Separation of the 13S Globulin in Sesame Seeds into Two Groups of Acidic and Basic Subunits, and Their Physicochemical Properties

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ABSTRACT

The 13S globulin in sesame seeds was composed of acidic and basic subunits. These subunits were separated into two fractions by column chromatography on DEAE-Sephadex A-50 containing urea and 2-mercaptoethanol—a fraction including the acidic subunits and a second fraction including the basic subunits, whose isoelectric points were pH 5.5–6.5 and 8.5–9.5, respectively, on estimation by gel isoelectric focusing. The molecular weights of the acidic and basic subunits were estimated as 30,500–33,500 and 20,000–24,500 daltons, respectively, by gel filtration and by sodium dodecyl sulfate (SDS) gel electrophoresis. The acidic and basic subunits were suggested to be present in equimolar amounts in the 13S globulin molecule by the densitometric scanning of the SDS gel and the molecular weight consideration. There were significant differences in the amino acid compositions of the acidic and basic subunits as illustrated by lower contents of threonine, serine, valine, methionine, lysine, and aspartic acid, and higher content of glutamic acid in the acidic than in the basic subunits. Glycine in the group of acidic subunits, and glycine and valine in the group of basic subunits, were detected as N-terminal amino acids.

The major fraction, α-globulin, represents about 70% of the total proteins in sesame seeds and a 13S globulin represents 95% of this α-globulin (Sinha and Sen 1962). In our earlier studies (Nishimura et al 1979, Okubo et al 1979), the 13S globulin, which was homogeneous by ultracentrifugation, gel electrophoresis, and immunodiffusion, was isolated by gel filtration on a Sepharose column from α-globulin fraction, and the physicochemical properties of the 13S globulin such as molecular weight, sedimentation coefficient, diffusion coefficient, partial specific volume, and amino acid composition were determined. Furthermore, by ultracentrifugal and slab gel electrophoretic analyses in the sytem with urea, it was confirmed that the 13S globulin in seeds had a subunit structure composed of acidic and basic subunits (Sinha and Sen 1962).

This paper deals with the separation of the 13S globulin into the two groups of acidic and basic subunits by column chromatography on DEAE-Sephadex A-50 containing urea, some physicochemical properties, and amino acid and N-terminal amino acid compositions of the separated groups.

MATERIALS AND METHODS

Materials

Representative white varieties of sesame seeds (*Sesamum indicum* L.) cultivated in Ethiopia, obtained from the Institute of Morinaga Seika Co., Kawasaki, Japan, were stored in the cold until used. The sample of purified 13S globulin was prepared from the α-globulin fraction by the methods described previously (Okubo et al 1979). The 13S globulin was dissolved in 0.05*M* tris-HCl buffer (pH 8.0) containing 6*M* urea and 0.05*M* 2-mercaptoethanol, and allowed to stand at room temperature for 1 hr to dissociate it into subunits. The approximate sedimentation coefficient of the globulin decreased 13S to 1.5S and eight bands were observed on gel electrophoresis (Okubo et al 1979).

DEAE-Sephadex Column Chromatography

A column (20 × 30 cm) of DEAE-Sephadex A-50 was equilibrated with 0.05*M* tris-HCl buffer (pH 8.0) containing 6*M* urea and 0.05*M* 2-mercaptoethanol. The urea-treated sample solution was applied to the column, followed by stepwise elution with the same buffer and then with the same buffer containing 0.5*M* sodium chloride (NaCl). Each 5-ml aliquot of effluent was collected with a fraction collector and the effluent corresponding to each peak was dialyzed and lyophilized.

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Gel Filtration

Sample proteins for gel filtration were reduced and carboxymethylated according to Crestfield et al (1963) as follows: 100 mg of the lyophilized proteins was dissolved in 10 ml of 0.15*M* tris-HCl buffer (pH 8.0) containing 8*M* urea, 0.10*M* 2-mercaptoethanol, and 1 m*M* EDTA and, after standing at 20°C for 4 hr, 0.135 g of iodoacetoamide and 0.117 g of 2-mercaptoethanol were added in the dark for 15 min. The modified proteins were dialyzed against water in the dark and lyophilized. A Sepharose 6B gel column (0.7 × 120 cm) was prepared according to Fish et al (1969). The eluting solvent for the column runs permitted a constant flow rate of 2.3 ml/hr/cm^2. The lyophilized proteins (2 mg) were dissolved in 0.2 ml of 6*M* guanidine·HCl. After standing for 3 hr at room temperature, blue dextran 2,000, DNP-alanine, and sucrose were added to 0.3, 0.05, and 10%, respectively. The sample solution was layered under the solvent on top of the gel. Fractions of 0.5 ml were collected and absorbances at 280 nm (blue dextran), 360 nm (DNP-alanine), and 220 nm (protein) were recorded. The elution positions were estimated based on weight determination (Fish et al 1969).

