New Aspects of Subunit Structure of Soybean Glycinin

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ABSTRACT

Glycinin, the most important storage protein from soybean, was isolated from an aqueous extract of defatted soymeal by cryoprecipitation and hydroxyapatite chromatography. After complete reduction and alkylation, four different acidic subunits were isolated by ion-exchange chromatography in the presence of urea. No attempt was made to isolate the basic subunits, which were treated as a group. Following partial reduction and alkylation three different intermediary subunits, each consisting of one acidic and one basic subunit held together by one or more disulfide bridges, were isolated. The fractions were characterized by polyacrylamide gel electrophoresis in urea, dansyl chloride for N-terminal analysis, and sodium dodecyl sulfate polyacrylamide gel electrophoresis for relative molecular mass determinations. Peptide mapping after tryptic hydrolysis, amino-acid analyses, and automated sequence analyses of the first 20–30 residues of the acidic subunits demonstrated a close similarity in their primary structures. In the preparation of the intermediary subunits, the presence of a "free" subunit identical to an acidic subunit was repeatedly observed. Glycinin appears to consist of two identical half molecules, each consisting of three intermediary subunits, and two additional acidic subunits that probably occupy the central hole of each half molecule.

In view of the interest in oilseed proteins, and particularly soyproteins, for application in food products, a more detailed understanding of their chemical structure is needed. This knowledge can help to better define and utilize their functional properties. A considerable amount of knowledge about glycinin, the major storage protein of the soybean (*Glycine max* (L) Merril), is already available in the literature.

Glycinin is generally considered to be composed of 12 subunits arranged in two identical or similar half molecules of six subunits each (Badley et al 1975, Catsimpoolas 1969, Kitamura and Shibasaki 1975). By means of preparative isoelectric focusing, Catsimpoolas (1969) isolated three acidic and three basic subunits from glycinin; relative molecular masses determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were approximately 37,000 for the acidic and 22,000 for the basic subunits (Catsimpoolas et al 1971). Badley et al (1975) presented a model of glycinin comprising two identical half molecules each consisting of a ring formed from three basic and three acidic subunits.

On the other hand, Kitamura and co-workers (Kitamura and Shibasaki 1975, Kitamura et al 1976) found that glycinin consists of four acidic and four basic subunits. Their basic subunits have relative molecular masses of 22,000, and three of their acidic subunits have relative molecular masses of 37,000, similar to those of Catsimpoolas et al (1971). Their fourth acidic subunit, however, has a relative molecular mass of 45,000. From these results they concluded that glycinin is composed of two similar but not identical half molecules. Kitamura et al (1976) also reported that each acidic and each basic subunit is linked by one or more disulfide bridges, forming intermediary subunits. Furthermore, they suggested that in glycinin the acidic and basic subunits are alternately arranged. Both the above groups of workers and Okubo et al (1969) report phenylalanine, leucine, or isoleucine as N-terminals for the acidic subunits and glycine for the basic subunits.

In a recent study on the subunits of glycinin from soybean cultivar CX 635-1-1-1, Moreira et al (1979) described the isolation of six polypeptides with acidic isoelectric points and four with basic isoelectric points. In addition to phenylalanine, leucine, and isoleucine as N-terminals of the acidic subunits, they found arginine as N-terminal for an acidic subunit, the first report in the literature. The basic polypeptides all had glycine at the N-terminals. Their sequence analysis also showed considerable homology between members of individual families of acidic and basic polypeptides. However, in their opinion, the glycinin polypeptide composition is more complex than previous reports have indicated.

Our findings have shown that acidic subunits occur as free subunits as well as subunits bound via intermediary subunits into ring systems.

However, the degree of homology of acidic and basic subunits is uncertain. We have tried to clear up this uncertainty by peptide mapping after tryptic digestion and by elucidating part of the N-terminal amino acid sequences. In addition, the intermediary subunits were isolated and characterized, the basic subunits being studied as a group.

MATERIALS AND METHODS

Soybean variety "Express Green" was used for all investigations. After being cracked and dehulled, the material was defatted with hexane and dried at room temperature. Bovine albumin, egg albumin, and cytochrome C were from Sigma Chemical Company and horse myoglobin from Serva Entwicklungs Labor. All other reagents were analytical grade, and those used for sequencing were of sequential grade.

For ultracentrifugal analysis an MSE analytical ultracentrifuge provided with a photoelectric scanner was used.

Preparation of Glycinin

The cold-insoluble fraction (CIF) of soymeal was prepared from approximately 200-g batches of defatted soymeal, using a meal-water ratio of 1:5 and an extraction temperature of 40° C, as described by Wolf and Sly (1967). Cryoprecipitation was done at 4° C for 18 hr, followed by centrifugation, after which the CIF was divided into convenient portions and frozen in the wet state at −20° C for further use. Yields of CIF were from 50 to 60 g. Portions of 10 g of wet-frozen CIF were fractionated on a 5 × 15-cm hydroxyapatite column (Biorad HTP) according to Wolf and Sly (1965), using a potassium phosphate buffer with a gradient from 0.03 to 0.4*M* (pH 7.6) containing 40 g of sodium chloride per liter as eluent at a flow rate of 70 ml/hr. The required gradient was produced with an LKB Ultrograd gradient mixer. Separation was completed in 16 hr at a column temperature of 15° C and fraction collector temperature of 5° C.

