Chemical and Physical Properties of 13S Globulin, the Major Protein in Sesame Seeds

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ABSTRACT

A 13S globulin fraction of sesame protein that was homogeneous by ultracentrifugation, gel electrophoresis, and immunodiffusion was found to have molecular weight of 361,000 to 399,000 from sedimentation coefficient ($S°_{20)w}$ = 12.80), viscosity ($|\eta|$ = 0.0325 dl/g), diffusion coefficient ($D_{20,w}$ = 3.46 × 10^{-7}), and partial specific volume (V = 0.718 ml/g). The integral numbers of amino acid residues per molecule calculated on the basis of 399,000 were as follows: Asp_{324}, Thr_{149}, Ser_{222}, Glu_{580}, Pro_{140}, Gly_{301}, Ala_{259}, $1/2Cys_{59}$, Val_{233}, Met_{57}, Ile_{152}, Leu_{226}, Tyr_{89}, Phe_{132}, His_{80}, Lys_{64}, Arg_{321}, Trp_{35}, and Amido-ammonia$_{408}$. Valine and glycine were found as the only N-terminal amino acids present.

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The major fraction, α-globulin, represents about 70% of the total proteins in sesame seeds and contains about 95% of a 13S globulin (Sinha and Sen 1962). As described previously (Okubo et al 1979), the 13S globulin, which was homogeneous by ultracentrifugation, gel electrophoresis, and immunodiffusion, was isolated from α-globulin by gel filtration on a Sepharose column.

This paper deals with some chemical and physical properties, molecular weight, amino acid composition, and N-terminal amino acids in the isolated 13S globulin.

MATERIALS AND METHODS

Materials

The sample of purified 13S globulin was prepared from α-globulin fraction by the method described in a previous paper (Okubo et al 1979). Potassium phosphate buffer (0.0026*M* KH_2PO_4, 0.0325*M* K_2HPO_4, pH 7.66) containing 0.02*M* 2-mercaptoethanol and 10% sodium chloride was used as the standard buffer.

Physical Analyses

Sedimentation analysis was done with a Hitachi UCA-1A ultracentrifuge at 55,430 rpm. Routine assay runs were made in standard buffer at 20° C. Viscosity measurement was made with an Ostwald viscometer at 20 ± 0.05° C. The densities of the solution and the solvent were measured at 20 ± 0.05° C using the Ostwald pycnometer with a capacity of 10 ml.

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Preparation of Antisera

Immunization was performed essentially as described by Catsimpoolas et al (1969). A 2% aliquot of the extracted whole proteins in the standard buffer without 2-mercaptoethanol, mixed and homogenized with an equal volume of Freund complete adjuvant (Difco), was used for intraperitoneal immunization of young adult white rabbits. The immunizing dosage was 1 ml the first week, 2 ml the second week, and 5 ml the third week. After a 30-day rest period, the rabbits were given a 5-ml booster injection. The animals were then subjected to test bleeding from the marginal ear vein, and bled after seven days from the carotid artery by cardiac puncture. The sera stood at 37° C for 1 hr, then overnight at 4° C to remove the clot, and was stored at −20° C after centrifugation (13,400 × *g*, 30 min, 4° C) and with addition of 0.001*M* sodium azid.

Chemical Analyses

Nitrogen determination was done by the micro-Kjeldahl method. Protein concentration was routinely determined by light absorption at 280 nm with a Hitachi model 124 spectrophotometer and turbidity at 410 nm. Phosphorus content was determined by the method of Allen (1940). The phenol-sulfuric acid method according to Dubois et al (1956) was used for determining total carbohydrate.

Amino Acid Analysis

The samples were hydrolyzed in vacuo for 24 and 72 hr at 110 ± 0.1° C with 6*N* hydrochloric acid. Amino acid analysis was performed with a Hitachi KLA-3B automatic amino acid analyzer. Threonine, serine, and tyrosine were corrected by extrapolation back 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

ultraviolet 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 al (1958).

N-Terminal Amino Acid Analysis

N-Terminal amino acids were determined by the sodium-2,4-dinitrophenolate (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 (TLC) 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

Chemical Composition

Analytical results of the 13S globulin indicated that the nitrogen content was 17.69; phosphorus, ash, and sugar were not detected. Therefore, the 13S globulin seemed to be a simple protein. The nitrogen content found was lower than that of α-globulin reported by Jones and Gersdorff (1927), but similar to that of legume proteins (Boulter 1971) considered to be typical globulins.

