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Review

Recent Progress in Research and Technology on Soybeans

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For a long time, it had been considered that soybean storage proteins play only a role as traditional nutrients and other soybean minor constituents such as isoflavones, saponins, trypsin inhibitors, phytic acid, lectin, etc., act as antinutrient factors. At present, however, these substances have all been recognized to have exciting roles in the prevention of heart disease, cancer, osteoporosis, etc. Besides these physiological effects, soybean storage proteins exhibit excellent functional properties physicochemically in food systems, such as gelation, binding, emulsification, fat and water absorption, etc. On the other hand, there are some substances having undesirable properties in soybeans, such as off-flavors, allergens, etc. Recently, there was a great progress in the research of a molecular basis on these functionalities, off-flavors, and allergenicities. By applying these results for soybean breeding, the creation of the new cultivars or lines having more improved properties is in progress. Another highlight in soybean research is the success of the crystallization of β-conglycinin and glycinin and the subsequent complete determination of their three-dimensional molecular structures through X-ray crystallographic analysis. This paper overviews these recent investgations.

Keywords: soybean, β-conglycinin, glycinin, physiological function, three-dimensional structure, isoflavone, lypoxygenase-free, allergen-less

1. Introduction

For more than 2000 years have people throughout East Asia consumed soybeans in the form of traditional soy foods, such as nimame (cooked whole soy), edamame (green fresh soy; Fukushima, 2000a), soy milk (Fukushima, 1994), tofu (Fukushima, 1981), kori-tofu (freeze-denatured and dry tofu; Fukushima, 1980 and 1994), abura-age (deep-fat-fried tofu; Fukushima, 1981), sufu or tofu-yo (fermented tofu; Fukushima, 1981 and 1985), soy sauce (Fukushima, 1985 and 1989), miso (Fukushima, 1985), natto (Fukushima, 1985), tempeh (Fukushima & Hashimoto, 1980), etc. In Western countries, soybeans had become to draw people's attention in 1960s as an economical and high quality vegetable protein source for humans. In the United States, new soy protein products were developed, such as soy flour, soy protein concentrates, soy protein isolates, and their texturized products. These soy products were introduced into Japan at the end of 1960s, but their consumption remains only 40,000 metric tons as products (see Table 1). The consumption of soybeans as foods in Japan is mostly to the traditional soy foods, for which about one million metric tons of soybeans and soybean meal are used, as shown in Table 1. The manufacturing techniques and equipments of these traditional soy foods had made a great progress through the technical innovations after the World War II and the modernization of the manufacturing had almost been achieved, until the end of 1980.

In Western countries, the history of soybeans for human consumption is only several decades, where the non-traditional protein products described above are mainly used as ingredients in formulated foods for their functional properties, such as water and fat absorption, emulsification, foaming, gelation, binding, etc. These soy foods have penetrated steadily into Western countries as healthy foods, but the growth is not so high as they expected, perhaps owing to their strong off-flavors associated with their products. However, the consumption of soy foods in the United States has begun to increase abruptly with a turning point in 1997 (Liu, 2000). Undoubtedly, this increase is due to the penetration of the recognition that soybeans possess the exciting physiological functions. Namely, numerous investigations during the 1990s put soybeans in the spotlight, where soybean storage proteins and soybean minor components traditionally considered to be antinutritional factors have been recognized to have exciting roles in the prevention of chronic disease. Furthermore, FDA authorized "Soy Protein Health Claim" on October 26, 1999, that 25 grams of soy protein a day may reduce the risk of heart disease. Since the market is very much responsive to this Health Claim, soy foods will penetrate rapidly into Western cultures and diets.

In the processing and utilization of soybeans, the following four points are very important. One is the nutritional and physiological aspects, the second is the functional properties working in food systems, the third is the unfavorable substances such as off-flavors, allergens, etc., and the forth is the creation of the beneficial cultivars and lines. This paper deals with the review on the recent progress in these subjects.

2. Physiological Functions of Soybeans and Soy Food Products

Reevaluation of nutritive value of soybean storage proteins The quality of soybean proteins has actually been undervalued until recently, because the protein efficiency ratio was based upon the growth of laboratory rats. Growing rats not only possess a much higher requirement for proteins than infants, but also a much higher need for certain amino acids than humans (Steinke, 1979). Particularly, the rat requirement for methionine

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Table 1. Consumption of traditional soy food products in 1998 in Japan.

