Major Proteins of Soybean Seeds. Subunit Structure of β-Conglycinin

Vu Huu Thanh* and Kazuo Shibasaki

β-Conglycinin, a major 7S soybean protein, possessed a 7S form ($s^0_{20,w}$ = 7.2) and a 9S form ($s^0_{20,w}$ = 10.7) at 0.5 and 0.1 ionic strength, respectively. The absorption coefficient ($A_{280\ nm}^{1\%}$) was found to be 4.16 cm^{-1}. The estimated molecular weight of the 7S form was in the range of 150 000 to 175 000, and that of the 9S form was 370 000. The protein consisted of six isomers (B_1 to B_6 conglycinins). Investigation on the subunit ratios in the six conglycinins indicated that the 7S form of the conglycinins had three subunits per molecule. The subunit structures of the six conglycinins are proposed as follows: B_1, $\alpha'B_2$; B_2, $\alpha\beta_2$; B_3, $\alpha\alpha'\beta$; B_4, $\alpha_2\beta$; B_5, $\alpha_2\alpha'$; and B_6, α_3. The proposal can explain similarities and differences in properties between the conglycinins and is consistent with their sugar, N-terminal, and amino acid compositions. From the consideration of symmetry of subunits in proteins, it is suggested that the 7S molecules may have a cyclic structure and the 9S form consists of two identical cyclic ensembles facing each other.

The storage proteins of legume seeds are composed of two major fractions, vicilin and legumin. The two types of protein have sedimentation coefficients of approximate 7S and 11S. Structural studies have revealed that legumin-type protein possesses an universal structure of 12 subunits which are packed in two hexagons, placed one on the other (Catsimpoolas, 1969; Badley et al., 1975; Kitamura et al., 1976; Derbyshire et al., 1976). However, the information on the quaternary structure of vicilin-type protein has been scarce. The existence of several vicilin proteins and the heterogeneity of most 7S preparations appear to make the elucidation of their structures a formidable problem (Derbyshire et al., 1976).

β-Conglycinin, which was identified with the major 7S soybean globulin (Koshiyama and Fukushima, 1976) or the 7S α-glycinin in a previous report (Thanh and Shibasaki, 1976a), is a vicilin-type protein. This protein has been fractionated into six isomers (B_1 to B_6 conglycinins) that are made up of three kinds of subunits (α, α′, and β) in varying proportions (Thanh and Shibasaki, 1976b). The subunits have been isolated and characterized (Thanh and Shibasaki, 1977). These results allow us to extend the study to the subunit structure of β-conglycinin.

In the present paper, we describe some physicochemical properties of the molecular forms. The molar subunit ratios in the six isomers are estimated from the data of gel electrophoresis and from N-terminal amino acid composition. From the consideration of the molecular weights of parent molecules and their subunits, and from the subunit ratios, we propose a trimeric structure for the 7S form and a hexameric structure for the 9S form of β-conglycinin. The proposed structures are discussed with reference to the properties of the six conglycinins.

MATERIALS AND METHODS

Protein Samples. β-Conglycinin, B_1 to B_6 conglycinins, and the α, α′, and β subunits were isolated and purified as previously described (Thanh and Shibasaki, 1976a,b, 1977). The samples for ultraviolet spectra determination were obtained from pooled fractions without freeze-drying. The other samples were freeze-dried at pH 7.0.

Analytical Methods. Ultraviolet absorption curves were recorded with a Hitachi 124 double-beam spectrophotometer. Absorption coefficients were determined in 0.5 ionic strength (I = 0.5) standard buffer (Wolf and Briggs, 1959) and in 0.02 M HCl. In the former case, protein concentration was on dried weight basis. In the latter case, protein concentration was estimated by the micro-biuret method as in a previous report (Thanh and Shibasaki, 1976b).

Diffusion constants were estimated by immunodiffusion method (Allison and Humphrey, 1960; Catsimpoolas et al., 1968). The gel media contained 1% agar in the standard buffers (0.5 and 0.1 ionic strength). Antisera were dialyzed against the buffers. Protein samples were dissolved in the buffers at 0.1% concentration.

Urea/sodium dodecyl sulfate (urea/SDS) gel electrophoresis was carried out as previously described (Thanh and Shibasaki, 1977). Polyacrylamide gels were stained with Coomassie Blue G-250 and scanned at 550 nm.

Ultracentrifugal analysis was carried out at 20 °C with a Hitachi UCA-1 ultracentrifuge at 55 430 rpm in the standard buffers.

