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# Isolation and Characterization of β-Globulin Low Molecular Weight Protein Fraction from Sesame Seed (*Sesamum indicum* L.)

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The low molecular weight protein fraction from the proteins of sesame seed has been isolated in a homogeneous form and is termed β-globulin or consesamin. The protein has an $S°_{20,w}$ of 2.0 ± 0.1, $D_{20,w}$ of $8 \times 10^{-7}$ cm²/s, and a partial specific volume of 0.725 mL/g. The intrinsic viscosity of the protein was determined to be 4.1 mL/g. The molecular weight determined by various approaches gives a value of 15000 ± 500. The evaluation of frictional ratios using Stokes radius and other hydrodynamic parameters indicates that the protein is elongated. The secondary structure of the protein indicates it to be rich in α helix. The protein is rich in acidic amino acids, especially glutamic acid, and also hydrophobic amino acids.

Sesame seed (*Sesamum indicum* L.) contains nearly 25% protein, and the defatted meal contains about 50% protein (Prakash and Narasinga Rao, 1986). Except for the presence of oxalates and phytic acid in the seed, there is no known toxic, antinutritional, or coloring principles in sesame seed (Prakash and Nandi, 1978). The work on sesame seed total proteins has mostly centered around nitrogen solubility and fractionation of proteins (Prakash, 1986). The total protein consists of mainly two fractions: One is the high molecular weight protein fraction, α-globulin (nearly 60%), which has been isolated and characterized under various solution conditions (Prakash and Nandi, 1976, 1977a–c, 1978; Prakash, 1985). Its quaternary structure is well established and is one of the seed proteins whose structure is well understood (Plietz et al., 1986). The other fraction, the low molecular weight protein fraction, β-globulin, constitutes nearly 25% of the total protein. No information is available on this protein fraction. In this study we report the isolation and char-

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acterization and the hydrodynamic properties of β-globulin.

## MATERIALS AND METHODS

**Materials.** Authentic varieties of *S. indicum* L. seeds were obtained locally. The chemicals and reagents used were as follows, with the sources of these materials shown in the parentheses: Sephadex G-100, Sepharose-2B, blue dextran (Pharmacia); guanidine hydrochloride (Gu·HCl), acrylamide, bisacrylamide (Sigma Chemical Co.); β-mercaptoethanol (Fluka); amido black and coomassie brilliant blue (Schwarz-Mann); bromophenol blue, ammonium persulfate, sodium chloride, sodium hydrogen phosphate, Ethylenediaminetetraacetic acid (BDH Chemicals); *N,N,N′,N′*-tetramethylethylenediamine (Koch-Light Laboratories Ltd.).

**Methods.** Dry sesame seeds were flaked in a flaking machine and defatted with *n*-hexane until the fat content was less than 1.0% (six washes). The defatted material was dried in a cabinet drier at 50 °C for 8 h to remove traces of hexane. This dried and defatted material was powdered to 60-mesh size in a Quadramat mill and used for routine extraction of protein.

**Isolation of the LMW Protein.** Phosphate buffer of pH 7.5 containing 1 M sodium chloride was added in the ratio of 1:10 to the flour and the resultant mixture stirred for 1 h with a Remi stirrer. The slurry was centrifuged at 6000*g* for 30 min, and the supernatant was diluted to 5.5 times with distilled water to remove α-globulin (Prakash and Nandi, 1978). The solution was allowed to stand at room temperature for 60 min, and α-globulin was removed by centrifugation at 6000*g*. To the clear supernatant was added powdered ammonium sulfate with constant stirring to 30% saturation. After thorough mixing, the solution was kept at 4 °C for 1 h. It was then centrifuged at 6000*g* for 30 min. The ammonium sulfate concentration of the supernatant was increased to 50% saturation with constant stirring. After 60 min of standing at 4 °C the solution was centrifuged at 6000*g*. The precipitate obtained was dissolved in extracting buffer and centrifuged, and the supernatant was dialyzed versus large amounts of distilled water with constant stirring. The dialyzed solution free of salts was lyophilized. The lyophilized material was stored in a desiccator at 4 °C and was used for all further studies.

**Protein Concentration.** The concentration of protein was determined by macro-Kjeldhal procedure (AOAC, 1984). Ultraviolet absorbance of the protein indicates that the absorption maximum is at 276 nm. This was related with the milligrams of nitrogen present in the protein sample, and a calibration curve was obtained relating milligrams of nitrogen and absorbance at 276 nm. A value of 7.24 was obtained for the $E^{1\%,1\ cm}_{276nm}$ for the protein.

All experiments were carried out in 0.06 M phosphate buffer, pH 7.5 (P buffer), unless otherwise stated.

