824–32

Jin T, Albillos SM, Guo F, Howard A, Fu T-J, et al. 2009a. Crystal structure of prunin-1, a major component of the almond (*Prunus dulcis*) allergen amandin. *J. Agric. Food Chem.* 57:8643–51

Jin T, Guo F, Chen Y, Howard A, Zhang Y. 2009b. Crystal structure of Ara h 3, a major allergen in peanut. *Mol. Immunol.* 46:1796–804

Johnson LA. 1999. Process for producing improved soy protein concentrate from genetically-modified soybeans. *U.S. Patent No.* 5936069

Jung R, Scott MP, Nam Y-W, Beaman TW, Bassüner R, et al. 1998. The role of proteolysis in the processing and assembly of 11S seed globulins. *Plant Cell* 10:343–57

Katsube T, Kang IJ, Takenaka Y, Adachi M, Maruyama N, et al. 1998. N-glycosylation does not affect assembly and targeting of proglycinin in yeast. *Biochim. Biophys. Acta* 1379:107–17

Kim C-S, Kamiya S, Sato T, Utsumi S, Kito M. 1990. Improvement of nutritional value and functional properties of soybean glycinin. *Protein Eng.* 3:725–31

Kimura A, Fukuda T, Zhang M, Motoyama S, Maruyama N, Utsumi S. 2008. 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. *J. Agric. Food Chem.* 56:10273–79

Kimura A, Tandang-Silvas MR, Fukuda T, Cabanos C, Takegawa Y, et al. 2010. Carbohydrate moieties contribute significantly to the physicochemical properties of French bean 7S globulin phaseolin. *J. Agric. Food Chem.* 58:2923–30

Kinsella JE. 1979. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* 56:242–58

Kohyama K, Murata M, Tani F, Sano Y, Doi E. 1995. Effects of protein composition on gelation of mixtures containing soybean 7S and 11S globulins. *Biosci. Biotech.Biochim.* 59:240–45

Kwanyuen P, Wilson RF, Burton JW. 1998. Soybean protein quality. In *Emerging Technologies, Current Practices, Quality Control, Technology Transfer and Environmental Issues*, ed. SS Koseoglu, KC Rhee, RF Wilson, p. 285. Champaign, IL: AOCS Press

Lauer I, Foetisch K, Kolarich D, Ballmer-Weber BK, Conti A, et al. 2004. Hazelnut (*Corylus avellana*) vicilin Cor a 11: molecular characterization of a glycoprotein and its allergenic activity. *Biochem. J.* 383:327–34

Maruyama N, Adachi M, Takahashi K, Yagasaki K, Kohno M, et al. 2001. Crystal structures of recombinant and native soybean β-conglycinin β homotrimers. *Eur. J. Biochem.* 268:3595–604

Maruyama N, Katsube T, Wada Y, Oh MH, Barba dela Rosa AP, et al. 1998. The roles of the N-glycans and extension regions of soybean β-conglycinin in folding, assembly and structural features. *Eur. J. Biochem.* 258:854–62

Maruyama N, Mikami B, Utsumi S. 2010. Globulin tanpakushitsu. In *Daitsu no Subete*, ed. K Kitamura, pp. 115–121. Tokyo, Jpn. Sci. Forum

Maruyama N, Mohamed Salleh MR, Takahashi K, Yagasaki K, Goto H, et al. 2002a. The effect of the N-linked glycans on structural features and physicochemical functions of soybean β-conglycinin homotrimers. *JAOCS* 79:139–44

Maruyama N, Mohamed Salleh MR, Takahashi K, Yagasaki K, Goto H, et al. 2002b. Structure-physicochemical function relationships of soybean β-conglycinin heterotrimers. *J. Agric. Food Chem.* 50:4323–26

Maruyama N, Mun LC, Tatsuhara M, Sawada M, Ishimoto M, Utsumi S. 2006. Multiple vacuolar sorting determinants exist in soybean 11S globulin. *Plant Cell* 18:1253–73