Glycinin fractions were pooled, dialyzed against water, and lyophilized. Ultracentrifugal analysis was performed in 0.5 ionic strength phosphate buffer (pH 7.6) containing 0.01*M* 2-mercaptoethanol at a protein concentration of approximately 2 mg/ml. The sedimentation pattern was recorded after about 30 min at 50,700 rpm.

Preparation of Acidic and Intermediary Subunits

Batches of 300–400 mg of glycinin after denaturation were completely reduced with 80–100 mg of dithioerythritol and carboxamidomethylated with about 120 mg of iodacetamide for 1.5 hr in the dark at room temperature, all reactions being performed under nitrogen. The procedure was essentially that of Koningsberg (1972). The modified protein was finally dialyzed

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against water in the dark, lyophilized, and used for isolation of the individual acidic subunits.

To obtain the intermediary subunits, 500 mg of glycinin was partially reduced in 50 ml of 0.4*M* sodium chloride and 0.01*M* 2-mercaptoethanol after adjusting the pH to 8.3 with solid Tris. Iodacetamide (675 mg) was added to this mixture and allowed to react for 20 min in the dark under nitrogen. The material was finally dialyzed in the dark and lyophilized.

Both the individual acidic subunits and the intermediary subunits were isolated by ion-exchange chromatography on diethylaminoethyl (DEAE)-Sephadex A-50 essentially as described by Kitamura and Shibasaki (1975). For the isolation of the individual acidic subunits, 300 mg of reduced and carboxamidomethylated glycinin was dissolved in 5 ml of starting buffer. Column size was 2.6 × 30 μm. Elution was for 24 hr at a flow rate of 20 ml/hr at 5°C. For the isolation of the intermediary subunits, 300 mg of partially reduced and carboxamidomethylated glycinin was dissolved in 5 ml of starting buffer. Column size was 2.6 × 30 μm. Elution was for 48 hr at a flow rate of 13 ml/hr at 5°C.

SDS-PAGE

SDS-PAGE was performed according to Weber and Osborn (1969), using gels (100 g/kg) containing bisacrylamide (26 g/kg) at pH 7.0. For determination of the relative molecular masses, the following proteins were used: horse myoglobin (17,800), bovine albumin (67,000), egg albumin (45,000), and cytochrome C (12,400). Protein bands were stained with Coomassie brilliant blue.

PAGE in the Presence of Urea at Alkaline pH

Alkaline urea-PAGE was performed, using a gel (50 g/kg) containing 50 g of bisacrylamide per kilogram (Cyanogum 41) at pH 8.7; the gel buffer was 0.04*M* Tris containing 7*M* urea. The pH was adjusted with citric acid (100 g/L). The electrode buffers contained 6 g of Tris and 28.8 g of glycine per liter (pH 8.5). Protein bands were visualized by staining with Coomassie brilliant blue.

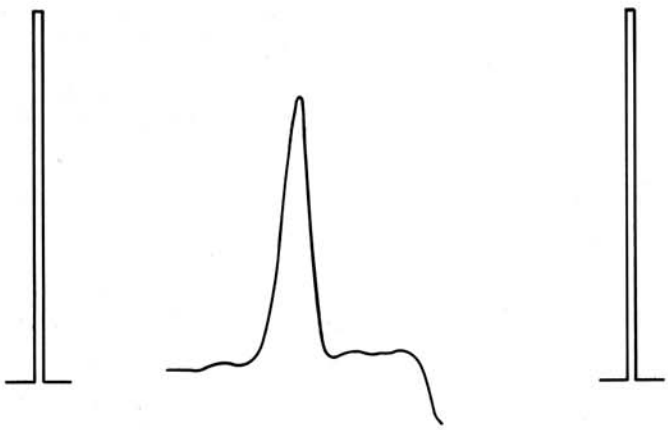

Fig. 1. Ultracentrifugal analysis of glycinin. Sedimentation is from right to left.