**Gel Filtration.** (*a*) *In Buffer.* Sephadex G-100 (medium) gel in 0.06 M phosphate buffer of pH 7.5 containing 0.15 M NaCl was packed into a glass column of dimension 1.5 × 200 cm. After proper equilibration with the solvent, 50 mg of the protein in 1 mL of the same buffer containing 5% sucrose was loaded on the column and eluted. Fractions of 2.8 mL were collected with use of an LKB automatic fraction collector. Protein concentration was monitored by measuring absorbance of the fractions at 276 nm in a Shimadzu UN-150-02 double-beam spectrophotometer.

(*b*) *In 6 M Gu·HCl.* Sepharose-2B gel was equilibrated with 0.06 M phosphate buffer, pH 7.5, containing 6 M Gu·HCl (Mann and Fish, 1972). The equilibrated gel was packed into a 70 × 1.5 cm glass column. A 20-mg portion of β-globulin in 0.5 mL of the buffer was loaded, and fractions were collected gravimetrically. The fractions were collected in preweighed tubes from which the weight of the fractions collected was obtained. By the knowledge of density of the solution, using a densimeter, Antan Paar DMA 55D, the exact volume was calculated. The absorbance at 276 nm was monitored. The column was calibrated with standard proteins, ribonuclease, β-lactoglobulin, ovalbumin, and bovine serum albumin according to the method of Ackers (1967).

**Polyacrylamide Gel Electrophoresis.** (*a*) *In Buffer.* Polyacrylamide gel electrophoresis (PAGE) was performed in P buffer using 10% acrylamide gel with 0.4% methylene bisacrylamide as the cross-linking agent. Samples of protein (10–100 μg) containing 10% sucrose and bromophenol blue (tracking dye indicator) were loaded on the gel. Electrophoresis was performed at constant current of 1 mA per gel initially and subsequently at 3 mA per gel. The gels were stained in 0.5% amido black or coomassie blue in 7.5% (v/v) acetic acid solution and destained in 7.5% acetic acid.

(*b*) *In SDS.* This was performed by the method of Weber and Osborn (1969) using a 12% acrylamide gel with 0.5% bisacrylamide as cross-linking agent from which the molecular weight of β-globulin was determined.

**Analysis.** Carbohydrate was estimated following the procedure of Montgomery (1961). The phosphorus content was estimated by following the procedure of Tausky and Shorr (1953) after the protein was digested successively in 10 N $H_2SO_4$ and perchloric acid. Proteolytic activity was determined with use of a 1.5% solution of denatured hemoglobin and a 1% solution of β-globulin by the procedure of Wejtowicz and Odense (1972). The polyphenol content was determined by following the procedure of Pomenta and Burns (1971). Phytic acid was estimated following the procedure of Latta and Eskin (1980). Hemagglutinin activity was determined according to the method of Liener and Hill (1953), using trypsinized erythrocyte solution.

**Velocity Sedimentation and Sedimentation Equilibrium.** Velocity sedimentation experiments were carried out at different protein concentrations in P buffer at room temperature (27 °C) in a Spinco Model E analytical ultracentrifuge equipped with phase-plate Schlieren optics and a rotor temperature indicator and control unit. A standard 12-mm single-sector KelF centerpiece was used, and photographs were obtained at regular intervals of time. Plates were read on a Zeiss microcomparator and $S_{20,w}$ values calculated by the standard procedure (Schachman, 1959).

**Diffusion Coefficient.** Diffusion experiments were conducted in a synthetic boundary cell at a speed of 5227 rpm in a Spinco Model E analytical centrifuge at 27 °C. Diffusion coefficient was calculated by the height area method (Kawahara, 1969).

**Viscosity.** Viscosity measurements were made on an Ostwald viscometer in buffer at a constant temperature of 26.5 ± 0.1 °C. The intrinsic viscosity [η] was determined from extrapolation of reduced viscosities determined at different protein concentrations to zero protein concentration (Yang, 1961).

**Ultraviolet Absorption Spectrum.** The ultraviolet absorption spectrum of protein solution was recorded in a Beckmann DU-8B recording spectrophotometer in the range from 370–240 nm in a 1-cm quartz cell at room temperature (27 °C).

**Fluorescence.** This was measured in an Aminco-Bowman spectrofluorimeter at room temperature (27 °C) using

protein solution having an absorbance of 0.075 at 276 nm.

**Partial Specific Volume.** The partial specific volume ($\bar{V}$) of the protein was determined in P buffer following the procedure of Prakash (1982), Lee and Timasheff (1974), and Prakash and Timasheff (1981) using an Antan Paar DMA 55D densimeter at 20 ± 0.01 °C.