Physical Characteristics

Viscosity measurements were made at five different concentrations of the globulin at 20 ± 0.05°C (Fig. 1). The value of intrinsic viscosity, $|\eta|$, was 0.0325 dl/g from inherent viscosity, $\ln \eta_{r/c}$.

The partial apparent specific volume, Va, of the globulin that was determined experimentally from equation 1 was 0.730 ml/g:

$$Va = 1/\rho_o[1 - (\rho - \rho_o)/w] \quad (1)$$

where ρ is the density of the solution, ρ_o that of the solvent, and w the protein concentration in grams per milliliter. The value is similar to that (0.735 ml/g) calculated from diffusion coefficient by Ventura and Lima (1963), but somewhat different from that (0.718 ml/g) calculated from the amino acid composition (Table I) by the following equation:

$$V = (Vi \times Wi)/Wi \quad (2)$$

where Wi is percent of weight of amino acid residue and Vi specific volume of amino acid.

The sedimentation coefficient, $S^0_{20,w}$, was 12.80S (Fig. 2). This value corresponds closely to 12.7S given by Ventura and Lima (1963) and 12.35S by Sinha and Sen (1962).

The diffusion coefficient of the globulin was immunologically estimated by the method of Allison and Humphrey (1960). Briefly, antigen and antibody are placed in troughs cut at a 90 degree angle in a uniform layer of agar. As shown in Fig. 3, precipitin occurred along a straight line inclined at an angle θ (43.1°) to the antigen trough. There is also a relation between θ, diffusion coefficient of antigen, Dg, and that of rabbit antibody, $Db = 3.8 \times 10^{-7}$ cm^2/sec (Greeth and Knight 1965):

$$\tan \theta = (Dg/Db)^{1/2} \quad (3)$$

Dg of the globulin in the standard buffer at 20° C was calculated to be 3.33×10^{-7} cm^2/sec. The value corrected with water as solvent was 3.46×10^{-7} cm^2/sec ($D_{20,w}$). This value is higher than 2.6×10^{-7} cm^2/sec estimated by Ventura and Lima (1963).

Molecular Weight Determination

Molecular weight of the 13S globulin was determined by the following two methods:

By Intrinsic Viscosity and Sedimentation Coefficient. The method of Scheraga and Mandelkern (1953) was used to determine the molecular weight that was calculated from the following formula (Allen 1940):

$$\text{Molecular Weight} = 4{,}690 \times S^{3/2} \times |\eta|^{1/2}/(1 - V\rho)^{3/2} \quad (4)$$

where S is sedimentation coefficient, $|\eta|$ intrinsic viscosity, V partial specific volume, and ρ density of medium. With $S = 12.80 \times 10^{-13}$, $\rho = 1.0782$, $|\eta| = 0.0325$, and $V = 0.718$, the molecular weight of the globulin was calculated to be 361,000.

TABLE I
Amino Acid Composition of the 13S Globulin

Amino Acid	g/100 g Protein	Integral Number[a]
Aspartic acid	10.81%	324
Threonine	4.43	149
Serine	5.84	222
Glutamic acid	21.40	580
Proline	4.02	140
Glycine	5.66	301
Alanine	5.78	259
Half-cystine[b]	1.78	59
Valine	6.82	233
Methionine	2.14	57
Isoleucine	5.00	152
Leucine	7.44	226
Tyrosine	4.04	89
Phenylalanine	5.43	132
Histidine	3.08	80
Lysine	2.34	64
Arginine	13.99	321
Tryptophan[c]	1.65	35
Amido ammonia	1.74	408
Total	113.40	3,831
Molecular weight calculated[d]		392,116

[a] Nearest integral number of residues per molecule; molecular weight = 399,000.
[b] As cysteic acid.
[c] By spectrophotometric method.
[d] Twelve moles of water was added from the results of subunits structure analysis, in which the 13S globulin was considered to be composed of 12 polypeptide chains (N. Nishimura, K. Okubo, and K. Shibasaki, unpublished).

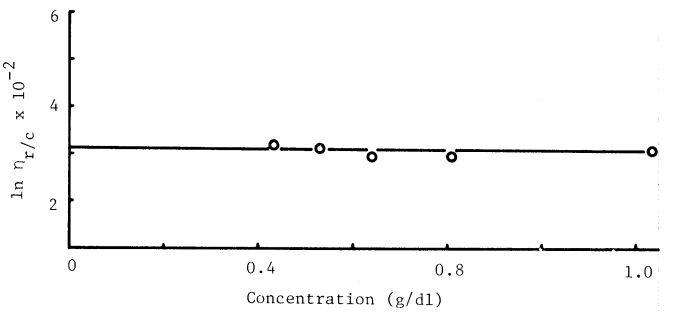

Fig. 1. Inherent viscosity ($\ln \eta_{r/c}$) of 13S globulin in standard buffer.