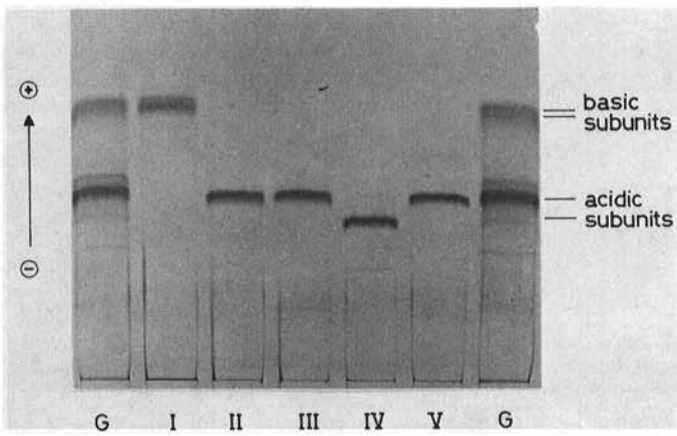

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reduced and carboxamidomethylated glycinin **(G)** and of isolated acidic subunits **(II–V).** Samples I–V correspond to the isolated fractions in Fig. 3.

PAGE in the Presence of Urea at Acidic pH

The acidic urea-PAGE system was essentially as described by Kitamura et al (1976), using a polyacrylamide gel (75 g/kg) containing bisacrylamide (50 g/kg). The gel solution consisted of acetic acid (100 g/L) and 7*M* urea, and the electrode buffers were acetic acid (100 g/L). Hydrogen peroxide (2.0 ml of a 30 g/L-solution per 150 ml of gel) was used as promoter and ascorbic acid (0.75 ml of a 150 g/L-solution per 150 ml of gel) as catalyst. The separated protein zones were stained with Coomassie brilliant blue.

For all gel electrophoresis experiments, the EC-460 electrophoresis cell from E-C Apparatus Corp. was used.

N-Terminal Analysis of the Isolated Subunits

Qualitative N-terminal analysis was done by the dansyl (DNS) chloride method as described by Gray (1972). The DNS-derivatives of the N-terminal amino acids were identified on double-coated micropolyamide plates (5 × 5 cm).

Amino Acid Analysis

Glycinin or individual acidic subunits (10–40 nmol) were hydrolyzed in 0.2 ml of 6*M* HCl containing phenol (10 g/L) for 24, 48, and 72 hr at 110° C, and the results were extrapolated to zero time of hydrolysis (Moore et al 1958). For the determination of tryptophan, samples were hydrolyzed with 0.2 ml of 4*M* methane sulfonic acid containing 3-(2-aminoethyl)-indole (2 g/L) according to Liu and Chang (1971). The hydrolysates were analyzed by a Rank Hilger Chromaspek amino-acid analyzer, using a single-column method according to the manufacturer's manual.

Tryptic Digestion and Peptide Mapping

Of each of the isolated acidic subunits, 5 mg was digested with TPCK-treated trypsin according to Van der Ouderaa et al (1973) at an initial enzyme-substrate ratio of 1:50. After 4 hr of incubation, the reaction was stopped by heating the sample for 2 min in a boiling water bath. Peptide mapping of the enzymatic digests was done by high-voltage paper electrophoresis (25 V/cm for 3 hr) in pyridine/acetic acid/water buffer (25:1:255 by volume, pH 6.5) in an apparatus cooled by Terpentol-15. Descending chromatography was done in the second dimension in butanol/acetic acid/pyridine/water (15:3:10:12 by volume, Waley and Watson 1953). The peptide bands were stained with a solution of 0.5 g of ninhydrin in pyridine/acetic acid/acetone (1:1:98 by volume).

Automatic Sequence Analysis of the First 20–30 Residues

Automatic Edman degradation (Edman and Begg 1967) was conducted with approximately 300 nmol of the carboxamidomethylated subunit in a model 890 C Beckman sequencer using an improved Quadrol protein program (Anonymous 1975). The resulting phenylthiohydantoin (PTH) derivatives of the amino acid residues were identified as described before by Iyengar et al (1979), using gas-liquid and thin-layer chromatography, and in some cases

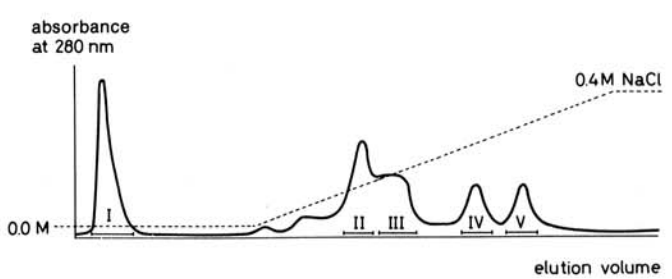

Fig. 3. Diethylaminoethyl-Sephadex A-50 column chromatography of carboxamidomethylated subunits **(I–V)** of glycinin. Dotted line = sodium chloride gradient, barred line = pooled fractions.

by means of the amino acid analyzer after back hydrolysis with hydroiodic acid (Mendez and Lai 1975). Asparagine and glutamine were directly determined as their PTH derivatives.

RESULTS

Purity of Glycinin

Chromatography of the CIF on hydroxyapatite yielded a pure glycinin fraction by ultracentrifugal analysis (Fig. 1) and by SDS-PAGE (Fig. 2). However, disulfide (S-S) polymers are probably present, as evidenced by gel-filtration on Biogel A 1.5 m with and without 2-mercaptoethanol.