**Amino Acid Analysis.** The amino acid analysis was carried out in an LKB α amino acid analyzer following the standard procedure of protein hydrolysis in 6 N HCl (24 h) (Spackman et al., 1958).

**Stokes Radius.** Stokes radius of the protein was determined by gel chromatography on Sephadex G-100 column using 0.06 M phosphate buffer of pH 7.5 containing 0.15 M NaCl. The dimension of the column was 1.5 × 200 cm. The column was equilibrated with the above buffer and calibrated with standard proteins, bovine serum albumin, ovalbumin, α-chymotrypsinogen, and ribonuclease. The Stokes radius $R_s$ was measured by the procedure of Ackers (1967).

**Circular Dichroism.** Circular dichroism (CD) measurements were made at room temperature (~27 °C) in a Jasco J20C automatic scanning spectropolarimeter equipped with xenon arc lamp. The instrument was calibrated with an aqueous solution of camphor-$d_{10}$-sulfonic acid, and the slits were programmed to yield a 1-nm bandwidth at each wavelength. Near-ultraviolet CD measurements were made in the range 250–360 nm using a 1-cm path length cell with a 2.1 mg/mL protein concentration. Far-ultraviolet CD measurements were made in 0.1-cm path length cell in the range of 200–260 nm with a protein concentration of 0.4 mg/mL.

The data were reduced to mean residue ellipticities with a value of 115 for the mean residue weight of β-globulin. The far-ultraviolet CD curves were curve-fitted by the Chen and Yang (1971) and Greenfield and Fasman (1969) procedure. The best fit curve was taken as the one having the least error and including most of the experimental points of the far-ultraviolet CD curves. The dichroic absorbance differences were averaged from two recordings, and the mean ellipticity was calculated from the averaged spectrum and with the knowledge of the amino acid composition of the protein.

**Molecular Weight Determination.** Molecular weight of β-globulin was determined from a calibration of sedimentation coefficient value and intrinsic viscosity in buffer using the Scheraga and Mandelkern equation (1953). Molecular weight by the Archibald approach to equilibrium and molecular weight by sedimentation and diffusion were determined as described by Schachman (1959). The molecular weight by sedimentation equilibrium was determined according to the procedure of Yphantis (1964).

**Hydrophobicity.** The average hydrophobicity and related terms were calculated according to the procedure of Bigelow (1967). The concerned hydrodynamic parameter has been investigated as a function of protein concentration and the desired parameter extracted after extrapolation of the data to zero protein concentration.

## RESULTS AND DISCUSSION

β-Globulin was found to be homogeneous, since it moved as a single band on polyacrylamide gel electrophoresis (Figure 1, inset b) and also eluted as single symmetrical peak in gel filtration (Figure 1). Analytical ultracentrifugation studies under a variety of conditions confirmed the homogeneity of the sample (Figure 1, inset a). Polyacrylamide gel electrophoresis also indicates that the protein preparation is homogeneous as it indicated only one band. SDS-PAGE also indicates a single band with a molecular weight of 14 000 ± 1000, indicating that the

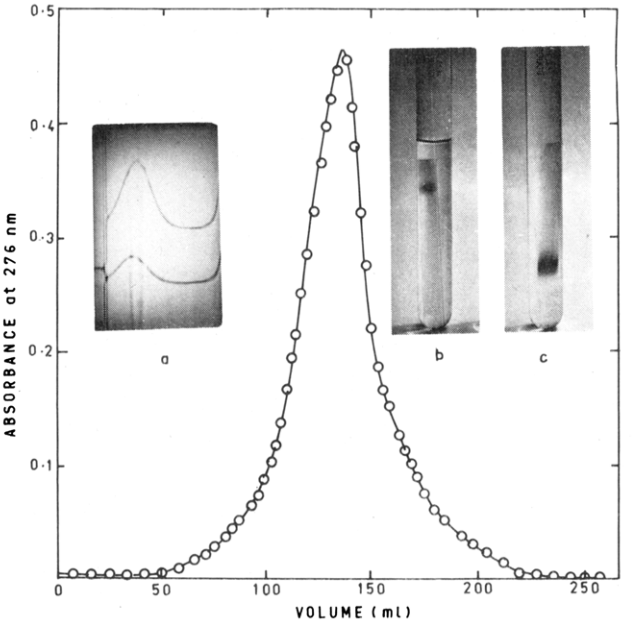


**Figure 1.** Gel filtration pattern of β-globulin on Sephadex G-100 column in P buffer containing 0.15 M sodium chloride. (a) Sedimentation velocity pattern of β-globulin in P buffer (time of photograph, 94 min after attaining two-thirds maximum speed of 59 780 rpm): upper, 22.5 mg/mL protein concentration; lower, 8.1 mg/mL protein concentration. (b) PAGE pattern of β-globulin in P buffer. (c) SDS-PAGE pattern of β-globulin.