Gel Electrophoresis

Slab gel electrophoresis in a system with urea was performed according to the method described previously (Okubo et al 1979). Isoelectric focusing gel electrophoresis was done by the methods of Catsimpoolas (1968), and Righetti and Drysdale (1974). A 5% acrylamide gel column containing 6*M* urea and 2% Ampholine was prepared in a glass tube (5 × 100 mm). Protein sample dissolved in 6*M* urea solution containing 2% Ampholine and 0.1*M* 2-mercaptoethanol was layered on top of the gel column. Isoelectric focusing was performed in the bath assembly used for disc electrophoresis separations. The upper bath as anode was filled with 5% phosphoric acid solution and the lower bath as cathode with 5% ethylenediamine solution. Electrofocusing was conducted for 4–10 hr at a constant of 5 mA per tube. After electrofocusing and removing the gel column from the glass tube, the gels were placed in 12% trichloroacetic acid for 2 hr. Staining of the electrofocused bands was performed with 0.02% Coomassie blue dye and destaining was done electrically in 7% acetic acid. pH was determined by slicing an unstained gel column, suspending the sliced sections in 1.0 ml of water for 4 hr, and measuring the pH with a microelectrode.

Sodium doceyl sulfate (SDS) gel electrophoresis was performed according to the method of Weber and Osborn (1969) on 10% polyacrylamide gel. Standard proteins for the estimation of molecular weight (mol wt) were used: γ-globulin (mol wt 50,000 H chain, 23,500 L chain), ovalbumin (mol wt 43,000), carboxypeptidase A (mol wt 35,000), chymotrypsinogen (mol wt 25,700), trypsin inhibitor (mol wt 21,500), myoglobin (mol wt 17,200), and lysozyme (mol wt 14,400). Densitometric scanning of the destained gel was obtained with Gelman ACD-15.

Amino Acid Analysis

The samples were hydrolyzed in vacuo for 24 and 72 hr at 110 ± 0.1°C with 6*N* HCl. Amino acid analysis was performed with a Hitachi KLA-3B automatic amino acid analyzer. Threonine, serine, and tyrosine were corrected by extrapolation to zero time. For valine and isoleucine, the 72-hr values were used. For cystine analysis, the protein oxidized with formic acid, and hydrogen peroxide was hydrolyzed by the method of Thompson (1954). Tryptophan content was measured by UV absorption according to the method of Bredderman (1974). Independent determination of the amide-ammonia in this protein was made by the modified procedure of Chibnall et all (1958).