Acidic Subunits of Glycinin

Figure 3 shows the separation of the carboxamidomethylated subunits of glycinin on DEAE-Sephadex. The fractions indicated were characterized with different PAGE systems and analyzed for their respective N-termini. Figure 2 shows the SDS-PAGE pattern of the five isolated fractions; two different relative molecular masses were found for the basic subunits. Relative molecular masses and N-termini are summarized in Table I. Fractions II–V (Fig. 3) are the acidic subunits, indicated as A1–A4 in Fig. 4. This electropherogram clearly shows that some acidic fractions are not homogeneous. However, just one N-terminal amino acid was found for each of these fractions, which indicates that microheterogeneity probably exists in the acidic subunits.

The four acidic subunits we isolated were not identical to those found by Kitamura et al (1976); subunits A3 and A4 from our experiments showed relative molecular masses of approximately 45,000 and 38,000 respectively, in contrast to the 38,000 and 45,000 reported by those authors.

Chemical Similarities Among the Acidic Subunits

Amino acid analyses (Table II) indicate a possible similarity among all the acidic subunits, which is, in fact, confirmed by the peptide maps of their tryptic digests (Fig. 5); two pairs of the acidic subunits are closely similar to each other, ie, A1 and A2 on the one hand and A3 and A4 on the other. To establish the extent of this similarity, a complete primary-structure elucidation would be necessary. So far, our work in this direction has revealed the amino acid sequence of the first 20–30 residues of the four subunits. Figure 6 shows the results of automatic Edman degradation of the individual acdic subunits; identical regions or residues in similar positions are indicated.

After conversion of the anilinothiazolinones into their PTH

TABLE I
Characteristics of Isolated Acidic Subunits and Total Basic Subunits

Fraction Number[a]	Relative Molecular Mass	N-Terminus and Nomenclature of Subunit	
I	21,500 and 20,200	Gly	basic
II	38,000	Phe	A1
III	38,000	Leu	A2
IV	45,000	Ile	A3
V	38,000	(Ile)[b]	A4

[a] Fraction numbers correspond to those of Fig. 3.
[b] After 18 hr of hydrolysis, a spot was found, the identity of which could not be established. After 65 hr of hydrolysis, Ile could be shown to be the N-terminus although it was rather faint compared to those of the other fractions.

TABLE II
Amino Acid Composition[a] of Acidic Subunits and Glycinin[b]

Amino Acid	Acidic Subunits				
	A1	A2	A3	A4	Glycinin
Asp	36	40	50	44	325
Thr	13	12	16	11	127
Ser	19	16	25	23	159
Glu	82	96	93	94	580
Pro	21	18	36	26	177
Gly	27	23	29	19	214
Ala	13	14	12	7	149
Cys[c]	4	5	2	1	41
Val	14	14	18	12	165
Met	3	5	3	1	25
Ile	16	15	13	10	126
Leu	19	17	25	14	203
Tyr	8	6	9	2	81
Phe	3	12	13	8	111
His	7	4	12	11	72
Lys	22	15	16	21	148
Arg	16	18	24	24	136
Trp	2	2	2	1	16
Total	335	332	398	329	2,840

[a] Numbers of residues per protein molecule.
[b] Relative molecular masses as found by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were used for calculating the composition of acidic subunits. A value of 320,000 was used for calculating the composition of glycinin.
[c] Determined as *S*-carboxymethylcysteine.

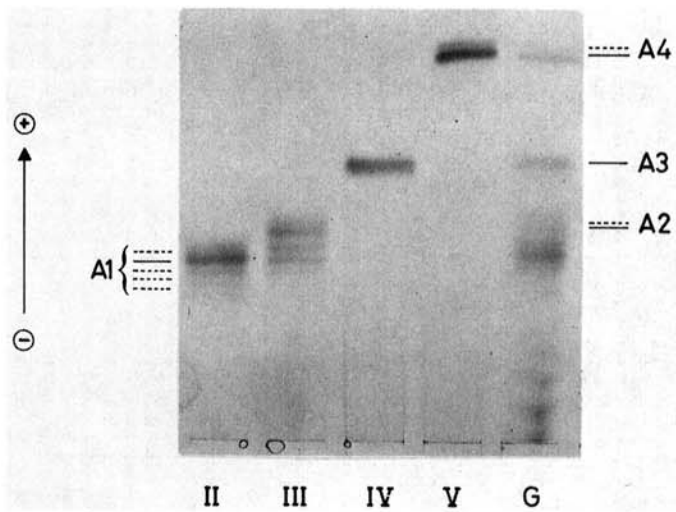

Fig. 4. Alakaline urea-polyacrylamide gel electrophoresis of isolated acidic subunits (**A1–A4**) of glycinin. **G** = reduced and carboxamidomethylated glycinin. Fractions **II–V** (carboxamidomethylated subunits) correspond to those of Fig. 3.