**Table I. Amino Acid Composition of β-Globulin**

| amino acid | mol/mol | amino acid | mol/mol |
|---|---|---|---|
| aspartic acid | 4 | methionine | 2 |
| threonine | 2 | isoleucine | 5 |
| serine | 4 | leucine | 6 |
| glutamic acid (includes glutamine) | 55 | tyrosine | 2 |
| | | phenylalanine | 2 |
| proline | 3 | histidine | 2 |
| glycine | 7 | lysine | 1 |
| alanine | 3 | arginine | 13 |
| cysteine | 3 | tryptophan[a] | 2 |
| valine | 3 | | |

[a] Spande and Witkop (1967) method.

protein is a single polypeptide chain (Figure 1, inset c). Table I shows the amino acid composition of the protein. The protein is rich in glutamine/glutamic acid, which constitutes nearly 55% of the amino acids. The protein is also rich in arginine, hydrophobic amino acids, and sulfur amino acids.

The various chemical, physicochemical, and hydrodynamic properties of β-globulin are summarized in Tables II and III. The protein has an ultraviolet absorbance maximum at 276 nm, indicating the presence of aromatic chromophores, especially tryptophan and tyrosine (Table I). The fluorescence emission maximum of β-globulin is at 336 nm, indicating that the fluorescence is predominantly due to tryptophan groups. On the basis of the model compound studies, it is indicative that the tryptophanyl residues are more exposed to bulk solvent (Mills and Creamer, 1975; Shifrin et al., 1971). The protein contains 1.8 g of tryptophan/100 g of protein. It also contains considerable amounts of tyrosyl residues (Table I). The noncontribution of tyrosyl groups has been confirmed as evidenced from the absence of any emission peak around 305-nm region, even when the protein is excited at 275 and 295 nm following the procedure of Weber and Young (1964).

From Table II it can be seen that the protein is practically free from carbohydrate, phytic acid, polyphenols

**Table II. Chemical and Physicochemical Properties of β-Globulin**

| property | value |
|---|---|
| absorption max | 276 nm |
| absorption coeff ($E^{1\%,\,1\,cm}_{276nm}$) | 7.24 |
| fluorescence emission max | 336 nm |
| fluorescence excitation max | 280 nm |
| N content | 16 g/100 g protein |
| P content | 0.06% |
| carbohydrate | not detectable |
| phytic acid | not detectable |
| polyphenols as chlorogenic acid | not detectable |
| hemagglutinin act. | 1 Hu unit = 250 μg |
| urease act. | not detectable |
| proteolytic act. (Kunitz unit) | |
| (a) in P buffer | 0.02 |
| (b) in P buffer + $1 \times 10^{-3}$ M EDTA | 0.35 |
| (c) in P buffer + $1 \times 10^{-5}$ M $CaCl_2$ | 0.11 |
| (d) in P buffer + $1 \times 10^{-4}$ M $MgCl_2$ | 0.08 |
| secondary structure | |
| (a) α helix | 35% |
| (b) β pleated | 45% |
| (c) aperiodic | 20% |
| hydrophobicity and related parameters | |
| (a) av hydrophobicity | 550 cal/residue |
| (b) NPS units | 0.33 |
| (c) C | 0.72 unit/residue |

**Table III. Hydrodynamic Parameters of β-Globulin**

| parameter | value |
|---|---|
| sedimentation coeff ($S°_{20,w}$) | 2.0 ± 0.1 S |
| diffusion coeff ($D°_{20,w}$) | $8 \times 10^{-7}$ cm²/s |
| intrinsic viscosity ($\eta$) | 4.1 ± 0.2 mL/g |
| partial specific vol | 0.725 ± 0.002 mL/g |
| nonideality coeff ($g$) | 0.01 mL/g |
| mol wt | |
| (a) approach to sedimentation equilibrium | 14900 ± 600 |
| (b) sedimentation and intrinsic viscosity | 18000 ± 1000 |
| (c) sedimentation equilibrium | 15000 ± 500 |
| (d) SDS-PAGE | 14000 ± 1000 |
| (e) min mol wt (from amino acid compn) | 15000 ± 1200 |
| (f) Gu·HCl (6 M) column chromatography | 16000 ± 1000 |
| size | |
| (a) Stokes radius | 12 ± 1 Å |
| (b) from sedimentation measmnts | 12 ± 1 Å |
| (c) radius of gyration | 8.64 Å |
| (d) $R_{min}$ | 16 Å |
| frictional ratios | |
| (a) from radius of equivalent sphere | <1 |
| (b) from sedimentation and mol wt | 1.3 |
| shape parameter | |
| (a) β function | $2.4 \times 10^6$ |
| (b) Perrin shape factor | 1.543 |
| (c) Simha shape factor (prolate) | 13.63 |
| axial ratio | |
| (a) prolate ellipsoid of revolution | 10 |