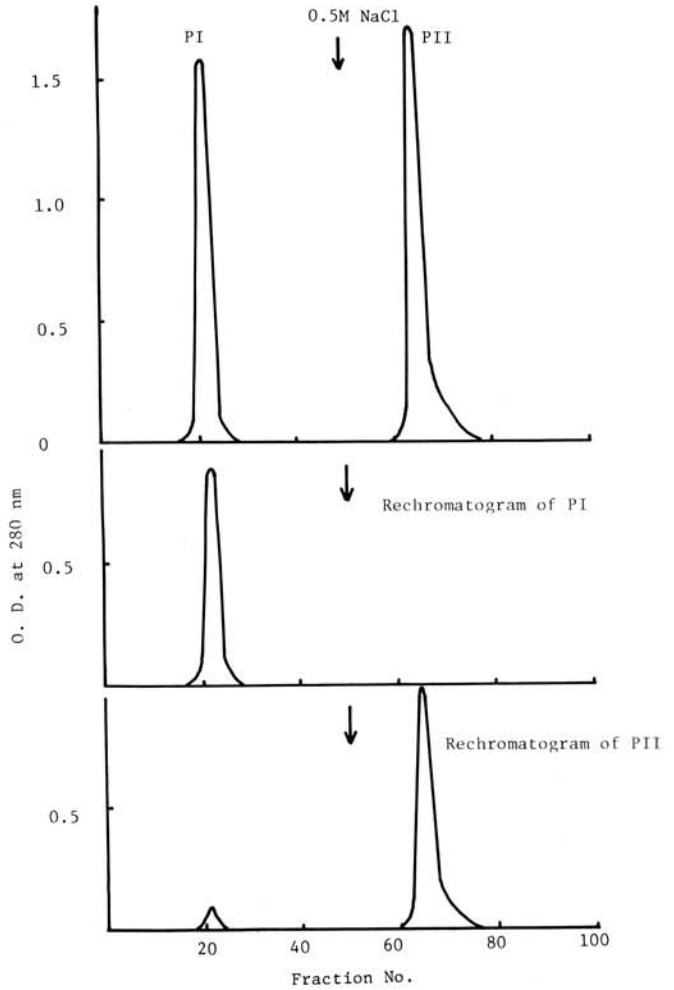

Fig. 1. Ion-exchange chromatogram of the dissociated 13S globulin on DEAE-Sephadex A-50 column (2.5 × 30 cm). (Buffer: 0.05*M* tris-HCl [pH 8.0] containing 6*M* urea and 0.05*M* 2-mercaptoethanol; flow rate: 20 ml/hr; volume of fraction: 10 ml; temperature: 5°C.)

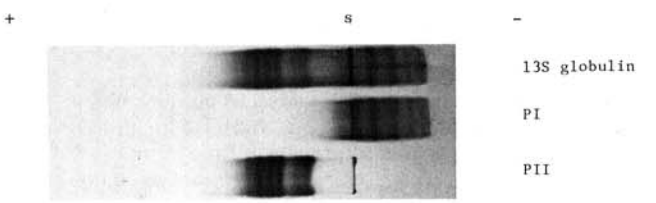

Fig. 2. Slab gel electrophoretic patterns of the fractions on DEAE-Sephadex A-50 column in Fig. 1. (Gel concentration: 7.0%; gel and sample buffer: 0.076*M* tris-citrate [pH 8.7] containing 7*M* urea and 0.02*M* 2-mercaptoethanol.)

N-Terminal Amino Acid Analysis

N-Terminal amino acids were determined by the DNP-amino acid method (Sanger 1945). DNP-protein was hydrolyzed with constant boiling HCl for 12 hr at 105 ± 0.1°C in sealed evacuated tubes. The fractionated ether-soluble DNP-amino acids were analyzed by thin-layer chromatography on silica gel G film by the method of Brenner et al (1961) using the "toluene" system for the first dimension and chloroform/benzyl alcohol/acetic acid (70:30:3) for the second dimension.

RESULTS

Separation of the 13S Globulin into Two Groups of Acidic and Basic Subunits

The 13S globulin was dissolved in 0.05*M* tris-HCl buffer (pH8.0) containing 6*M* urea and 0.05*M* 2-mercaptoethanol to dissociate it into subunits. The sample solution was applied to the column of DEAE-Sephadex A-50 equilibrated with the same buffer, followed by stepwise elution with the same buffer and then buffer containing 0.5*M* NaCl. As shown in Fig. 1., two peaks, PI unabsorbed on the DEAE-Sephadex A-50 column and PII absorbed on the column, were obtained. The gel electrophoretic patterns of the peaks obtained by rechromatography are shown in Fig. 2. PI and PII corresponded to several bands migrating nearer to the slot and faster to the cathode, respectively. The electrophoretic pattern by the densitometric method (not shown in the figure) and the elution profile at 280 nm in Fig. 1 show that the area ratio of PI and PII is approximately 1:1. This indicates that the molar ratio of the total basic to total acidic subunits in the 13S globulin molecule is approximately 1:1.