	Soybeans[a)]	Soybean meal[b)]	Total
Tofu and its derivatives	496,000	0	496,000
Kori-tofu	28,000	0	28,000
Natto	128,000	0	128,000
Miso	162,000	0	162,000
Soy sauce	26,300	157,600	183,900
Soy milk	4,200	0	4,200
Major traditional products (Total above)	844,500	157,600	1,002,100
Non-traditional products (Soy proteins)		4,000 (as product)	4,000 (as product)
Food use total	1,032,000	401,000[c)]	1,433,000[c)]

Source: [a)]Shokuhin Sangyou Shinbunsha and [b)]Ministry of Agriculture, Forestry, and Fishery. [c)]Including non-food meal other than feeds.

Table 2. Patterns of amino acid requirements and soybean amino acid composition.

Amino acid (mg/g protein)	Pattern of requirement				Amino a. comp. of soybeans
	3–4 Mo.	2–5 Yr.	10–12 Yr.	Adult	
His	26	19	19	16	27
Ile	46	28	28	13	48
Leu	93	66	44	19	78
Lys	66	58	44	16	61
Met + Cys	42	25	22	17	26
Phe +Tyr	72	63	22	19	90
Thr	43	34	28	9	35
Trp	17	11	9	5	13
Val	55	35	25	13	48
Total (Including His)	460	339	241	127	426
Total (Minus His)	434	320	222	111	399

Source: Joint FAO/WHO/UNU Expert Consultation (1985).

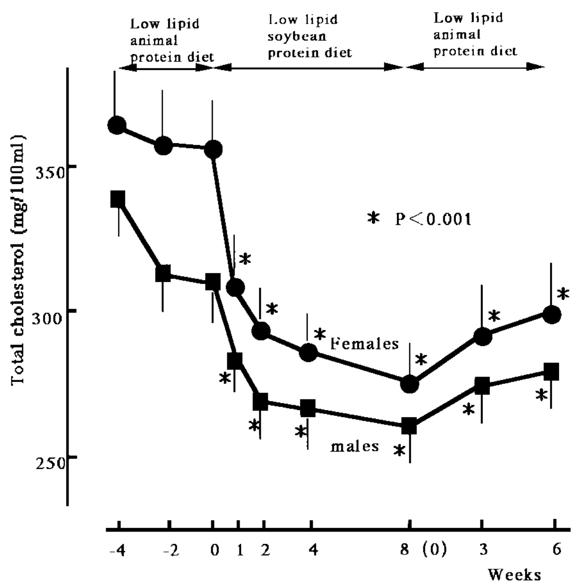


Fig. 1. Total cholesterol levels in type II patients treated with soy protein diets. Mark (*) indicates highly significant difference from mean plasma lipid levels during the term before soy protein diets. Source: Descovich *et al.* (1980).

is about 50% higher (Sarwar, 1985). According to the Report of a Joint FAO/WHO/UNU Expert Consultation in 1985, the amino acid requirements are different depending upon human age and methionine is not a limiting amino acid for soybean proteins, except infants (see Table 2; Fukushima, 1991a). Both the World Health Organization (WHO) and the United States Food and

Table 3. Meta-analysis of effects of soy protein intake.

	No. of studies	No. of subjects	Change (mg/dl)	95% CI[a)]	Change (%)
Total cholesterol	38	730	–23.2	–32.9~–13.5	–9.3
LDL cholesterol	31	564	–21.7	–31.7~–11.2	–12.9
HDL cholesterol	30	551	+1.2	–3.1~+5.4	+2.4
VLDL cholesterol	20	255	–0.4	–4.6~+3.9	–2.6
Triglyceride	30	628	–13.3	–25.7~–0.3	–10.5

[a)]Confidence interval. Source: Anderson *et al.* (1995).

Drug Administration (FDA) adopted the protein digestibility corrected amino acid score (PDCAAS) as the official assay for evaluating protein quality. Soybean proteins have a PDCAAS of 1.0, indicating that it is able to meet the protein needs of children and adults when consumed as the sole source of protein at the recommended level protein intake of 0.6 g/kg body wt. (Young, 1991). It is now concluded that the quality of soybean proteins is comparable to that of animal protein sources such as milk and beef.

Physiological functions of soybean storage proteins Formerly, soybean proteins had been considered to play only a role as traditional nutrients. In the latter half of 1970s, however, it was found that soybean proteins have a hypocholesterolemic effect. As shown in Fig. 1 (Descovich *et al.*, 1980), the animal proteins in the diet are exchanged with soybean proteins, the serum cholesterol is lowered markedly. Since then, numerous investigations on the hypocholesterolemic effect of soybean proteins have been carried out. According to a meta-analysis of 38 separate studies involving 743 subjects, the consumption of soy protein resulted in significant reduction in total cholesterol