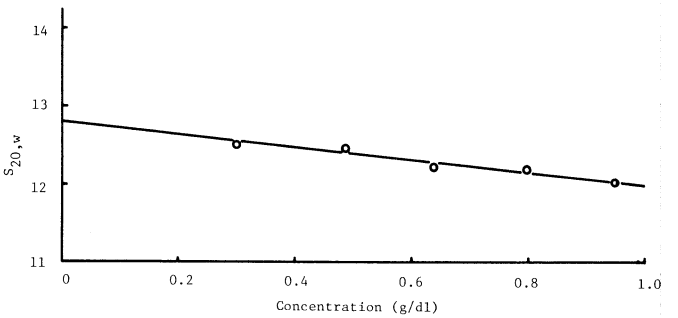

Fig. 2. Sedimentation coefficient of 13S globulin.

By Diffusion and Sedimentation Coefficient. The molecular weight was calculated from the following well-known formula (Dubois et al 1956):

$$\text{Molecular Weight} = RT/(1 - V\rho) \times S/D \qquad (5)$$

where R is gas constant, T absolute temperature, and D diffusion coefficient. With $R = 8.314 \times 10^7$ ergs/mole/degree, $T = 293.15$ and $D = 3.46 \times 10^{-7}$ cm^2/sec, the molecular weight of the globulin was calculated to be 399,000. This value is smaller than 450,000 as estimated from the sedimentation and diffusion coefficients by Ventura and Lima (1963). This discrepancy is apparently derived from the difference between the value of the diffusion coefficient estimated by them and that in this experiment. As summarized in Table II, the value estimated centrifugally by them may be small in comparison with the soybean glycinin as a typical globulin in legumes (Koshiyama and Fukushima 1976).

Frictional Ratio

As described for soybean 11S globulin by Koshiyama and Fukushima (1976), the frictional ratio of the molecule, f/f_o, was calculated by the following formulas introducing the observed values of sedimentation coefficient (S), diffusion coefficient (D), partial specific volume (V), and molecular weight (M) in the relationship of S and D, S and M, and D and M:

$$f/f_o = 1/\eta \times (RT/DN)^{2/3} \times [(1 - V\rho)/162\pi^2 VS]^{1/3} \qquad (6)$$

$$f/f_o = (1 - V\rho)/6\pi\eta S \times (4\ M^2/3VN^2)^{1/3} \qquad (7)$$

$$f/f_o = RT/6\pi\eta ND \times (4\pi N/3VM)^{1/3} \qquad (8)$$

where η is the viscosity of solvent (1.3045×10^{-2} poise), N the Avogadro's number (6.023×10^{23}), R the gas constant (8.314×10^7 ergs/mole/degree), T the absolute temperature, and ρ the density of the medium (1.0782 g/ml). With $D = 3.46 \times 10^{-7}$ cm^2/sec, molecular weight = 399,000 dalton and S = 12.80, f/f_o was found to be 1.06 using any of the three equations. f/f_o was calculated to be less than 1.00 from the above equations if molecular weight = 361,000 were used instead of 399,000. Therefore, 399,000 is considered to be a closer value to the actual molecular weight.

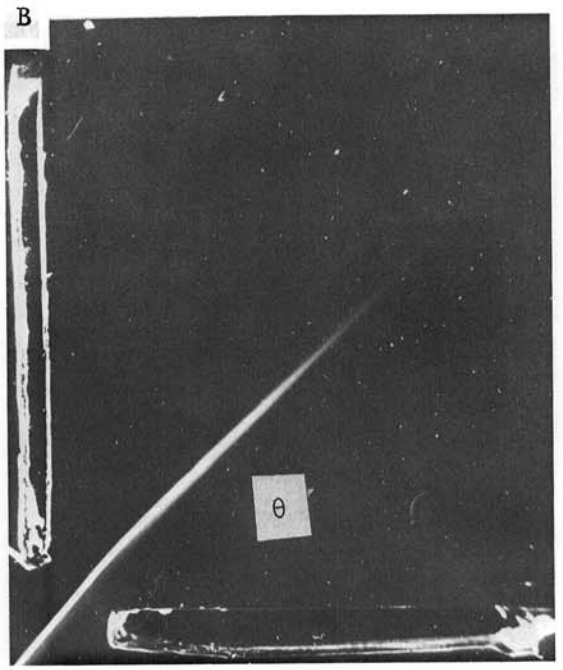

Fig. 3. Double gel gradient diffusion of the 13S globulin against 13S antiserum for the determination of the diffusion coefficient at 20°C in standard buffer. A, 13S globulin; B, 13S antiserum.