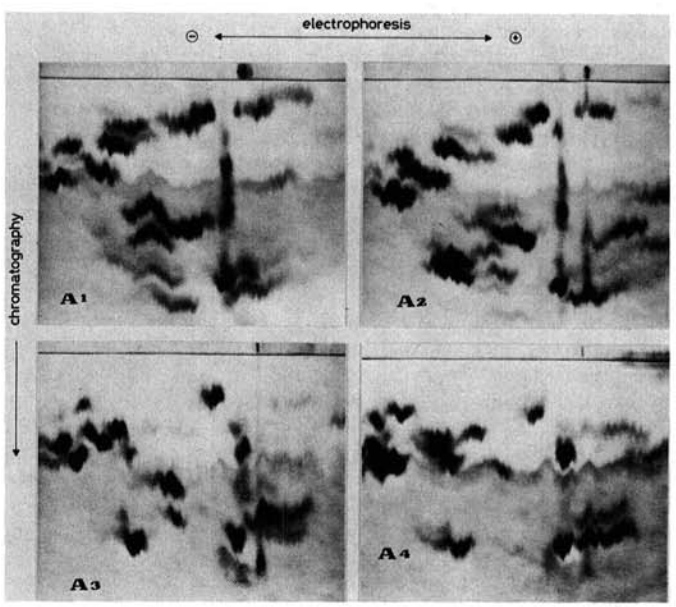

Fig. 5. Peptide maps of tryptic hydrolysates of carboxamidomethylated individual acidic subunits (**A1–A4**).

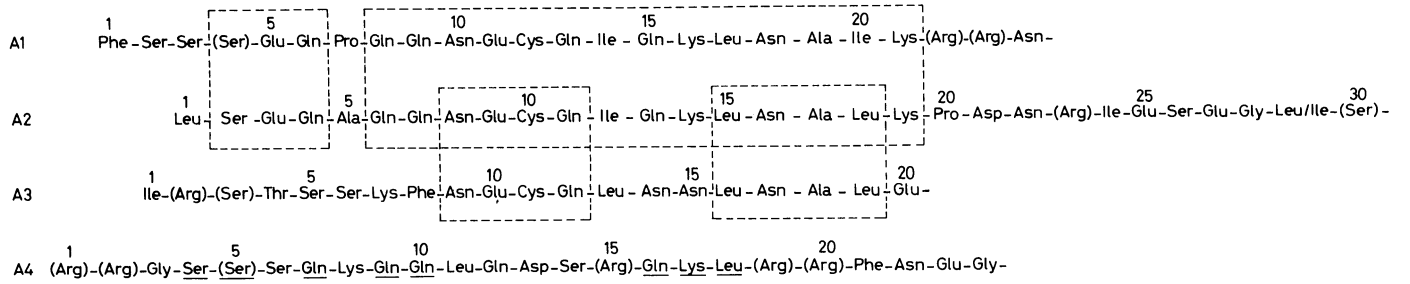

Fig. 6. N-Terminal amino acid sequence of the acidic subunits **(A1–A4).** Identical regions of A1–A3 are enclosed within a frame. The underlined residues in A4 are the same as those in similar positions in A1. Arginine is shown in parentheses because it was not detected directly.

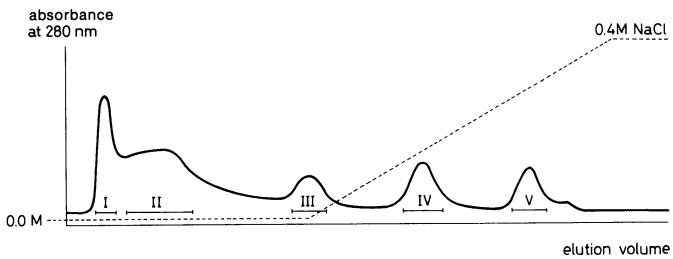

Fig. 7. Diethylaminoethyl Sephadex A-50 column chromatography of carboxamidomethylated intermediary subunits **(I–V).** Dotted line = sodium chloride gradient, barred line = pooled fractions.

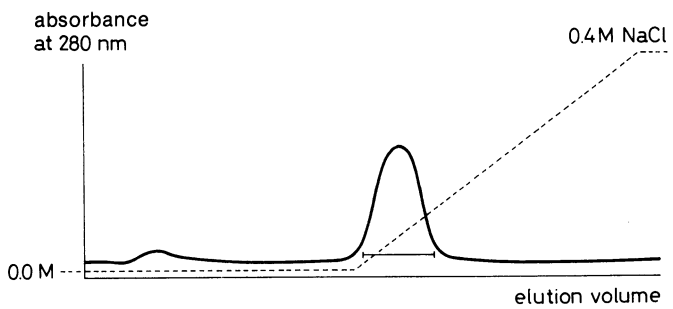

Fig. 8. Rechromatography of fraction II of Fig. 7 on diethylaminoethyl-Sephadex A-50. Dotted line = sodium chloride gradient, barred line = pooled fraction.