Amino acid composition was determined in a Hitachi KLA-2 amino acid analyzer after hydrolysis with a large excess (1000 volumes) of redistilled 6 N HCl at 110 °C for 20 h in sealed evacuated tubes.

RESULTS

Physicochemical Properties. *Ultraviolet Absorption.* The isolated B_1 to B_6 conglycinins in the phosphate buffers (I = 0.5 and 0.05, pH 7.6) showed the absorption spectra similar to that obtained by Koshiyama (1968b). No differences between the absorption curves of the isolated conglycinins were observed. They all showed absorption maxima at 278 nm and at 232–234 nm region, a minimum at 250 nm, and four shoulders at 253, 258, 265, and 269 nm. The absorption ratios of 280 to 260 nm were in the range of 1.61 to 1.63, and those of 278 to 250 nm were in the range of 2.38 to 2.34. The absorption coefficients at 280 nm determined in the standard buffer (I = 0.5) were not significantly different from those estimated in 0.02 M HCl (Table I). These values are lower than the reported value of 5.47 (Koshiyama, 1968a). Higher ratios of A_{280nm} to A_{260nm} in this study may indicate that the conglycinins, purified by ion-exchange chromatography, are free from other ultraviolet-absorption materials and therefore account for the lower absorption coefficents observed.

Sedimentation Coefficient. The sedimentation coefficients of β-conglycinin measured in the standard buffers (I = 0.5 and 0.1) at 20 °C and infinite dilution, s^0_{20}, were 6.65 and 10.2 S, respectively. These values corrected to water as solvent, $s^0_{20,w}$ = 7.20 and 10.7 S, were slightly lower than those obtained by Koshiyama (1968a,c) and Roberts and Briggs (1965).

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Table I. Physicochemical Properties of β-Conglycinin

	β-Conglycinin	Isomers of β-conglycinin		
		Group A	Group B	Group C
Absorption,[a] $A_{280nm,1\%}$ 1 cm	4.16	3.91,3.81	4.05,3.88	4.27,4.20
Sedimentation,[b] $S^0_{20,w}$, S	7.20 (10.70)			
Diffusion,[b] $D_{20,w}$, $cm^2\ s^{-1} \times 10^{-7}$	4.52 (2.68)			
N-terminals[c]	Val, Leu	Val (1) Leu (2)	Val (2) Leu (1)	Val (3)
Molecular weight,[d] A	175 000			
B	150 000 (370 000)			
C		141 000 (282 000)	156 000 (312 000)	171 000 (342 000)

[a] In standard buffer ($I = 0.5$). [b] In standard buffers ($I = 0.5$ and 0.1), value in parentheses was obtained at $I = 0.1$. [c] Composition in the 7S form. [d] Molecular weight by sedimentation Stokes' radius (A), sedimentation-diffusion (B), and from subunit sizes (C); values in parentheses were those of the 9S form.

Table II. Amino Acid Composition and Carbohydrate Content of B_1 to B_6 Conglycinins[a,b]

	Conglycinins						
	Group A		Group B		Group C		
Amino acid	B_1	B_2	B_3	B_4	B_5	B_6	α′ subunit
Asx	11.97	13.67	11.22	11.41	11.01	11.17	12.02
Thr	2.42	2.44	2.34	2.34	2.22	2.16	2.27
Ser	7.17	7.08	6.87	7.03	6.90	6.80	6.85
Glx	22.35	22.26	24.02	24.30	26.18	25.80	26.42
Pro	4.91	4.78	5.52	5.70	5.89	6.16	4.39
Gly	4.78	4.64	4.77	4.53	4.62	4.45	4.87
Ala	5.22	5.26	4.87	4.82	4.57	4.62	4.31
½-Cys.	0	0	0	0	0	0	0
Val	4.23	4.02	4.18	4.05	3.65	3.76	3.98
Met	c	c	c	c	c	c	0.27
Ile	4.57	4.56	4.72	4.76	4.40	4.72	4.17
Leu	9.02	9.24	8.50	8.60	7.94	8.28	7.24
Tyr	2.51	2.38	2.41	2.39	2.26	2.28	2.23
Phe	5.54	5.42	5.09	5.06	4.79	4.77	4.60
His	2.27	1.44	1.83	1.30	1.73	1.07	3.61
Lys	6.27	6.01	6.68	6.44	6.70	6.35	6.55
Arg	6.74	6.79	6.97	7.26	7.14	7.60	6.22
Carbohydrate							
A	3.95	3.83	4.27	4.57	5.11	5.36	5.03
B	4.00	4.05	4.61	4.65	5.11	5.15	

[a] Amino acid compositions are expressed as mole percent of protein. [b] Carbohydrate content (sum of mannose and glucosamine) is expressed as percent of protein on moisture-free basis: A, experimental values; B, calculated from subunits. [c] Not detected.