N-Terminal Amino Acids

Valine and glycine were the only ether-soluble N-terminal amino acids found in the 13S globulin by TLC. No water-soluble N-terminal amino acids were found.

Amino Acid Composition

Results of amino acid analyses are shown in Table I. Moisture content of the lyophilized globulin was 6.23% and nitrogen content was 17.69% on a dry basis. Nitrogen recovery was 99.35% and total weight of amino acid residues was 97.97 g per 100 g protein. Integral numbers of amino acid residues per molecule were calculated on the basis of molecular weight of 399,000. From the nearest integral numbers of residues, molecular weight was calculated as 392,116 dalton. The percentage of glutamic acid and aspartic acid in the amide form in the globulin was 42.5.

Ultraviolet Absorption

The ultraviolet absorption curve of the 13S globulin in standard buffer shows absorption maximum at 278.5 nm with $E^{1\%}_{1cm} = 8.83$ and minimum at 249.5 nm.

The physical properties of the globulin are summarized in Table I.

DISCUSSION

Ventura and Lima (1963), using a major globulin (fraction 1) of sesame seed that contained approximately 5% of a faster component according to the ultracentrifugal pattern, determined the molecular weight of the 13S globulin as 450,000 by diffusion and sedimentation coefficient. In the present experiments, however, a molecular weight of 399,000 was obtained using the same method. This discrepancy should depend mainly on the very marked difference between diffusion coefficient (3.46×10^{-7}) in this paper and that (2.6×10^{-7}) of Ventura and Lima (1963). Therefore, adjusting the 450,000 value reported by Ventura and Lima (1963)

TABLE II
Summarized Physical Properties of the 13S Globulin

Properties	Method and Symbol	Value	Literature
Isoelectric point	Turbidity	pH 5.2	...
Sedimentation coefficient	$S^o_{20,w}$	12.80S	12.7S[a]
Intrinsic viscosity	$\|\eta\|$	0.0325 dl/g	...
Partial specific volume	V	0.718 ml/g	0.735 ml/g[b]
	Va	0.734 ml/g	...
Diffusion coefficient	$D_{20,w}$	3.46	2.6[a]
Frictional ratio	f/f_o	1.06	1.50[a]
Absorption maximum		278.5 nm	280 nm[b]
Absorption minimum		249.5 nm	...
Absorbance	$E^{1\%}_{1cm}$ at 280 nm	8.83	10.8[b]
Molecular weight	$S^o_{20,w} \times \|\eta\|$	361,000	...
	$S^o_{20,w} \times D_{20,w}$	399,000	450,000[a]

[a] From Ventura and Lima (1963).
[b] From Sinha and Sen (1962).

TABLE III
Comparison of Content in Sulfur-Containing Amino Acids (g/100 g Protein)

	13S Globulin	Glycinin[a]	Legumin[b]	Arachin[c]
Methionine	2.14	1.22	0.71	0.51
Half-Cystine	1.78	1.51	0.65	0.42
Total	3.92	2.73	1.36	0.93

[a] From Fukushima (1968).
[b] From Boulter (1971).
[c] From K. Yotsuhashi, Thesis, Tohoku University, 1972.

for the difference in sedimentation and diffusion coefficient yields a value of 341,000, which is closer to the lower molecular weight (361,000) determined in the present paper. On the other hand, the axial ratio of the molecule was calculated to be 2.5 (53 × 131 Å) from frictional ratio (1.06) in this paper, assuming no hydration and oblate ellipsoid (Bradbury 1970). From the gel filtration with Sepharose 6B, the K_d value of 0.47 found for the 13S globulin in our previous paper (Okubo et al 1979) agrees closely with the value calculated by using 399,000 for the molecular weight and 2.5 for the axial ratio.

The 13S globulin is richer in aspartic acid, glutamic acid, arginine, and leucine than the representative globulin legumin (Boulter 1971). It is of particular interest that the 13S globulin has a higher content of sulfur-containing amino acids than legumin (Table III), suggesting a higher nutritional value when mixed with soybean proteins.

The 13S globulin has a subunit structure composed of acidic and basic subunits, which were observed as at least eight bands on the gel electrophoresis (Okubo et al 1979). The polypeptide chains of the subunits have either glycine or valine as N-terminal amino acid, because only two kinds of N-terminal amino acid were detected.

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