Maruyama N, Prak K, Motoyama S, Choi S-K, Yagasaki K, et al. 2004a. Structure-physicochemical function relationships of soybean glycinin at subunit levels assessed by using mutant lines. *J. Agric. Food Chem.* 52:8197–201

Maruyama N, Sato R, Wada Y, Matsumura Y, Goto H, et al. 1999. Structure-physicochemical function relationships of soybean β-conglycinin constituent subunits. *J. Agric. Food Chem.* 47:5278–84

Maruyama N, Tecson-Mendoza EM, Maruyama Y, Adachi M, Utsumi S. 2007. Molecular design of soybean proteins for enhanced food quality. In *Functional Foods and Biotechnology*, ed. K Shetty, G Paliyath, AL Pometto, RE Levin, pp. 25–50. New York: Taylor & Francis

Maruyama Y, Maruyama N, Mikami B, Utsumi S. 2004b. Structure of the core region of the soybean β-conglycinin α′ subunit. *Acta Cryst.* D60:289–97

Mohamed Salleh MR, Maruyama N, Adachi M, Hontani N, Saka S, et al. 2002. Comparison of protein chemical and physicochemical properties of rapeseed cruciferin with those of soybean glycinin. *J. Agric. Food Chem.* 50:7380–85

Mohamed Salleh MR, Maruyama N, Takahashi K, Yagasaki K, Higasa T, et al. 2004. Gelling properties of soybean β-conglycinin having different subunit compositions. *Biosci. Biotechnol. Biochem.* 68:1091–96

Molina MI, Petruccelli S, Añon MC. 2004. Effect of pH and ionic strength modifications on thermal denaturation of the 11S globulin of sunflower (*Helianthus annuus*). *J. Agric. Food Chem.* 52:6023–29

Momma K, Hashimoto W, Yoon H-J, Ozawa S, Fukuda Y, et al. 2000. Safety assessment of rice genetically modified with soybean glycinin by feeding studies on rats. *Biosci. Biotechnol. Biochem.* 64:1881–86

Moreno FJ, Clemente A. 2008. 2S albumin storage proteins: What makes them food allergens? *Open Biochem. J.* 2:16–28

Mori T, Maruyama N, Nishizawa K, Higasa T, Yagasaki K, et al. 2004. The composition of newly synthesized proteins in the endoplasmic reticulum determines the transport pathways of soybean seed storage proteins. *Plant J.* 40:238–49

Mori T, Nakamura T, Utsumi S. 1981. Gelation mechanism of soybean 11S globulin: formation of soluble aggregates as transient intermediates. *J. Food Sci.* 47:26–30

Mori T, Nakamura T, Utsumi S. 1986. Behavior of intermolecular bond formation in the late stage of heat-induced gelation of glycinin. *J. Agric. Food Chem.* 34:33–36

Mori T, Saruta Y, Fukuda T, Prak K, Ishimoto M, et al. 2009. Vacuolar sorting behaviors of 11S globulins in plant cells. *Biosci. Biotechnol. Biochem.* 73:53–60

Motoyama T, Amari Y, Tandang-Silvas MR, Cabanos C, Kimura A, et al. 2010. Development of transgenic rice containing a mutated β subunit of soybean β-conglycinin for enhanced phagocytosis-stimulating activity. *Peptides* 31:1245–50

Müntz K. 1998. Deposition of storage proteins. *Plant Mol. Biol.* 38:77–99

Nakamura T, Utsumi S, Kitamura K, Harada K, Mori T. 1984a. Cultivar differences in gelling characteristics of soybean glycinin. *J. Agric. Food Chem.* 32:647–51

Nakamura T, Utsumi S, Mori T. 1984b. Network structure formation in thermally induced gelation of glycinin. *J. Agric. Food Chem.* 32:349–52

Nakamura T, Utsumi S, Mori T. 1986a. Mechanism of heat-induced gelation and gel properties of soybean 7S globulins. *Agric. Biol. Chem.* 50:1287–93