Diffusion Constant. At $I = 0.5$ where β-conglycinin has a 7S sedimenting form, the angle θ formed by the precipitating line and the antigen trough was in the range of 47–48°. At $I = 0.1$, the 9S form of β-conglycinin had an angle θ of 40 ± 1°. The calculated $D_{20,w}$ values are presented in Table I.

Molecular Weight. The molecular weight of the 7S form of β-conglycinin estimated from the sedimentation coefficient s^0_{20} and Stokes' radius was 175 000. The following values were used for the calculation: solvent viscosity, 1.034×10^{-2} poise; solvent density, 1.016 g mL^{-1}; and partial specific volume of the protein, 0.725 mL g^{-1}. The value s^0_{20} was taken as the sedimentation coefficient of the protein in the standard buffer ($I = 0.5$) at 20 °C and at zero concentration (6.65×10^{-13}). The Stokes' radius was taken as 59 Å (Koshiyama, 1968b). This value seems reasonable since gel filtration suggested that β-conglycinin and the 11S soybean globulin were similar in Stokes' radius (Thanh et al., 1975). The Stokes' radius of the 11S globulin has been determined as 58.5 Å (Badley et al., 1975) and 59 Å (Koshiyama, 1972).

Calculation from the values of $s^0_{20,w}$ (7.20 and 10.7×10^{-13}) and diffusion constant ($D_{20,w} = 4.52$ and 2.68×10^{-7} $cm^2\ s^{-1}$) gave a molecular weight of 150 000 for the 7S and 370 000 for the 9S form of β-conglycinin. The obtained molecular weights are slightly lower than those of previous reports (Koshiyama, 1968a,c).

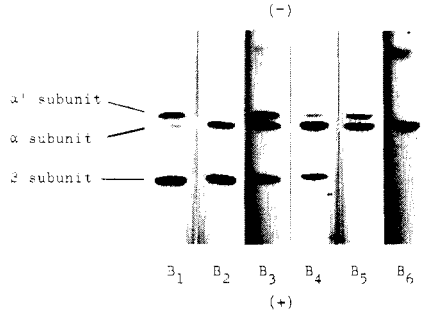

Figure 1. Fractionation of the subunits of B_1 to B_6 conglycinins on urea/SDS gels. The direction of migration is from the top. Electrophoresis was carried out in 5% gel containing 8 M urea and 0.1% SDS at 3 mA for 6.5 h.

Amino Acid Composition. Table II shows the amino acid composition of B_1 and B_6 conglycinins. For a convenient comparison, the composition of α′ subunit analyzed under identical conditions is also presented. The composition of α subunit can be taken from B_6 conglycinin

Table III. Subunit Ratio in B_1 to B_5 Conglycinins

Conglycinins	$\alpha,\alpha'/\beta$ ratio			α/α' ratio, urea/SDS[b]	Proposed composition
	Urea/AcOH[a]	Urea/SDS[b]	N-termini[c]		
B_1	0.48	0.47	0.51		$\alpha'\beta$ (1:2)
B_2	0.50	0.53	0.55		$\alpha\beta$ (1:2)
B_3	1.98	1.80	1.55	1.39	$\alpha\alpha'\beta$ (1:1:1)
B_4	2.48	1.89	2.21		$\alpha\beta$ (2:1)
B_5				2.06	$\alpha\alpha'$ (2:1)
Total β-conglycinin		1.37	1.40	1.54	

[a] Estimation on urea/acetic acid gels. [b] Estimation on urea/SDS gels. [c] Calculation from N-terminal amino acid composition.

which is a homooligomer of α subunit (Figure 1). Half-cystine and methionine in the conglycinins were not detected. The conglycinins had some characteristics of vicilin-type protein: they were rich in glutamate, aspartate, leucine, and arginine, and very low in methionine and cystine. The three groups A, B, and C of the conglycinins differed greatly in the content of some amino acids. From groups A to C, the contents of glutamate, proline, lysine, and arginine gradually increases whereas the contents of hydrophobic amino acids (alanine, valine, leucine, and phenylalanine) decreases. Within one group, the α'-containing conglycinins (B_1, B_3, and B_5) had a higher content of histidine.

Subunit Structure. The molecular weights 150000 to 170000 of β-conglycinin (7S form), and 57000 and 42000 of its subunits (Thanh and Shibasaki, 1977) suggest that the 7S form is composed of three subunits. However, the subunit structure of the protein could only be determined if the subunit compositions of its isomers are known.