(9.3%), LDL cholesterol (12.9%), and triglycerides (10.5%), with a small but insignificant increase (2.4%) in HDL cholesterol (see Table 3; Anderson *et al*., 1995). In linear regression analysis, the threshold level of soy intake at which the effects on blood lipids became significant was 25 g. Thus, soy protein represents a safe, viable, and practical nonpharmacologic approach to lowering cholesterol. It is clear that soybean storage proteins possess the hypocholesterolemic effect in themselves, because the plasma total cholesterol of the rats fed casein-cholesterol diets was reduced by 35 and 34% by the administration of purely isolate β-conglycinin and glycinin, respectively (Lovati *et al*., 1992). The exact mechanism of the cholesterol reduction has not been established fully. Some suggest that cholesterol absorption and/or bile acid reabsorption is impaired, when soybean proteins are fed, while others propose that changes in endocrine status, such as alteration in insulin to glucagon ratio and thyroid hormone concentrations, are responsible (Potter, 1995).

In addition to the cholesterol-lowering effects described above, soybean proteins suppress the lipogenic enzyme gene expression in the livers of genetically fatty rats (Wistar fatty rats), indicating that dietary soybean proteins are useful for the reduction of body fats (Iritani *et al*., 1996).

Physiologically active fragments derived from soybean storage protein molecules It has been suggested that some hydrophobic polypeptides produced through proteolytic hydrolysis of soybean proteins, which bind well to bile acids, are involved in the hypocholesterolemic effect of soybean proteins (Iwami *et al*., 1986; Sugano *et al*., 1988). Minami *et al*. (1990) found that the A_{1a} and A_2, the acidic polypeptides of the glycinin subunits $A_{1a}B_{1b}$ and A_2B_{1a}, strongly combine to bile acids. Further, they obtained the peptide fraction of Ile (114)–Arg (161) with 48 amino acid residues through a tryptic digestion of A_{1a} peptide. This peptide has a high hydrophobicity and provides a binding site to bile acids.

Besides these, there are many physiologically active peptide fragments derived from storage protein molecules, which have antioxidative activities (Chen *et al*., 1995), the inhibitory action for angiotensin converting enzymes (Kawamura, 1997), or the promoting action for phagocytosis (Yoshikawa *et al*., 1993), as shown in Table 4.

Physiological functions of soybean minor components Another importance in the physiological action of soybeans is that soybean minor components have exciting roles in the prevention of chronic disease (see Table 5). Hitherto, these minor components, such as isoflavones, saponins, trypsin inhibitors, phytic acid, lectin, etc., were thought to be antinutritional factors, but now they are recognized to have preventing effects on cancer (Messina & Barnes, 1991). Among these, isoflavones (mainly genistein and daidzein) are particularly noteworthy, because soybeans are the only significant dietary source of these compounds. Isoflavones have not only anticarcinogenic activities, but also the preventive effects on osteoporosis (Anderson & Garner, 1997) and the alleviation of menopausal symptoms (Albertazzi *et al*., 1998).

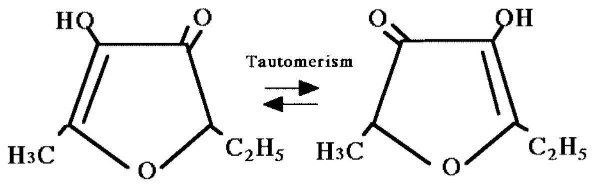


Fig. 2. 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone. Abbreviated as HEMF.

Physiologically functional substances produced by microorganisms contained in fermented soy foods The physiologically functional substances described above are the components originally contained in soybeans. Besides these substances, however, some of the traditional fermented soy foods, such as soy sauce, miso, natto, and tempeh, have the physiologically active substances which are produced by microorganisms. Particularly, it is very interesting that HEMF (see Fig. 2), the key flavor component in Japanese-style fermented soy sauce, has quite a strong anticarcinogenic activity (Nagahara *et al*., 1992). HEMF is biosynthesized through the pentose-phosphate cycle by the yeast during the fermentation (Sasaki, 1996). Therefore, it is not present at tamari-type soy sauce, because of the lack of yeast fermentation. HEMF is a very unique compound which is not contained in foods other than Japanese-style fermented soy sauce and miso (Sasaki, 1996). The HEMF content in miso is low, namely around 0–7 ppm (Sugawara, 1991; Hayasida, 1999),

Table 4. Physiologically active peptide fragments from soybean storage protein.