Nakamura T, Utsumi S, Mori T. 1986b. Interactions during heat-induced gelation in a mixed system of soybean 7S and 11S globulins. *Agric. Biol. Chem.* 50:2429–35

Nielsen NC, Evans DE, Wilcox JR. 1992. Special purpose soybean varieties. *New Crop News*. **http://www.hort.purdue.edu/newcrop/newcropsnews/92-2-1/soybean.html**

Osborne TB. 1924. *The Vegetable Proteins*. London: Longmans

Prak K, Nakatani K, Katsube-Tanaka T, Adachi M, Maruyama N, Utsumi S. 2005. Structure-function relationships of soybean proglycinins at subunit levels. *J. Agric. Food Chem.* 53:3650–57

Prak K, Nakatani K, Maruyama N, Utsumi S. 2007. C-terminus engineering of soybean proglycinin: improvement of emulsifying properties. *Protein Eng. Design Selection* 20:433–42

Shewry PR, Halford NG. 2002. Cereal seed storage proteins: structures, properties and role in grain utilization. *J. Experimental Bot.* 53:947–58

Shewry PR, Napier JA, Tatham AS. 1995. Seed storage proteins: structures and biosynthesis. *Plant Cell* 7:945–56

Soyatech, LLC. 2010. *Soy facts*. **http://72.32.142.180/soy_facts.htm**

Soyatech, LLC, SPINS Inc. 2005. *Soyfoods: The U.S. Market 2005*. **http://www.soyatech.com/pdf/soyfoods05.pdf**

Taikawa F, Sakuta C, Choi S-K, Tada Y, Motoyama T, Utsumi S. 2008. Co-expression of soybean glycinins A1aB1b and A3B4 enhances their accumulation levels in transgenic rice seed. *Plant Cell Physiol.* 49:1589–99

Tandang MR, Adachi M, Inui N, Maruyama N, Utsumi S. 2004. Effects of protein engineering of canola procruciferin on its physicochemical and functional properties. *J. Agric. Food Chem.* 52:6810–17

Tandang MR, Atsuta N, Maruyama N, Adachi M, Utsumi S. 2005. Evaluation of the solubility and emulsifying property of soybean proglycinin and rapeseed procruciferin in relation to structure modified by protein engineering. *J. Agric. Food Chem.* 53:8736–44

Tandang-Silvas MR, Fukuda T, Fukuda C, Prak K, Cabanos C, et al. 2010. Conservation and divergence on plant seed 11S globulins based on crystal structures. *Biochim. Biophys. Acta* 1804:1432–42

Utsumi S. 1992. Plant food protein engineering. *Adv. Food Nutr. Res.* 36:89–208

Utsumi S, Gidamis AB, Kanamori J, Kang IJ, Kito M. 1993. Effects of deletion of disulfide bonds by protein engineering on the conformation and functional properties of soybean proglycinin. *J. Agric. Food Chem.* 41:687–91

Utsumi S, Kinsella JE. 1985. Structure-function relationships in food proteins: subunit interactions in heat-induced gelation of 7S, 11S and soy isolate proteins. *J. Agric. Food Chem.* 33:297–303

Utsumi S, Kitagawa S, Katsube T, Higasa T, Kito M, et al. 1994. Expression and accumulation of normal and molecular designed soybean glycinins in potato tubers. *Plant Sci.* 102:181–88

Utsumi S, Maruyama N, Satoh R, Adachi M. 2002. Structure-function relationships of soybean proteins revealed by using recombinant systems. *Enzyme Microbial Technol.* 30:284–88

Utsumi S, Matsumura Y, Mori T. 1997. Structure-function relationships of soy proteins. In *Food Proteins and Their Applications*, ed. D Damodaran, A Paraf, pp. 257–91. New York: Marcel Dekker

Visschers RW, de Jongh HHJ. 2005. Disulphide bond formation in food protein aggregation and gelation. *Biotech. Adv.* 23:75–80