derivatives and extraction with ethyl acetate, PTH-Arg and PTH-His were the only derivatives expected in the water layer. When no derivatives could be identified in the ethyl acetate layer by gas-liquid or thin-layer chromatography, the water phases were subjected to back hydrolysis with hydroiodic acid and the resultant free amino acids determined in the usual way by amino acid analysis. However, on analysis of the residues in question, a component eluting precisely in the place of arginine was observed. It could not be unequivocally established as arginine because of the unusually large peak height in relation to the yield of the amino acid residues from neighboring steps in the sequence. No histidine could be detected in the fractions concerned either; therefore, the only inference is that arginine is present in these steps.

The N-terminus of A4 appears to be arginine according to automatic sequence analysis. Earlier analysis by means of DNS-Cl had indicated that it might be isoleucine. Arginine might have been overlooked because, on dansylation of a protein, all ϵ-NH_2 groups of lysine in the chain are modified, and therefore a considerable amount of ϵ-DNS-Lys in addition to the N-terminal amino acid is found after hydrolysis. In our solvent system, this derivative has about the same mobility on a micropolyamide plate as does DNS-Arg. The high concentration of ϵ-DNS-Lys may quite possibly interfere with the detection of DNS-Arg.

Intermediary Subunits

The concept and possible existence in seed proteins of the so-called "intermediary subunits," with intermolecular disulfide bridges between individual subunits, was first proposed by Wright and Boulter (1974) in their studies on legumin, the major storage protein of *Vicia faba* L (broadbean) seeds. In the study of the quaternary structure of proteins containing disulfide bonds, isolation and study of the constituent intermediary subunits is obviously of importance because it could provide at least a partial answer to the question of the protein structure. According to Kitamura et al (1976), intermediary subunits of glycinin are formed from one basic and one acid subunit being linked together by disulfide bonds.

TABLE III
N-Terminal Analysis of Isolated Intermediary Subunits

Fraction Number[a]	N-Termini
I	Ile, Phe, Gly
II	Phe, Gly
III	Leu, Gly
IV	Ile, Gly
V	(Ile)[b]

[a] Fraction numbers correspond to those of Fig. 7.
[b] As with fraction V of Table I, which is the acidic subunit A4, a spot was found after 18 hr of hydrolysis that could not be identified with certainty. After 65 hr of hydrolysis, a faint spot indicated that Ile could be the N-terminus.

We had to use a procedure for preparing the intermediary subunits different from that described by Kitamura et al (1976) because of the probability of S-S polymers in our preparation of glycinin. In the presence of 0.01 *M* 2-mercaptoethanol at neutral pH and an ionic strength of >0.35, the glycinin monomer is stable (Wolf and Briggs 1958), and, whereas the S-S bridges responsible for polymer formation are reduced, the S-S bonds holding the acidic and basic subunits to form intermediary subunits are intact and evidently protected from the reducing agent. Addition of iodacetamide to the monomer in this condition modifies the sulfhydryl groups resulting from intermolecular S-S bond cleavage. Because the dissociation of glycinin into the intermediary subunits is done in the presence of urea, no reducing agent must be present at the same time.

Ion-exchange chromatography of the carboxamidomethylated intermediary subunits on DEAE-Sephadex A 50 in the presence of 8 *M* urea yielded five fractions (Fig. 7). Fraction II, which was not well separated from fraction I, was rechromatographed with 0.05 *M* buffer and elution time of 24 hr (Fig. 8). N-terminal determination of the five fractions by means of the dansylation technique revealed the residues indicated in Table III.

Fraction I was obviously a mixture of intermediary subunits and was not characterized further. Fractions II–V were subjected to SDS-PAGE; Fig. 9 shows the electropherogram. Fractions II–IV are pure intermediary subunits, which were also indicated by N-terminal analysis (Table III). Fraction V corresponds to an acidic subunit (relative molecular mass ~ 38,000), which obviously is not part of an intermediary subunit but is present as a "free" subunit in the structure of glycinin.

The intermediary subunits after reduction and carboxamidomethylation were analyzed by the three electrophoresis systems: SDS-PAGE, alkaline urea-PAGE, and the acidic-urea-PAGE

(Fig. 10). From Fig. 10A and B, we can conclude that the intermediary subunits in fractions II, III, and IV are made up of acidic subunits A1, A2, and A3, respectively, and Fig. 10C shows that these are combined with the basic subunits, B2, B1, and B3, respectively. The results concerning the intermediary subunits are summarized in Table IV. Clearly, the acidic subunit A3, which has a relative molecular mass of 45,000, is covalently linked with basic subunit B3 and thus forms part of the glycinin molecule, it cannot be considered a minor contaminant, as one might conclude after a casual observation of the SDS-PAGE pattern of total glycinin.