	Peptide fragments	Protein source
Antioxidant activities	VNPHQN[a]	β-Conglycinin
	LVNPHDHQN[a]	β-Conglycinin
	LLPHH[a]	β-Conglycinin
	LLPHHADADY[a]	β-Conglycinin
	VIPAGYP[a]	β-Conglycinin
	LQSGDALRVPSGTTYY[a]	β-Conglycinin
Inhibition of angiotensin-converting enzymes	FVIPAGY[b]	α,α'(β-Conglycinin subunit)
	ASUDTLF[b]	α,α' (β-Conglycinin subunit)
	DQTPRVF[b]	$A_5A_4B_3$ (Glycinin subunit)
	YRILEF[b]	α'(β-Conglycinin subunit)
Promoting action of phagocytosis	MITLAIPVNKPGR[c]	α'(β-Conglycinin subunit)
	HCQRPR[d]	$A_{1a}B_{1b}$ (Glycinin subunit)
	QRPR[d]	$A_{1a}B_{1b}$ (Glycinin subunit)

Source: [a]Chen *et al*. (1995); [b]Kawamura (1997); [c]Tanaka *et al*. (1994); and [d]Yoshikawa *et al*. (1993).

Table 5. Physiological functions of minor components contained in soybeans.

Isoflavones	Anticarcinogenic activities[a], prevention of cardiovascular diseases[b], prevention of osteoporosis[c], antioxidant activeties[d], and alleviation of menopausal symptoms[e].
Saponins	Anticarcinogenic activities[a),f),g)], hypocholesterolemic effects[f], Inhibition of platelet aggregation, HIV preventing effects (group B saponin)[h], and antioxidant activities (DDMP saponin)[i].
Phytosterol	Anticarcinogenic activities[a].
Phytic acid	Anticarcinogenic activities[a),f)].
Lectin (Hemagglutinin)	Activation of lymphocytes (T cell)[h] and aggregating action of tumor cells[h].
Nicotianamine	Inhibitor of angiotensin-converting enzymes[j), k)].
Protease inhibitors	Anticarcinogenic activities[a),f)].

[a]Hawrylewicz *et al*. (1995); [b]Setchell and Cassidy (1999); [c]Anderson and Garner (1997); [d]Yoshiki and Okubo (1997); [e]Albertazzi *et al*. (1998); [f]Messina and Barnes (1991); [g]Rao and Sung (1995); [h]Harada (1999); [i]Yoshiki and Okubo (1995); [j]Kinoshita *et al*. (1993); and [k]Kinoshita *et al*. (1994).

whereas that in Japanese-style fermented soy and sauce is very high, namely around 230 ppm. HEMF is effective when it is fed to mouse at 4 mg/kg body wt./day, indicating that it is a potent anticarcinogen. As another physiological effect, fermented soy sauce has the activity to inhibit angiotensin I-converting enzyme, but this activity is mostly ascribed to nicotianamine (Kinoshita *et al*., 1994), which is the constituent of soybeans (see Table 5).

Natto made through the fermentation by *Bacillus natto* has a strong fubrinolytic activity, which is due to the enzyme named nattokinase, produced by *Bacillus natto* (Sumi *et al*., 1987). Natto also has anti-tumor-promoting activity, because the extract prevented the reduction of dye transfer caused by a typical tumor promoter of 12-O-tetradecanoylphorbol-13-acetate (Takahashi *et al*., 1995). Besides these, natto possesses the activities to inhibit angiotensin I-converting enzyme, as soy sauce does (Okamoto *et al*., 1995). The substances responsible for these activities have not been elucidated yet. However, it is certain that nicotianamine does not relate to these activities, because it disappears during the fermentation of natto (Kinoshita *et al*., 1994).

Table 6. Functional properties of soybean storage proteins and their subunits working physicochemically in food systems.

Functionality	Proteins or subunits	Property or its difference
Gel formation	β-Conglycinin	Transparent, soft, but rather elastic gel.
	Glycinin	Turbid, hard, and not so fragile gel.
	A_2B_{1a} subunit	A_2 polypeptide relates to gel hardness.
	A_3B_4 subnunit	A_3 polypeptide relates to gel hardness.
	$A_5A_4B_3$ subunit	$A_5A_4B_3$ subunit relates to the easiness of gel formation.
Thermal stability	Soybean storage proteins	β-Conglycinin<Glycinin
	β-Conglycinin subunits	α<α'<β
Emulsification	Soybean storage protein	β-Conglycinin>Glycinin
	β-Conglycinin Subunits	α≧α'≫β

Source: Utsumi *et al*. (1997).

3. Physicochemical Functions of Soybean Storage Proteins

Approximately 90% of the proteins in soybeans exist as storage proteins, which mostly consist of β-conglycinin and glycinin. β-Conglycinin (Koshiyama, 1965; Catsimpoolas & Ekenstam, 1969; Koshiyama & Fukushima, 1976a) has the sedimentation coefficients of 7S, whereas glycinin (Mitsuda *et al*., 1965) has that of 11S. Besides β-conglycinin, there are two kinds of globulins which have the sedimentation coefficient of 7S. They are γ-conglycinin (Catsimpoolas & Ekenstam, 1969; Koshiyama & Fukushima, 1976b) and basic 7S globulin (Yamauchi *et al*., 1984). However, these two 7S globulins are minor components which account for less than a few percent. The major storage proteins of β-conglycinin and glycinin possess a variety of functional properties physicochemically for food applications as shown in the introduction. These functional properties are ascribed to the intrinsic physicochemical characteristics which are based on the molecular structures. Therefore, this chapter focuses on recent developments in the structure-function relationship of β-conglycinin and glycinin.