Wieser H. 2007. Chemistry of gluten proteins. *Food Microbiol.* 24:115–19

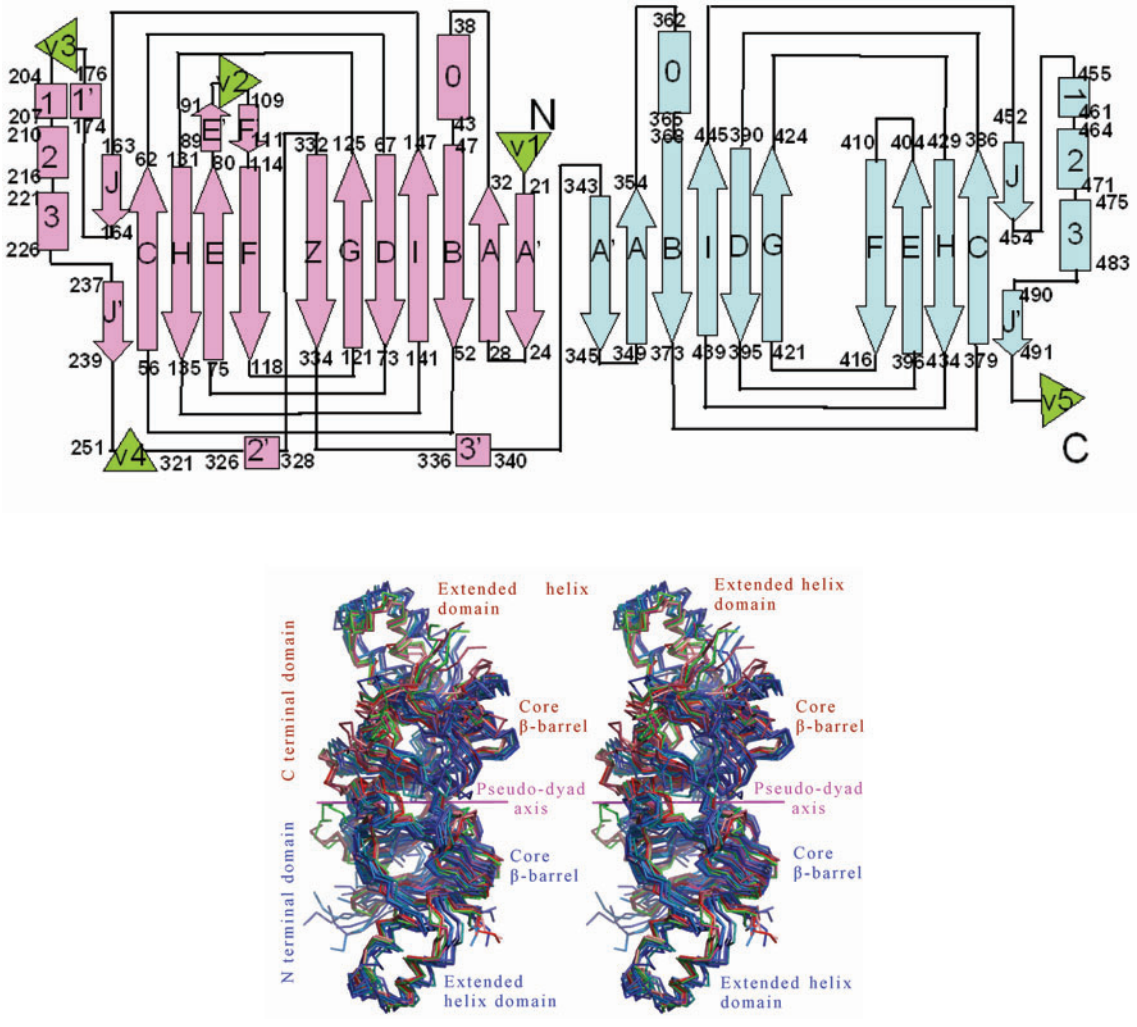



**Figure 2**

Schematic diagram of the secondary structures in mature glycinin A3B4 (PDB id: 2D5H) (*top*) (from Maruyama et al. 2010 with permission from the publisher). The N-terminal and C-terminal domains are in pink and cyan, except for the variable regions (v1–v5), which are represented by green triangles. N and C indicate the N- and C-terminal ends. 7S and 11S globulin monomers are superimposed (*bottom*) (from Tandang-Silvas et al. 2010 with permission from the publisher).