DISCUSSION

The Free Acidic Subunit

Our experiments indicated that a free acidic subunit was present in our glycinin preparation. By "free" we mean that it is not covalently linked through S-S bond(s) to any other component of glycinin; thus the subunit is not a part of an intermediary subunit. None of the research groups that have worked on the subunit composition of glycinin have so far reported the presence of such a subunit. We have repeatedly found this free acidic subunit, evidenced by the characteristics of fraction V (Figs. 7 and 9), during the preparation of intermediary subunits from glycinin.

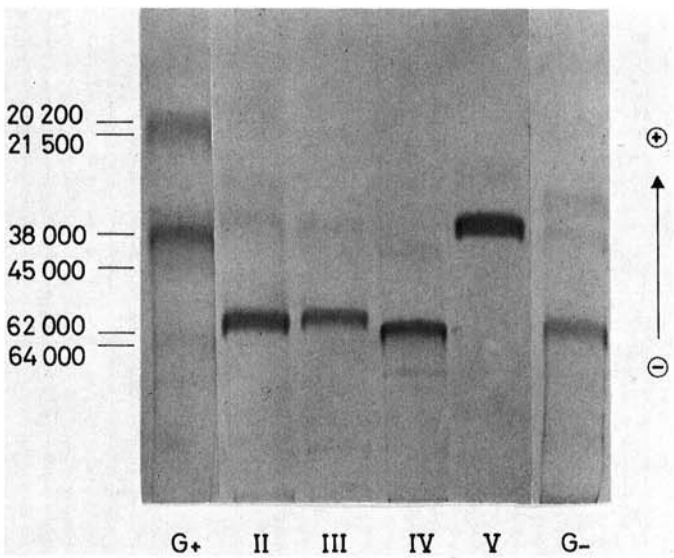

Fig. 9. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of intermediary subunits (**II–V**). G+ = glycinin with 2-mercaptoethanol, G− = glycinin without 2-mercaptoethanol. Samples II–V correspond to isolated fractions in Fig. 7.

The electron microscope picture of glycinin published by Badley et al (1975), quite clearly shows that the center of the half molecule contains protein. Although the authors contend that the lightness is due to the stain's difficulty in reaching the center of the molecule, we consider this picture to show clearly the presence of a free subunit. Figure 10C shows that all basic subunits liberated from the intermediary subunits are present in the total basic fraction. Also, the total basic fraction does not show evidence of any extra basic subunit; therefore no basic partner exists to pair with the free acid subunit.

To reconcile our findings with the presence of an extra pair of free acidic subunits, probably occupying the central hole formed by the two hexagons of subunits one on top of the other, we have to consider the following. Our estimates of the molecular masses of the individual acidic, basic, and intermediary subunits are based only on their comparative mobility in the SDS-PAGE system. SDS-PAGE does not always give a correct estimate of the molecular mass of a polypeptide (Weber et al 1972). The good agreement between electrophoretic mobilities in gels and molecular masses was rather surprising in view of the fact that the proteins examined doubtlessly differ substantially in net charge and probably in conformation in simple salt solutions. Although the heavy binding of SDS to denatured proteins swamps out even relatively large differences in intrinsic net charge, too much confidence should not be placed in a molecular mass determination, especially if its amino acid composition deviates significantly from the average composition for proteins used as standards.

According to Ochiai-Yanagi et al (1977), reevaluation of the molecular masses by 6*M* guanadine gel chromatography gave the values 28,000 and 18,000 for the acidic and basic subunits, respectively. These results were supported by results of equilibrium

TABLE IV
Characteristics and Composition of Intermediary Subunits

Fraction Number[a]	Relative Molecular Masses of Subunits			N-Termini and Nomenclature of Subunits			
	Intermediary	Basic	Acid	Basic		Acidic	
II	62,000	21,500	38,000	Gly	B2	Phe	A1
III	62,000	20,200	38,000	Gly	B1	Leu	A2
IV	64,000	20,200	45,000	Gly	B3	Ile	A3

[a] Fraction numbers correspond to those of Fig. 7.