as chlorogenic acid, and urease activity. However, the protein has 0.06% phosphorus in it and also detectable amount of hemagglutinin activity. The protein also shows considerable amount of proteolytic activity (Table II), which is enhanced to a large extent in presence of EDTA; other cations like $Ca^{2+}$ and $Mg^{2+}$ do not appear to have significant effect on the proteolytic activity as compared to the enhancement observed in presence of EDTA. It may be noted that the major protein fraction, α-globulin, the high molecular weight protein, does not have any proteolytic activity. This may have a role to play during the germination of seeds, when the high molecular weight protein may act as a substrate releasing the needed amino acids for the growth of seedlings through the specific enzyme action from the low molecular weight group of proteins (Prakash and Narasinga Rao, 1986). Also the absence

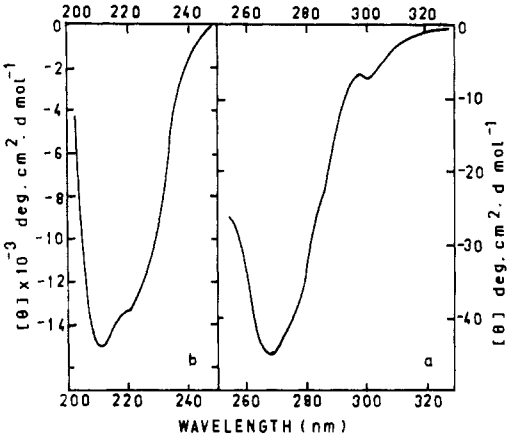


**Figure 2.** Circular dichroic spectra of β-globulin in P buffer in the 200–330-nm region: (a) near-ultraviolet region, 257–330 nm; (b) far-ultraviolet region, 200–250 nm.

of undesirable constituents like trypsin inhibitor and phytic acid may facilitate a better germination profile.

**Secondary Structure.** The near-ultraviolet CD spectrum shows a (Figure 2a) minimum at 268 nm for the protein β-globulin. The CD minimum of the protein at this particular wavelength is indicative of the specific orientation of the chromophores in an unusual hydrophobic interior (Strickland, 1974). The shoulder at 296 nm probably originates from tryptophan residues alone. However, in assigning the CD bands especially in the regions 250–260 nm, one has to exercise a sufficient amount of caution not to overlook the contributions from the dihedral angle of disulfide, the vicinal interactions, and other covalent bond angles. Similar results are obtained with the low molecular weight protein fraction concarmin from safflower seeds (Sridhara and Prakash, 1987).

Figure 2b shows the far-ultraviolet CD spectrum of β-globulin in the above buffers in the region 200–250 nm. The spectrum is characterized by a major negative band around 211 nm and a well-defined shoulder at 222 nm. This is indicative of a typical α-helix-rich protein CD spectrum (Greenfield and Fasman, 1969). As evidenced, the protein appears to be rich in α-helical structure. A further insight into the determination and quantitation of various transitions in the system was delineated from a conformational analysis of the far-ultraviolet CD spectrum by the Greenfield–Fasman procedure (1969). From the above curve-fitting procedure, β-globulin has about 35% α-helix, 45% β-structure, and 20% aperiodic structure. The α-helical and β-structure contents of β-globulin reported here are very similar to the values reported for the low molecular weight proteins from other oilseeds like mustard, sunflower, and safflower (Prakash and Narasinga Rao, 1986). It can be seen from the CD spectra that the CD minimum around 210 nm is greater in intensity than the one at 222 nm. If this can be a clue along with quantitative data from curve fitting, the protein can be considered as belonging to the (α + β) group of proteins of Levitt and Chothia (1976) and Blake and Johnson (1984) classification. From the above results it can be seen the low molecular weight protein is quite different from the high molecular weight protein in its secondary structure belonging to the (α + β) protein class as compared to the later, which probably comes under (β + coil) class of proteins (Prakash and Narasinga Rao, 1986).