Basic structures of β-conglycinin and glycinin molecules β-Conglycinin is a glycoprotein and a trimer with a molecular mass of 150–200 kDa. Major subunits are α' (72 kDa), α (68 kDa), and β (52 kDa) (Thanh & Shibasaki, 1977). Besides these, there is a minor subunit called γ in β-conglycinin (Thanh & Shibasaki, 1977). The amino acid sequences of these subunits are similar each other (Hirano *et al*., 1987). Each of α' and α subunits possesses one cysteine residue (-SH) near the N-termini, whereas β subunit does not possess any cysteine residue (Utsumi *et al*., 1997). No cystine residues (-SS-) exist in these subunits. β-Conglycinin exhibits molecular heterogeneity, where six molecular species are identified as $\alpha'\beta_2$, $\alpha\beta_2$, $\alpha\alpha'\beta$, $\alpha_2\beta$, $\alpha_2\alpha'$, and α_3 (Thanh & Shibasaki, 1978; Yamauchi *et al*., 1981). In addition, Yamauchi *et al*. (1981) found another species of β_3. β-Conglycinin trimers cause association or dissociation depending upon the pHs and ionic strengths of the solution (Thanh & Shibasaki, 1979).

Glycinin is a hexamer with a molecular mass of 300–380 kDa. Each subunit is composed of acidic (~35 kDa) and basic (~20 kDa) polypeptides, which are linked together by a disulfide bond (Staswick *et al*., 1984). In glycinin, five subunits are identified as $A_{1a}B_{1b}$ (53.6 kDa), A_2B_{1a} (52.4 kDa), $A_{1b}B_2$ (52.2 kDa), $A_5A_4B_3$ (61.2 kDa) and A_3B_4 (55.4 kDa), which are classified into group I ($A_{1a}B_{1b}$, A_2B_{1a} , $A_{1b}B_2$) and group II ($A_5A_4B_3$, A_3B_4) by the extent of the homology (Nielsen, 1985; Nielsen *et al*., 1989). Each subunit in group I has two cysteine and three cystine residues, whereas that in group II has two cysteine and two cystine residues (Utsumi *et al*., 1997). Glycinin subunits exhibit polymorphism, that is, there are some amino acid replacements in the same kind of subunit among soybean cultivars (Mori *et al*., 1981; Utsumi *et al*, 1987). Moreover, glycinin exhibits molecular heterogeneity, because the molecule is a hexamer with different subunit composition (Utsumi *et al*., 1981). Glycinin hexamers dissociate to their constituent polypeptides, subunits, and half-molecules, depending upon pHs, ionic strengths, and heating temperatures (Wolf & Briggs, 1958; Mori *et al*., 1982).

Physicochemical functionalities and three-dimensional structures of protein molecules The difference of the functionalities on the gel formation, thermal stability, and emulsification, in soybean storage proteins and their subunits is shown in Table 6 (Utsumi *et al*., 1997). The mechanisms on the gel formation of β-conglycinin (Nakamura *et al*., 1986) and glycinin (Mori *et al*., 1982; Nakamura *et al*., 1984) are studied in details. Glycinin forms a turbid, hard, and not fragile gel, whereas β-conglycinin forms a transparent, soft, but rather elastic gel, in 100°C heating (Utsumi *et al*, 1997). The A_2 polypeptide of glycinin A_2B_{1a} subunit closely relates to gel turbidity, whereas the A_3 polypeptide of the A_3B_4 subunit relates to the gel hardness. The hardness of glycinin gel increases in proportion to the content of A_3 polypeptide. The $A_5A_4B_3$ subunit relates to the easiness of gel formation, because of the easy cleavage of the hydrophobic bonds between the A_5 and A_4 acidic chains during heating. β-Conglycinin is more unstable thermally than glycinin, but the emulsifying and emulsion-stabilizing abilities of β-conglycinin are much stronger than those of glycinin.

The physicochemical functions of proteins depend upon their three-dimensional structures substantially. The polypeptide chains of the protein molecules are unfolded through the heat treatment of soybeans and as a result the amino acid side resi-

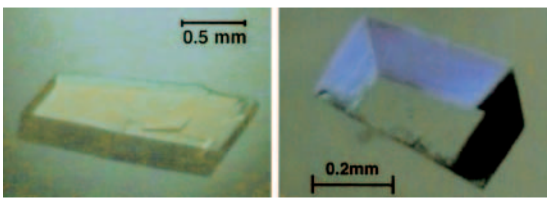
Table 7. Number of cysteine and cystine in each subunit of β-conglycinin and glycinin.