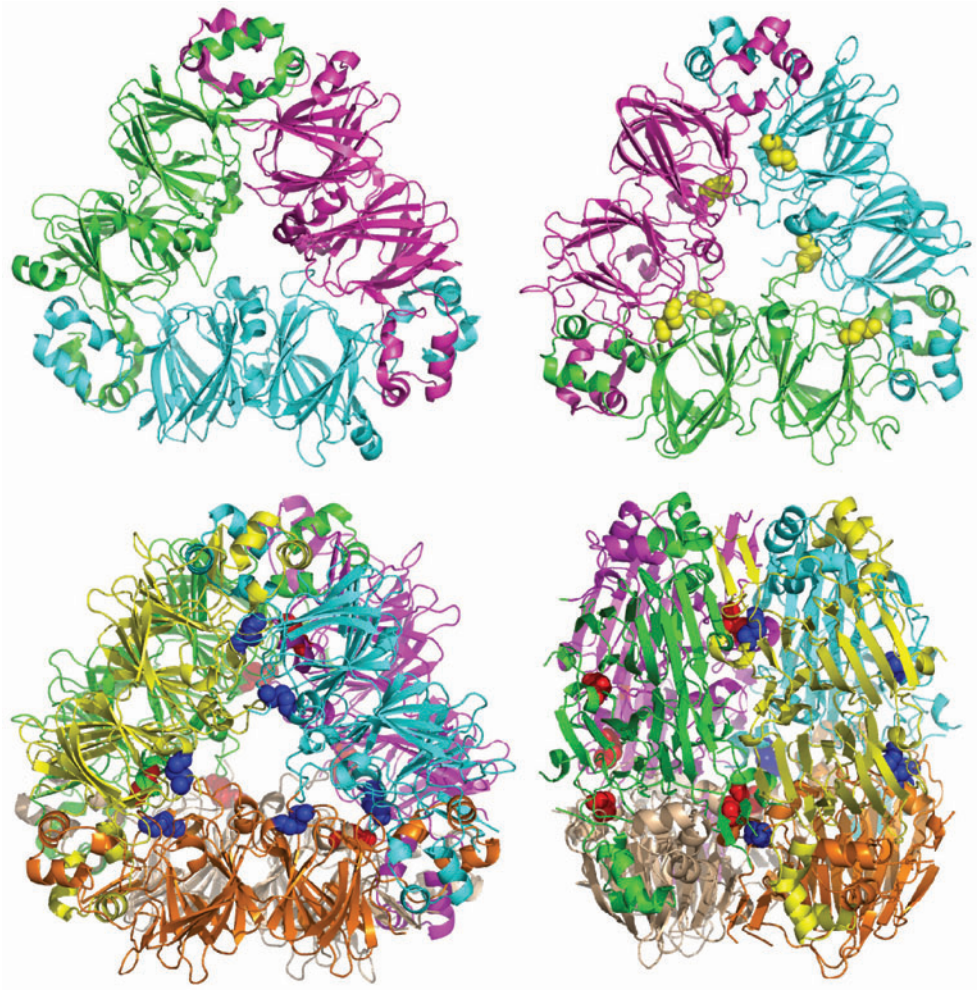


**Figure 3**

The cartoon models of β-conglycinin α′ core (*top left*, PDB id 1UIK), proA3B4 (*top right*, PDB id 2D5H), and mature A3B4 (PDB id 2D5F) at threefold axis (*bottom left*) and side view (*bottom right*). The disulfide bridges are designated by spheres. Images were generated using the program Pymol.

Annual Review
of Food Science
and Technology

Volume 2, 2011

# Contents

**Errata**

An online log of corrections to *Annual Review of Food Science and Technology* articles may be found at http://food.annualreviews.org