**Hydrophobicity and Related Parameters.** The average hydrophobicity and related parameters like NPS and C are shown in Table II. In general, the hydrophobicity and NPS values are low as compared to other proteins that

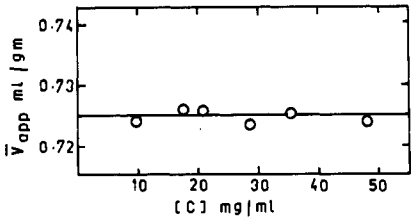


**Figure 3.** Concentration dependence of apparent partial specific volume of β-globulin in P buffer.

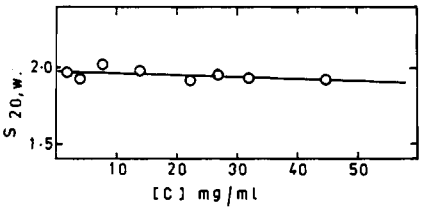


**Figure 4.** Concentration dependence of $S_{20,w}$ value of β-globulin in P buffer.

are rich in α-helical content. The protein appears to be less hydrophobic. A high $C$ value of 0.72 unit per residue probably has a major contribution from the glutamic and glutamine residues, which constitute nearly 55% of the amino acids of the protein (Table I).

**Partial Specific Volume.** The concentration dependence of apparent partial specific volume of β-globulin is presented in Figure 3. The value after extrapolating to zero protein concentration is 0.725 ± 0.002 mL/g. This value is taken as the partial specific volume of protein in the native condition. The partial specific volume was also evaluated from amino acid composition by the Cohn and Edsall (1943) method, by which a value of 0.720 mL/g was obtained for the protein. This value obtained is in excellent agreement with the experimentally determined value. However, since it is believed that the experimental value takes into consideration many other parameters like electrostriction and vicinal interactions in solution, the experimental value of $\bar{V}$ is used for all further calculations.

**Sedimentation Velocity.** The sedimentation velocity pattern of β-globulin is shown in Figure 1, inset a. This indicates a single, symmetrical, rather broad peak. The sedimentation coefficient value determined as a function of protein concentration is shown in Figure 4. From the figure, $S°_{20,w}$ of 1.95 ± 0.10 was obtained for β-globulin. The concentration dependence showed the usual negative slope and was analyzed according to the standard equation (Schachman, 1959). This analysis yielded a value for the nonideality coefficient $g$ = 0.01 mL/g. This value is well within normal limits for a "fairly" globular protein that shows a normal negative slope of sedimentation coefficient versus protein concentration.

**Diffusion Coefficient.** The diffusion coefficient was also determined following the same logistics of velocity sedimentation. A value of $8 \times 10^{-7}$ cm$^2$/s was obtained for β-globulin and is used in all calculations. The value of the diffusion coefficient is higher compared to that of α-globulin of sesame seeds.

**Viscosity.** Figure 5 shows a plot of reduced viscosity as a function of protein concentration over the protein concentration range 1.5–4.7%. Linear least-squares fit of the data gives an intercept of 0.041 dL/g. It is a well-known fact in synthetic polypeptide chemistry that the macromolecules of globular shape have an intrinsic viscosity of 0.03 dL/g (Tanford, 1961). When this is extrapolated to the biological macromolecules, the above data show that the β-globulin cannot be considered as a true globular protein. Further results support this fact.

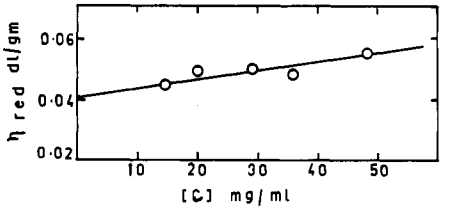


**Figure 5.** Concentration dependence of reduced viscosity of β-globulin in P buffer.

**Molecular Weight.** The molecular weight of β-globulin as determined by various hydrodynamic approaches, SDS-PAGE, and amino acid composition are listed in Table III. The value indicates an average molecular weight of 15 000. Especially from two different kinds of techniques like sedimentation equilibrium, which has a firm thermodynamic basis, and SDS-PAGE, the value obtained is 15 000. From these results the molecular weight of β-globulin can be taken as 15 000 ± 1000.

**Stokes Radius and Frictional Ratio.** The Stokes radius ($R_s$) of protein was determined experimentally from gel filtration and also calculated from sedimentation values (Tanford et al., 1974) and is shown in Table III. From gel filtration a value of 1.2 nm was obtained. The value obtained from sedimentation coefficient correlates excellently to the value obtained experimentally. However, the value obtained by using the diffusion coefficient was found to be on the higher side (Table III). It is difficult to explain this anamoly at this point. However, since the value obtained from gel filtration is the experimental value, this value is taken as the working value for the purposes of calculation. At this point it is relevant to mention that the $R_{min}$ was also calculated on the assumption of β-globulin as a nonhydrated sphere. This gave a value of 16 Å, which agrees fairly well with the experimentally determined value. The difference in the value between experimentally calculated and $R_{min}$ could be due to the fact that from the earlier data we see that the protein β-globulin appears more as an elongated molecule. In Table III is reported the approximate radius of gyration calculated for a sphere (Piltz, 1973). However, this can be experimentally and accurately determined by small-angle X-ray diffraction at very high protein concentration (Plietz et al., 1986).