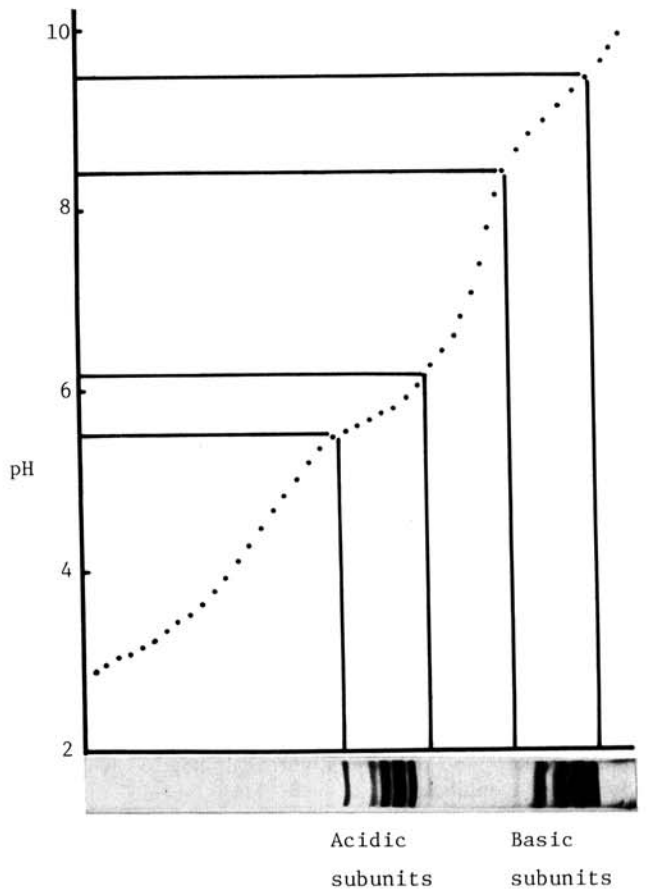

Fig. 3. Estimation of isoelectric point by gel isoelectric focusing. (Gel concentration: 5.5%; gel buffer: 2.0% Ampholine containing 6*M* urea; sample buffer: 2.0% Ampholine containing 6*M* urea and 0.05*M* 2-mercaptoethanol.)

Isoelectric Focusing and Isoelectric Points of the Subunits

Figure 3 shows the isoelectric focusing pattern and pH gradient of the dissociated 13S globulin in the pH range between 3 and 10. The basic and acidic subunits were focused between pH 8.5 and 9.5 and between pH 5.5 and 6.5, respectively. The isoelectric points of these main subunits (average of three experiments) were pH 9.4, 9.3, 9.1, and 8.8 in the basic subunits, and pH 5.9, 5.8, 5.7, and 5.5 in the acidic subunits.

Molecular Weight Estimation of the Subunits

Standard proteins and the carboxyaminomethylated fractions including acidic and basic subunits, respectively, were gel-filtered on Sepharose 6B column in 6*M* guanidine·HCl. The distribution coefficients, Kd, were calculated from the elution profile and, as shown in Fig. 4, the calibration curve was obtained. From the curve, the molecular weights of the acidic and basic subunits were estimated to be 31,000 and 20,000 daltons, respectively.

The SDS gel electrophoretic pattern shown in Fig. 5 indicates that the acidic subunits gave three bands corresponding to molecular weight of 33,500, 31,500, and 30,500, and that the basic subunits gave one band corresponding to molecular weight of 24,500.

Amino Acid Composition

The amino acid compositions of the 13S globulin and fractions including the acidic and basic subunits are shown in Table I. These fractions and globulin had some characteristics of legumin-type proteins (Derbyshire et al 1976)—they were rich in glutamate, aspartate, leucine, and valine, and low in tryptophan and cysteine. However, there were significant differences in the amino acid compositions of the fractions in that the fractions including the acidic subunits were richer in glutamic acid, but lower in threonine, serine, valine, lysine, methionine, and aspartic acid than the fractions including the basic subunits. Each average value of amino acid contents in the acidic and basic subunits is almost equal to that in the 13S globulin, indicating that the acidic and basic subunits should be present in equimolar amounts in the 13S globulin molecule.

N-Terminal Amino Acid

The results of the amino terminal analysis are shown in Table I. The fraction including the acidic subunits contained only glycine as N-terminal amino acid, but the fraction including the basic subunits contained glycine and valine as N-terminal amino acids.