	Subunit	Cysteine (-SH)	Cystine (-SS-)
β-Conglycinin	α'	1	0
	α	1	0
	β	0	0
Glycinin	$A_{1a}B_{1b}$	2	3
	A_2B_{1a}	2	3
	$A_{1b}B_2$	2	3
	A_3B_4	2	2
	$A_5A_4B_3$	2	2

Source: Utsumi *et al*. (1997).

dues buried inside a molecule are exposed on the surface. The exposed -SH or hydrophobic residues combine the protein molecules through -SH, -SS- interchange reaction or hydrophobic bonding, respectively. In this case, it is very important that these active residues are present at an accessible location of the molecules each other. Table 7 shows the numbers of -SH and -SS- groups in each subunit. The larger numbers of -SH groups and their topology in glycinin make glycinin gel much harder and more turbid in comparison with β-conglycinin gel, whereas the higher hydrophobicity and more easily unfolded structure in β-conglycinin make its emulcifying ability much stronger than that of glycinin (Utsumi *et al*., 1997).

In order to improve these functional properties, it is necessary to know about the theoretical relations between the functional properties and the three-dimensional structures of the molecules. The research on the three-dimensional structures of soybean storage proteins started 35 years ago. We investigated the three-dimensional structures of β-conglycinin and glycinin molecules through optical rotatory dispersion (ORD), circular dichroism (CD), infrared absorption spectra, ultra-violet difference spectra, deutration studies, etc. (Fukushima; 1965, 1967, and 1968). However, these methods are the indirect ones for the measurement of three-dimensional structures. For a direct and complete analysis of three-dimensional structures, soybean proteins must be crystallized, followed by a X-ray crystallographic analysis. The complete amino acid sequence of molecular subunits of soybean storage proteins has been determined in early 1980s through the sequence analysis of full-length cDNA and a genomic clone (see the review of Fukushima, 1988, 1991a, and 1991b). For a long time, however, the X-ray analysis of soybean proteins have not been carried out, because the molecular heterogeneities in both β-conglycinin and glycinin obstructed their crystallization. Utsumi's group has overcome these difficulties by using a special soybean variety, of which β-conglycinin molecules or glycinin molecules are composed of the same kinds of subunits [β homotrimer (3β) in β-conglycinin and A_3B_4 homohexamer (6 A_3B_4) in glycinin]. Thus, they have succeeded in the crystallization of both β homotrimer β-conglycinin and A_3B_4 homohexamer glycinin (see Fig. 3) and in the subsequent X-ray crystallographic analysis of the three-dimensional structures of their molecules (Maruyama *et al*.,1999; Adachi *et al*., 1999). The schematic diagrams of the polypeptides are shown in Fig. 4 and 5 (Fukushima, 2000b). The success of the complete analysis of the three-dimensional structures should be mentioned to be epoch-making in knowing the mechanisms of the functionalities of soybean proteins, because most of the properties of proteins are ascribed to the conformation of the molecular surface in the three-dimensional structures of the molecules. Furthermore, the



(A) (B)

Fig. 3. The crystals of β-conglycinin β homotrimer (A) and glycinin A_3B_4 homohexamer (B) (By the courtesy of Dr. S. Utsumi).

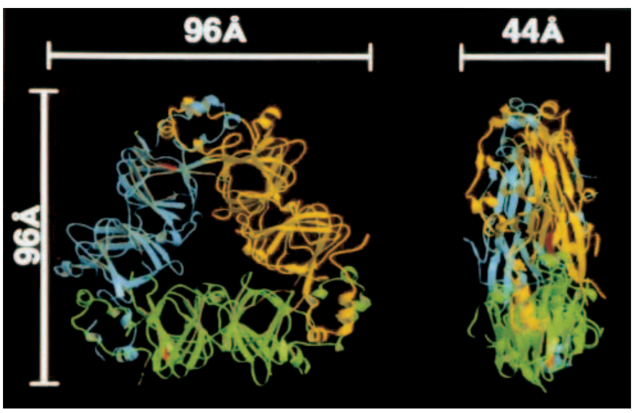


Fig. 4. Three-dimensional molecular structures of β-conglycinin β homotrimer (By the courtesy of Dr. S. Utsumi).