Frictional ratios were determined from the $S°_{20,w}$ molecular weight, partial specific volume, intrinsic viscosity, and hydrated volume. The values are shown in Table III. A frictional coefficient of 0.74 is obtained if the molecule is assumed as sphere, indicating the protein to be definitely elongated, which confirms the earlier viscosity results. From these hydrodynamic parameters, the shape parameter, such as β function, and axial ratio were also calculated. This gives a value of $2.4 \times 10^6$ for the β function. This value of β function automatically eliminates an oblate model for the protein since the limiting value of β for an infinitely thin oblate ellipsoid is $2.15 \times 10^6$ (Van Holde, 1971). Thus, any molecule with a larger β must be prolate. On the basis of these values other shape parameters such as Perrin shape factor and Simha parameter were also calculated and are presented in Table III along with the axial ratio.

**Homogeneity of Polypeptide Chain.** The number of polypeptide chains in β-globulin was analyzed in the presence of SDS by polyacrylamide gel electrophoresis (PAGE) in the presence of a disulfide reducing agent. The results (Figure 1, inset c) indicate that the protein is a single polypeptide chain of 15 000 molecular weight moving very close to the standard protein ribonuclease. This was further substantiated by measurement of sedimentation

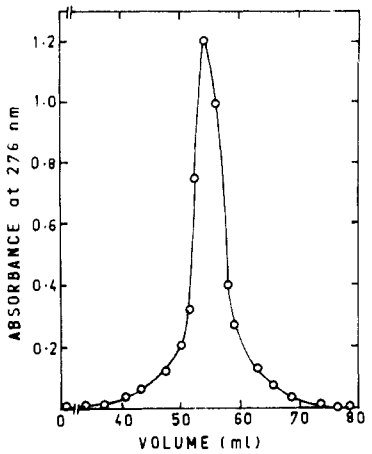


**Figure 6.** Gel filtration pattern of β-globulin in Sepharose-2B in P buffer containing 6 M Gu·HCl.

coefficient of protein as a function of protein concentration in 6 M Gu·HCl in the presence of reducing agent. This gave a value of 1.85. This value is close to the value obtained in the buffer. To confirm that the protein is made of a single polypeptide chain, it was denatured in 6 M Gu·HCl and loaded on a Sepharose-2B column equilibrated with 6 M Gu·HCl. The result showed a single symmetrical peak in the gel filtration profile (Figure 6). The selected fractions of the peak over the Gaussian distribution curve were dialyzed to remove Gu·HCl and electrophoresed on PAGE and SDS-PAGE. The results indicated a single band of same molecular weight and equal mobility in all of the experiments, confirming that the protein is made of a single polypeptide chain. The molecular weight of the protein in 6 M Gu·HCl was estimated from standard protein markers and yielded a value of 16 000 ± 1000 analyzed according to the procedure of Mann and Fish (1972).

In most of the oilseed proteins the general pattern of 11S, 7S, and 2S being present is a common feature (Prakash and Narasinga Rao, 1986). The 2S fraction of soybean, called α-conglycinin, is isolated and shown to consist of low molecular weight proteins differing in sedimentation values. Similarly, conarachin I constitutes nearly 30% in the groundnut protein, sediments as a 2S component, and is made up of a group of low molecular weight proteins. The low molecular weight protein from sunflower seed (Sripad, 1986; Prakash and Narasinga Rao, 1986) and mustard seed (Gururaj Rao et al., 1978) appears to be basic in nature and rich in cysteine and lysine along with a high content of α-helix structure. The low molecular weight protein fraction from safflower seed has been isolated and characterized and is shown to be rich in glutamic acid with a high content of α-helix structure (Sridhara and Prakash, 1987). These data are compiled and the generalities brought out among the various low molecular weight proteins from several oilseeds (Prakash, 1988). The above data on β-globulin fit in with the general concept that these low molecular weight proteins are rich in α-helix structure and have considerable amount of proteolytic activity with unusually high contents of sulfur and acidic amino acids, except for the fact that β-globulin from sesame seed does not appear to contain other protein fractions along with it. This may be due to the fact that it is quite possible that in sesame seeds also, as in many other oilseeds, there could be number of low molecular weight proteins and the above specific procedure might have isolated only one of them, i.e. β-globulin. In this study the protein was prepared by a nondenaturing procedure, and hence we assume that the parameters observed and calculated truly represent the native molecule. Also in most of the hydrodynamic studies, it is preferable to perform the experiments near the isoelectric pH of the protein in order to reduce the charge effects. Since the solubility of β-globulin was rather low, at the isoelectric pH, the studies were carried out at a pH and ionic strength wherein a reasonable working concentration of the protein could be obtained. The interpretation of overall shape of protein depends very much upon the definition of the limiting axial ratio, intrinsic viscosity, various radii parameters, molecular weights, and radius of equivalent sphere. If one looks at the data presented in Table III, one could classify β-globulin as an elongated protein tending more toward a rod shape for the molecule rather than a globular shape for the molecule, exposing most of the amino acids to the bulk solvent. It is, however, important to note that the dimensions of β-globulin described in this investigation are only an approximation, assuming either spherical or equivalent ellipsoidal models. Stringent caution must be exercised in interpretations and in describing the real particle.