Contribution of α, α', and β subunits to the structure of B_1 to B_6 conglycinins could be estimated on urea/SDS gels (Figure 1). The molar subunit ratios were calculated from the densitometer tracings of the gels and from a consideration of the subunit molecular weight (carbohydrate prosthetic groups were excluded). Thus, the $\alpha,\alpha'/\beta$ ratios were the corresponding scanning-area ratios multiplied by 40500/54000. The $\alpha,\alpha'/\beta$ ratios were also calculated from data of urea/acetic acid gels. The results from the two electrophoresis systems are in good agreement (Table III). They indicate that α (and/or α') and β subunits were present in a molar ratio of 1:2 in the molecule of β_1 and B_2 conglycinins, and in a molar ratio of 2:1 in B_3 and B_4 conglycinins.

The α/α' ratio in Table III was taken as the densitometric area ratio since the two subunits have the same molecular weight and a similar carbohydrate content (Thanh and Shibasaki, 1977). Conglycinins B_3 and B_5 were found to contain α and α' in an approximate molar ratio of 1:1 and 2:1, respectively. Thus, the subunit ratio in B_1 to B_5 conglycinins could be summarized as follows: B_1, $\alpha'\beta$ (1:2); B_2, $\alpha\beta$ (1:2); B_3, $\alpha\alpha'\beta$, (1:1:1); B_4, $\alpha\beta$ (2:1); and B_5, $\alpha\alpha'$ (2:1).

The proposed subunit ratios were supported by the result of N-terminal amino acid composition. Since α and α' subunits have valine and "tyrosine" as N-terminal amino acid, and β subunit has leucine (Thanh and Shibasaki, 1977), the molar ratio (valine + tyrosine)/leucine in B_1 to B_4 conglycinins represents its $\alpha,\alpha'/\beta$ subunit ratio. Calculation from previous data (Thanh and Shibasaki, 1976b) yielded the ratios which are consistent with the results of gel electrophoresis (Table III).

DISCUSSION

From the molecular weights of β-conglycinin and its subunits, and from the subunit composition in each of its isomers, we propose the subunit structure of the six conglycinins as follows: B_1, $\alpha'\beta_2$; B_2, $\alpha\beta_2$; B_3, $\alpha\alpha'\beta$; B_4, $\alpha_2\beta$; B_5, $\alpha_2\alpha'$; and B_6, α_3. Based on these structures, the six conglycinins can be classified into three groups: group A (B_1 and B_2) which has two β subunits per molecule (7S); group B (B_3 and B_4), one β subunit; and group C (B_5 and B_6), no β subunit.

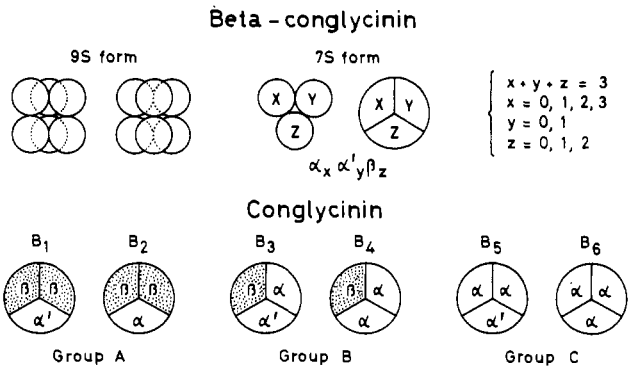

Figure 2. Schematic representation of the subunit structure of β-conglycinin and its six isomers.

The general structure of the 7S form of β-conglycinin can be written as $\alpha_x\alpha'_y\beta_z$ where x, y, and z representing the number of the corresponding subunits in the oligomers should fit the equation $x + y + z = 3$, and have one of the following values: 0, 1, 2, 3 (x); 0, 1 (y); and 0, 1, 2 (z). Figure 2 shows a schematic representation of the subunit structure of β-conglycinin and its isomers, B_1 to B_6 conglycinins.

The present proposal can shed light on the multiplicity of β-conglycinin and can explain different properties of the conglycinins. The molecular weights of the three groups A, B, and C, calculated from the subunit sizes (Table I), are in the order C > B > A, which may account for the order of elution C, B, A from gel filtration column (Thanh et al., 1975). The elution order B_1 to B_6 of the conglycinins by DEAE-Sephadex A-50 (Thanh and Shibasaki, 1976b), which imply a gradual increasing in negative charge in the order B_1, B_2, ... B_6, is in accord with the proposed structures. These structures, which show an increase (from B_1 to B_6) in the contribution of α and α' subunits to the molecules of the conglycinins, suggest a corresponding increase in the contribution of negative charge since the isoelectric points of the subunits are in the order $\alpha < \alpha' < \beta$ (Thanh and Shibasaki, 1977).