TABLE I
Amino Acid Compositions of the 13S Globulin and the N-Terminal Amino Acids of the Acidic (Glycine) and Basic (Glycine and Valine) Subunits

Amino Acid	13S Globulin	Acidic Subunits (g/100 g protein)	Basic Subunits
Aspartic acid	10.81	9.84	12.03
Threonine	4.43	3.72	5.59
Serine	5.84	4.73	7.88
Glutamic acid	21.40	27.83	15.55
Proline	4.02	4.35	3.96
Glycine	5.66	5.71	4.92
Alanine	5.78	5.44	6.56
Half-cystine	1.78[a]	1.46[b]	1.70[b]
Valine	6.82	5.34	8.56
Methionine	2.14	1.71	3.21
Isoleucine	5.00	4.75	5.07
Leucine	7.44	7.08	7.93
Tyrosine	4.04	3.65	4.64
Phenylalanine	5.43	5.83	4.65
Histidine	3.08	3.03	2.42
Lysine	2.34	1.49	3.03
Arginine	13.98	14.52	13.41
Tryptophan[c]	1.65	1.52	1.78
Amide ammonia	1.74	1.99	1.62
Total	113.38	113.99	114.51

[a]As cysteic acid.
[b]As carboxymethyl cysteine.
[c]By spectrophotometric method.

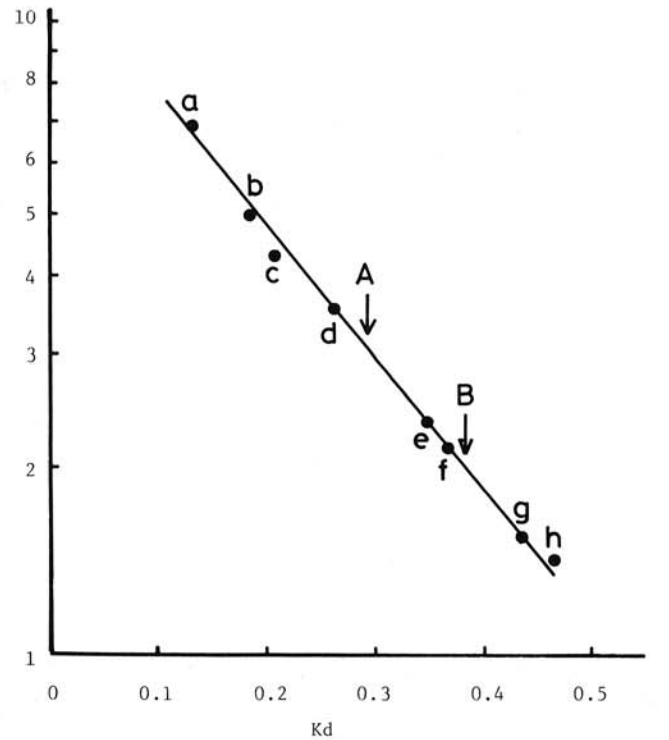

Fig. 4. Estimation of molecular weight by gel filtration on Sepharose 6B column in 6*M* guanidine · HCl. **a**, Bovine serum albumin; **b**, γ-globulin, H chain; **c**, ovalbumin; **d**, pepsin; **e**, γ-globulin, L chain; **f**, trypsin inhibitor; **g**, hemoglobin; **h**, lysozyme. **A**, Molecular weight of acidic subunits = 31,000; **B**, molecular weight of basic subunits = 20,000.