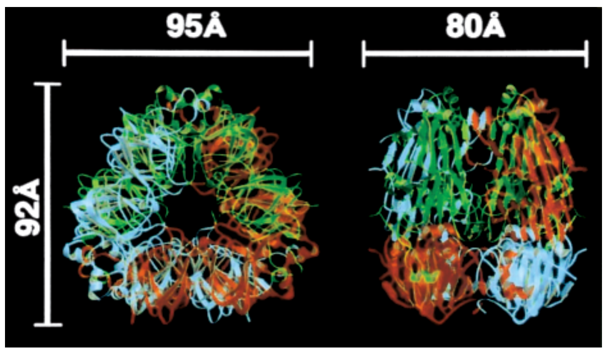


Fig. 5. Three-dimensional molecular structures of glycinin A_3B_4 homohexamer (By the courtesy of Dr. S. Utsumi).

Table 8. Contents of secondary structures contained in soybean storage proteins molecules.

	β-Conglycinin		Glycinin	
	X-ray[a)]	CD[b)]	X-ray[a)]	CD[b)]
α-Helix	10	5	8	5
β-Structure	33	35	36	35
Disordered structure	57	60	56	60

[a)]Values obtained by X-ray crystallographic method. [b)]Values obtained by circular dichroism method.

elucidation of the detailed three-dimensional structures enables us the theoretical modifications of the molecules, leading into the improvement of soybean protein properties through the protein engineering. Table 8 shows the comparison between the X-ray data of Utsumi's group (Fukushima, 2000b) and our ORC and CD data (Fukushima; 1965, 1967, and 1968; Koshiyama & Fukushima, 1973) on the per cent of the secondary structures. It is very interesting that the results of X-ray analysis are in good accordance with the results by our indirect CD method around 30 years ago.

4. Quality Improvement of Soy Food Products

Off-flavors and allergenic proteins in soybeans The most difficult problem limiting the expanded use of soy protein products in the Western countries is strong off-flavors associated with these products. There are two types of off-flavors. One is grassy and beany flavors and the other is bitter, astringent, and chalky flavors. The grassy-beany flavors are developed through the action of the three kinds of lipoxygenases 1, 2, and 3 present at soybeans. The bitter, astringent, and chalky flavors are caused by saponins and isoflavones (Okubo *et al*., 1992). The off-flavors of isoflavones are enhanced by the hydrolysis into their aglycones through the action of three kinds of β-glucosidases A, B, and C in soybeans (Matsuura & Obata, 1993). Thus, both lipoxigenases and β-glucosidases contained in soybeans play an important role in the production or enhancement of the off-flavors. Moreover, the lipid hydroperoxides produced by lipoxygenases oxidize the free -SH groups of soybean proteins, resulting into the decrease of their gel-forming ability (Fukushima, 1994). For a long time, a variety of attempts to remove or mask these off-flavors have been done through the operation during processing. However, it was impossible to remove or mask the off-flavors to a satisfactory extent by these methods.

Table 9. Major allergenic proteins in soybeans[b].

Protein assignment	Molecular wt. (k Da)	Frequency[a] (%)
Gly m Bd30 k	30	65.2
Gly m Bd28 k	28	23.2
α subunit of β-conglycinin	68	23.2
β subunit of β-conglycinin	45	10.1

[a]Detection frequency among 69 soybean-sensitive patients with atopic dermatitis. [b]Source: Ogawa *et al*. (1991).

Another unbeneficial substance other than off-flavors in soybeans is allergenic proteins. The major allergenic proteins in soybeans are shown in Table 9 (Ogawa *et al*., 1991). It is noticeable that the two of the three subunits of β-conglycinin have allergenic proteins. It is impossible to remove all of these major allergens through usual treatments or processing.

Genetic improvement of soybeans In the last two decades, the various soybean mutant genes which control the production of enzymes, allergenic proteins, storage proteins, etc. have been identified in the world soybean germplasm. Using these mutants, the commercially available soybean cultivars without having undesirable substances or with the beneficially modified composition of storage proteins have been bred. As an example, there is the cultivar "Kunitz" [Illinois Agricultural Experiment Station (AES)] lacking Kunitz's soybean trypsin inhibitor (Bernard *et al.* 1991) or the cultivar "Ichihime" (Kyushu AES) lacking all of the lipoxygenases 1, 2, and 3 (Nishiba *et al*., 1995). The development of lipoxygenase-free cultivar will be beneficial for the production of non-traditional soy products, since Western people are very sensitive for beany flavors. However, the soybean cultivars lacking the β-glucosidases have not been developed yet, which are the enzymes enhancing the off-flavors by changing the isoflavones into their aglycones.