The differences in amino acid composition between the conglycinins (Table II) may be correlated to the proposed structures. Previous report (Thanh and Shibasaki, 1977) indicated that β subunit had lower contents of glutamate, proline, lysine, and arginine and higher contents of hydrophobic amino acids than α and α' subunits. Therefore, the increasing in the contents of glutamate, proline, lysine, and arginine and the decreasing of hydrophobic amino acids in the three groups A, B, C reflect an increasing in the content of α and α' subunits of its, which is in accord

with the subunit structures. Besides, since α′ subunit has three times the histidine content of α subunit, we may relate the relatively high content of histidine in B_1, B_3 and B_5 conglycinins to the presence of α′ subunit in their structures.

The proposed structures are in agreement with N-terminal amino acid composition and carbohydrate content of the conglycinins. Calculation of the carbohydrate content (mannose and glucosamine) from that of the subunits (Thanh and Shibasaki, 1977) gave values that are consistent with the experimental values (Table II). The immunological relationship between the conglycinins, attributable to their structural relationship (Thanh and Shibasaki, 1976b, 1977), is also consistent with the structure proposal. A further support for the structures is furnished by investigations on self-association of the subunits and reconstitution of the conglycinins from their subcomponents, which will be discussed in a subsequent report.

These structures, presenting three subunits per molecule at first, seem to be uncommon. Most of protein oligomers have an even number of subunits. Of the 300 proteins of which the subunit compositions have been established (Klotz et al., 1975), only ten have three subunits per oligomers and only four of them (troponin, $\alpha_2\beta$; bovine procarboxypeptidase A, $\alpha\beta\gamma$; molybdoferredoxin, $\alpha_2\beta$; and cysteine synthetase, $\alpha_2\beta$) are composed of nonidentical subunits. The present proposal becomes common as we look at the "dimer" form (9S form) of β-conglycinin which would possess a hexameric structure, i.e., an even number of subunits.

Taking into consideration that only one type of geometry, namely a cyclic structure, is possible for the arrangement of monomers in a homotrimer (Klotz et al., 1975), from the structure α_3 of B_6 conglycinin, we can speculate that the 7S molecules might have a cyclic structure. The 9S molecules, "dimer" of the 7S, therefore, might be composed of two cyclic ensembles packed one on top of another. This may be the only possible type of geometric arrangement in a dimer (Klotz et al., 1975).

In the light of a recent report which showed clearly that the acidic and basic polypeptides in glycinin (the 11S soybean globulin) are linked by disulfide bond(s) (Kitamura et al., 1976), the glycinin molecules would be considered to consist of six subunits (12 polypeptide chains) packed in two cyclic ensembles. On the basis of the consideration, the molecular model of β-conglycinin appears to be similar to that of glycinin. Whether the subunit structure presented here is an universal structure of vicilin-type proteins deserves further investigations.

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Major Proteins of Soybean Seeds. Reconstitution of β-Conglycinin from Its Subunits

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Isolated α, α′, and β subunits of β-conglycinin were unfolded in phosphate buffer containing 6 M urea. Upon removal of urea, self-association of the subunits occurred. The self-association and the reconstitution of β-conglycinin from the three subunits were investigated by disc electrophoresis and ultracentrifugal analysis. The α subunit reassociated to form a 7S protein identical with B_6 conglycinin. Most of α′ subunit recombined to build a 7S aggregate which had no ability to dimerize at 0.1 ionic strength. The β subunit associated to form a 16S aggregate at 0.05 ionic strength, pH 8.4. The six molecular species of β-conglycinin (B_1 to B_6) could be reconstituted by mixing the three subunits in urea solution, and subsequently dialysis of the solution against phosphate buffer. A combination of α and α′ subunits produced B_5 conglycinin. Mixtures of α and β subunits gave B_2, B_4, and B_6 conglycinins; α, α′, and β gave B_3; and α′ and β gave B_1 conglycinin. The results are discussed with regard to the ten possible molecular species ($\alpha_x\alpha'_y\beta_z$) of β-conglycinin.

Recent studies have revealed that a number of proteins have the ability of renaturation (Anfinsen and Scheraga, 1975; Baldwin, 1975). Proteins were unfolded by urea or guanidine hydrochloride and by reduction of disulfide bonds. Refolding could be achieved under appropriate conditions by reoxidation of the sulfhydryl groups and removal of the denaturing reagents. On the other hand, some proteins could be reconstituted from their subunits

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