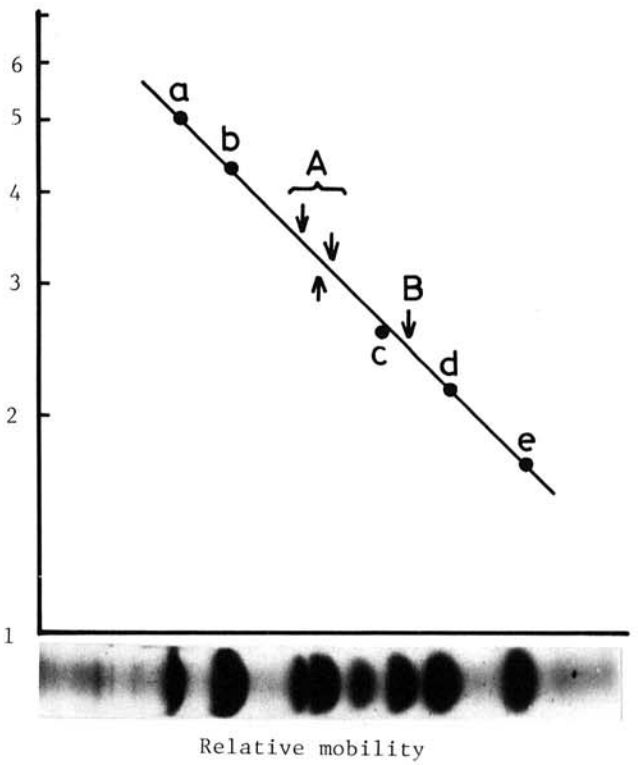

Fig. 5. Estimation of molecular weight by sodium dodecyl sulfate gel electrophoresis. **a**, γ-Globulin, H chain; **b**, ovalbumin; **c**, chymotrypsinogen; **d**, trypsin inhibitor; **e**, myoglobin. **A**, Molecular weight of acidic subunits = 33,500, 31,500, 30,500; **B**, molecular weight of basic subunits = 24,500.

TABLE II
Comparison in Subunit Structure between 13S Globulin and Legumin

Globulin	Molecular Weight of Subunit		Ratio and No. of Acidic/Basic Subunits	Estimated Molecular Weight of Globulin
	Acidic	Basic		
13S Globulin (sesame seed)	33,500[a]	24,500[a]	6:6	338,000[a]
	31,500[a]			
	30,500[a]			
	31,000[b]	20,000[b]		306,000[b]
Glycinin (soybean)[c]	35,500[a]	21,600[a]	6:6	355,600[a]
	42,000[a]			
	28,500[b]	18,500[b]		282,000[b]
	28,000[d]	18,000[d]		276,000[d]
Legumin (*vicia faba*)[e]	37,000[a]	20,100[a]	6:6	351,000[a]
	35,800[a]	20,900[a]		
		23,800[a]		
Arachin (pea nut)[f]	29,000[d]		12	348,000[d]

[a]By sodium dodecyl sulfate gel electrophoresis.
[b]By gel filtration in 6*M* guanidine•HCl.
[c]From Kitamura et al (1976) and Ochiai-Yanagi et al (1977).
[d]By sedimentation diffusion method.
[e]From Derbyshire et al (1976).
[f]From Yotsuhashi (1974).

DISCUSSION

It is well known that the main globulin in legume seeds such as *Pisum sativum, Vicia faba, Cicer arietinum, Glycine max,* and *Arachis hypogaea* can be broken down by treatment with urea or SDS into subunits, which are then detected on gel electrophoretic patterns with urea or SDS (Derbyshire et al 1976). The 13S globulin in sesame seeds also can be fragmented by treatment with urea into smaller fragments, which was detected on the gel electrophoretical pattern with urea (Fig. 2) or SDS (Fig. 3). Therefore, the fragments dissociated from the 13S globulin with urea are regarded as the subunits of the 13S globulin. Unabsorbed and absorbed fractions of the dissociated 13S globulin on DEAE-Sephadex A-50 column, an anion exchanger, showed acidic and basic bands on the gel electrophoretic and electrofocusing patterns (Figs. 1, 2, and 3). Furthermore, in addition to the physicochemical properties of the 13S globulin as described in a previous report (Nishimura et al 1979), the equimolar composition and the molecular weights of the acidic and basic subunits in the 13S globulin indicate an interesting similarity with the legumin-type globulins (Derbyshire et al 1976, Kitamura et al 1976, Ochiai-Yanagi et al 1977, Yotsuhashi 1974) in the Leguminosae as shown in Table II. In spite of the fact that this main storage protein of sesame seeds belongs to a different family than the Leguminosae, the subunit structure of the 13S globulin is suggested to be quite similar to that of the legumin-type globulin. Higher isoelectric points of the basic subunits should be based on the lower contents of glutamate than the 13S globulin and acidic subunits (Table I). Accordingly, this suggests that the basic subunits are located inside the 13S globulin molecule, similarly to the glycinin in soybean seeds (Kitamura et al 1976).

As shown in Table II, the molecular weight of the 13S globulin was estimated to be 338,000 and 306,000 on the basis of equimolar contents of the acidic and basic subunits in the globulin and the molecular weight of the subunits. Either of these values is smaller than the values of 361,000 and 399,000 in our previous report (Nishimura et al 1979), and the value closest to true molecular weight of the 13S globulin is estimated by using each of purified subunits after determination of kinds and numbers of the subunits in the globulin.

Generally, sesame protein is regarded as a methionine-rich protein for processed foods. Interestingly, however, the basic subunits have a higher content of methionine than the 13S globulin and the acidic subunits (Table I).

ACKNOWLEDGMENT

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