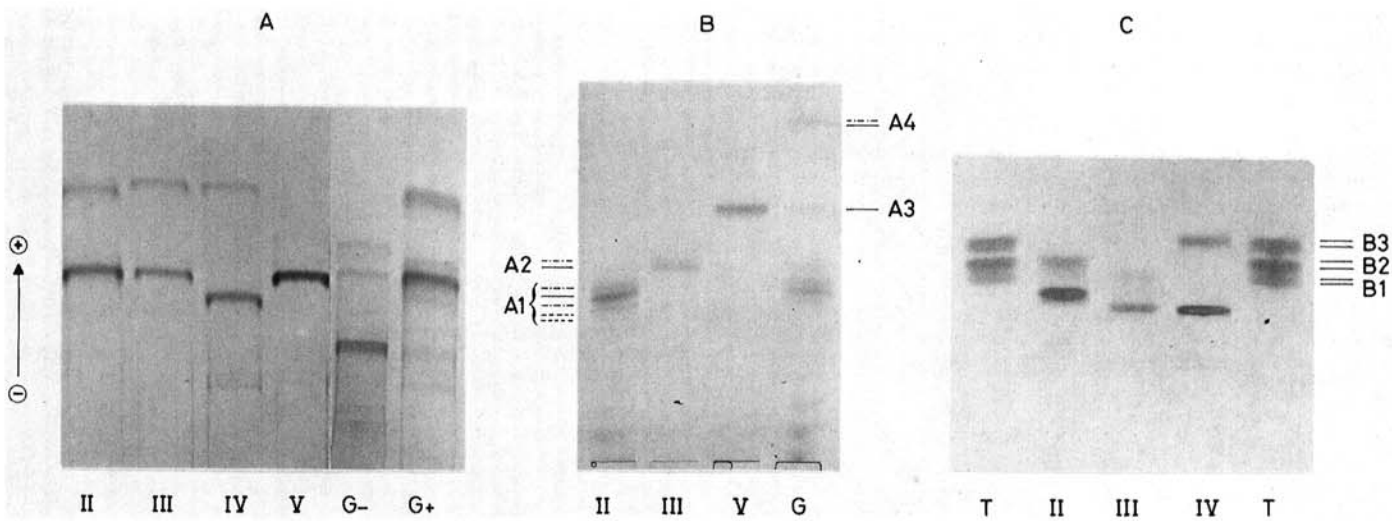

Fig. 10. Polyacrylamide gel electrophoresis of reduced and carboxamidomethylated intermediary subunits (**II–V**): **A,** by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE); **B,** by alkaline urea-PAGE; **C,** by acidic urea-PAGE. Samples II–V correspond to isolated fractions in Fig. 7 after further reduction and carboxamidomethylation. **G** = reduced and carboxamidomethylated glycinin, **G+** = glycinin with 2-mercaptoethanol, **G−** = glycinin without 2-mercaptoethanol, **T** = total basic fraction (fraction I of Fig. 3).

sedimentation in the same solvent. They further state that previously reported values seem to be overestimated, especially for the acidic subunits. Because of the high percentage of acidic amino acids, the conformation of the SDS-protein polypeptide complexes deviates from those of proteins usually employed as standards for molecular mass estimates.

Comparison of the N-terminal amino acid sequences of our acidic subunits (Fig. 6) with those of similar acidic subunits reported by Moreira et al (1979) shows excellent agreement between the sequences of A1, A2, and A4. Although the sequences of the A3 subunits appear to have very little similarity, shifting our sequence to the left by a couple of places produces quite remarkable similarity, ie, Thr(4) to Glu(20) of our sequence agrees exactly with Thr(2) to Glu(18) of that reported earlier (Moreira et al 1979). Thus we can say with greater confidence that acidic subunit A4 from our glycinin, the so-called free acidic subunit, is a part of the total molecule.

If we assume relative molecular masses of 28,000 and 18,000 for the acidic and basic subunits, respectively, as published by Ochiai-Yanagi et al (1977), and approximately 42,000 for subunit A3 instead of 45,000, we can compute the total relative molecular mass of glycinin as follows: A1 (28,000) + B2 (18,000) = 46,000; A2 (28,000) + B1 (18,000) = 46,000; A3 (42,000) + B3 (18,000) = 60,000; plus A4 (28,000), giving 152,000 for a half molecule of glycinin and 304,000 for two halves. With two free acidic subunits of 28,000 each, the total relative molecular mass is 360,000. This figure is clearly an acceptable one for the molecular mass of glycinin because it compares favorably with the published figures of 320,000–360,000 given by most authors.

Basic Subunits

In the present work, the basic subunits were not isolated as such and characterized individually. However, besides being studied as a group with the unabsorbed fraction from the separation of the acidic subunits on the DEAE-Sephadex column (Fig. 3, Table I), they were studied as constituents of the intermediary subunits, from which they could be characterized after cleaving the S-S bonds (Fig. 10A and C). Table IV summarizes the characteristics of the individual basic subunits. The nomenclature of the basic subunits is analogous to that of the acidic; for instance, B1 is the least basic and B3 the most basic subunit. All basic subunits consist of doublets similar to those of some of the acidic subunits (Fig. 10C).

The model of Kitamura et al (1976) is difficult to reconcile with our findings. Kitamura and Shibasaki (1975) suggest that the differences in glycinin subunits among varieties of soybean are due to polymorphism. However, our own investigations[1] on a number of well-defined varieties, a field type and a vegetable type, did not show any significant difference between these types nor between these and the "Express Green" variety studied here. Therefore glycinin probably does not occur in polymorphic forms.

CONCLUSION

From our findings we conclude that glycinin consists of two identical half molecules, each consisting of three different intermediary subunits, and two additional acidic subunits that probably occupy the central hole of each half molecule.

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[1] Unpublished results.

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