There was some progress on the removal of allergenic proteins recently. The cultivar with a high ratio of glycinin to β-conglycinin was developed by the group of Tohoku National AES, named Tohoku 124. This cultivar lacks the two major allergenic proteins of 28K and α subunit, while it still possesses allergenic proteins of 30K and β-subunit (Samoto, 1996). Fortunately, the 30K protein can be removed easily by centrifugation, which is bound to IgE antibodies most strongly and frequently. Another group of Kyushu National AES found the wild soybean line, named QT_2, which lacks all of β-conglycinin (Hajika *et al*., 1998). This line grows normally and produces successive generations, indicating the possibility to breed the soybean varieties, of which storage proteins are mainly composed of glycinin without containing any β-conglycinin. Using this QT_2 line, they obtained the line lacking all the subunits of β-conglycinin by back-crossing with Fukuyutaka. This line contains only glycinin as storage proteins and that it lacks the three major allergenic proteins of 28K, α, and β subunits (Takahashi *et al*., 2000). This had so good field performance as Fukuyutaka in the on-campus experiment and was named Kyu-kei 305. Kyu-kei 305 should be mentioned to be the variety with the least quantities of allergens so far. Besides these,

Table 10. Hardness of tofu gel made from soybeans with different ratio of both 11S/7S proteins and glycinin subunit compositions.

Breeding line	Glycinin subunit			11S/7S in soy milk	Breaking stress of tofu gel
	Group I	$A_5A_4B_3$	A_3B_4		
Enrei (control)	+	–	+	58/42	9,891
EnB_2-111	+	+	+	66/34	9,989
EnB_2-110	+	+	–	62/38	8,955
EnB_2-101	+	–	+	57/43	10,171
EnB_2-100	+	–	–	45/55	7,162
EnB_2-011	–	+	–	52/48	6,791
EnB_2-010	–	+	–	33/67	4,835
EnB_2-001	–	–	+	25/75	5,381
EnB_2-000	–	–	–	12/88	3,002

Crops: Enrei (control), 380 kg; and others, 384–441 Kg/10 a. Protein content of seeds: Enrei (control), 42%; and others, 39.3–40.7%. Source: Yagasaki *et al.* (1999).

the eight isogenic breeding lines with a different ratio of glycinin to β-conglycinin have been obtained by back-crossing, using Enrei as a recurrent parent in Nagano Chushin Experiment Station (Yagasaki *et al*., 1999). In each of these lines, not only the ratio of glycinin to β-conglycinin, but also the subunit composition of glycinin is varied systematically (see Table 10). The breaking stress of tofu gels made from the soybeans of these lines increases markedly with the increase of the contents of glycinin and its A_3B_4 subunit. The yields and protein contents of the soybean seeds in these lines are substantially the same as the parent Enrei, indicating the possibility of the breeding of a practical cultivar.

Now that the three-dimensional structures have been elucidated completely in the molecules of β-conglycinin and glycinin, it is possible to improve the qualities of soybean storage proteins both physiologically and physicochemically through a genetic modification. For instance, Kim *et al*. (1990) made a modified $A_{1a}B_{1b}$ gene which has four additional methionyl residues near the C-termini. This modified $A_{1a}B_{1b}$ is excellent in both gelling and emulsifying properties. If this gene is introduced into the soybean lines lacking β-conglycinin such as Kyu-kei 305, the resultant transgenic soybeans are expected to have beneficial functional properties in food systems, together with a high content of methionine. We can also improve the properties of the storage proteins by introducing various kinds of physiologically active peptides into the molecules on the basis of the three-dimensional structures. In addition, transgenic rice (Momma *et al*., 1999) and potato (Utsumi *et al*., 1994) with glycinins have already been bred. On soybean oil, the genetic modification of the fatty acid composition has been carried out for the increase of the oil stability. As a result, soybean lines with oleic, linoleic, and linolenic acids of 85, 3, and 3%, respectively, have been developed (Krebbers *et al*., 1997).

5. Conclusion

For the long time, the removal of off-flavors has been a primary concern in research and technology on the utilization of soybeans for non-traditional foods. At present, however, the concern is switching over to the physiological active substances as well as the physicochemical functions of soybeans in food systems. For instance, isoflavones were considered simply as undesirable substances having strong off-flavors, but now their image has been changed to the useful substances with an excellent preventive or alleviating effect to cancer, osteoporosis, and menopausal symptoms, etc. However, it should be considered that the isoflavones are the substances having both favorable and unfavorable properties for the development of new soy products. The creation of new soybean cultivars is one of the most effective methods to obtain new soy products with a preferable character, because the desirable or undesirable components of soybeans can be controlled essentially at a DNA level, in either conventional breeding or modern genetic engineering. Now that the preventive effects of soybeans on chronic diseases have been proved scientifically, the future of soybeans must be bright. It is no doubt that the year of 2001, is surely the dawn of the innovative era for the technology of soybeans.

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