**Registry No.** $N_2$, 7727-37-9; P, 7723-14-0; proteinase, 9001-92-7.

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# Thermal Gelation of Oat Globulin[1]

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The thermal gelation properties of oat globulin were studied under different conditions of temperature, protein concentration, pH, and ionic strength. Differential scanning calorimetry shows that oat globulin heated under conditions inducing gelation was not extensively denatured and exhibited highly cooperative transition characteristics. The chemical forces involved in gel formation were investigated by measuring the gel hardness under the influence of neutral salts, reducing agents, denaturants, and water-miscible solvent. Some fatty acid salts were effective in improving the gelling property of oat globulin near neutral pH.

Thermal coagulation and gelation are important functional properties of food proteins. Gels act as a medium for holding water, lipids, flavors, and other ingredients and impart unique rheological and textural qualities to food systems (Kinsella, 1979). As defined by Hermansson (1979), thermal coagulation is the random interaction of protein molecules to form aggregates, while gelation involves the formation of a three-dimensional network exhibiting a certain degree of order. Most heat-coagulable proteins are of animal origin, and the failure of most plant proteins to gel has been attributed to relatively high heat stability of the proteins (German et al., 1982).

Oat globulin, the major protein fraction in oats, has a quaternary structure very similar to that of soy 11S globulin (glycinin), a heat-coagulable protein. It is made up of six acidic and six basic polypeptides, and each acidic polypeptide is linked to a basic polypeptide by disulfide bonds to form a subunit. The six subunits are linked through noncovalent forces to form the hexamer (Derbyshire et al., 1976; Neilsen, 1985; Brinegar and Peterson, 1982). Our previous report (Ma and Harwalkar, 1987) shows that when a dilute oat globulin solution was heated at high ionic strength, both soluble and insoluble aggregates were formed, but the coagulation behavior was found to be significantly different from that of soy glycinin. While the heat-induced gelation of soy glycinin and the chemical forces involved have been extensively studied (Catsimpoolas and Meyer, 1970, 1971; Shimada and Matsushita, 1980; Mori et al., 1982, 1986; Babajimopoulos et al., 1983; Utsumi and Kinsella, 1985), little is known about the thermal gelation of oat globulin. The present work was conducted to study the gelling properties of oat globulin under various environmental conditions and to elucidate the mechanism and molecular forces involved in gel formation.

## MATERIALS AND METHODS

**Materials.** Oats (variety Sentinel) were grown at the Central Experimental Farm, Ottawa, Canada. Dehulled groats were ground in an Alpine pin mill (Model 160 Z) and defatted by Soxhlet extraction with hexane.

**Preparation of Oat Globulin.** Globulin was extracted from defatted ground groats by the Osborne fractionation procedure (Osborne and Mendel, 1914). Groats were mixed with 1.0 M NaCl at a solvent to solid ratio of 10:1 and stirred at room temperature for 30 min. The slurry was centrifuged at 20000*g* for 30 min, and the residue was reextracted twice with 1.0 M NaCl. The combined supernatant was dialyzed exhaustively against distilled water at 4 °C, and the precipitated globulin was recovered by centrifugation and freeze-dried.

**Heat Treatment.** Dispersions of oat globulin were prepared at different protein concentrations with distilled water. Varying amounts of NaCl and other additives were added as solids, and the pH of the dispersions was then adjusted by the addition of 1 N HCl or NaOH. Mixing was by magnetic stirring in most cases, but for preparations containing reagents that are not readily dispersible in aqueous solution, e.g., fatty acid salts, mixing was by a Biosonik ultrasonic homogenizer (Bronwill Scientific, Rochester, NY) at low-energy output. The samples were then subjected to vacuum evacuation to remove dissolved air. Aliquots (4.0 mL) of samples were pipetted into 1.2 × 4 cm glass tubes. The tubes were covered with glass marbles and heated in a water bath at preset temperature. After heating, the tubes were immediately cooled by immersing in an ice bath.

**Measurement of Gel Hardness.** Hardness of the heated samples was measured with an Ottawa Texture

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