Isolation and Characterization of α-Globulin of Sesame Seed (*Sesamum indicum* L.)

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The major protein fraction, α-globulin, of sesame seed (*Sesamum indicum* L.) has been isolated. The homogeneity (~95%) of the isolated protein was determined by polyacrylamide gel electrophoresis, sedimentation velocity measurements, DEAE–cellulose chromatography, and gel filtration experiments. The different chemical and physicochemical properties of the protein and its amino acid composition are reported here.

The chemical and physicochemical studies of the nutritionally important proteins of sesame seed (Block and Bolling, 1951; Joseph et al., 1962; Sastry et al., 1974) available in the literature are mostly concerned with the isolation of total protein, its solubility at different pHs, and fractionation of different proteins present in it (Jones and Gersdroff, 1927; Adolph and Lin, 1936; Nath and Giri, 1957a,b; Salem and Bekheit, 1964; Guerra and Park, 1975). The study on the protein fraction α-globulin, the major constituent (~65–70%) of the protein in the sesame seed, has been mostly on the electrophoresis, sedimentation velocity measurements, and amino acid composition (Nath et al., 1957; Sinha and Sen, 1962; Ventura and Lima, 1963). Recently from this laboratory, we have reported the association–dissociation, aggregation, and denaturation phenomenon of α-globulin in various solution conditions (Prakash and Nandi, 1976, 1977a,b,c; Lakshmi and Nandi, 1977) to understand the structural characteristics of this protein.

Sesame α-globulin isolated by different workers (Nath and Giri, 1957a; Ventura and Lima, 1963) showed both high and low molecular weight fractions as contaminants. Further, the homogeneity of α-globulin isolated by the previous workers has not been tested critically. In this communication, we report the isolation of sesame α-globulin of ~95% purity as evidenced from various physicochemical techniques. A few of its properties are also reported here.

EXPERIMENTAL SECTION

Materials. White variety sesame seed was obtained from the local market and identified as *Sesamum indicum* L. during the flowering stage of the plant.

The chemicals and reagents used were as follows: the source of these materials is shown in the parenthesis. Sepharose 6B-100 and DEAE–cellulose (Sigma Chemicals); hemoglobin substrate (Worthington Biochemicals); bisacrylamide (Koch-Ligh Laboratories); TEMED and β-mercaptoethanol (Fluka); coomassie brilliant blue (Schwarz-Mann); bromophenol blue, ammonium persulfate, sodium chloride potassium hydrogen phosphate (BDH Chemicals); trichloroacetic acid (Sarabhai M. Chemicals); and sodium dodecyl sulfate (Hindustan Levers). Sodium dodecyl sulfate (SDS) was recrystallized twice from aqueous ethanol.

Methods. The sesame seeds were soaked in water for 6 h after which they were scrubbed to remove the hull. The dehulled and dried seeds were flaked, followed by defatting by extraction with solvent *n*-hexane, with a solvent to flaked seed ratio of 1:1. The process of extraction was repeated at least six times and a meal containing less than 3% fat was obtained. This defatted meal was air-dried in a cabinet drier at 45 °C for 6 h after which it was powdered in a microatomizer and passed through a 60-mesh sieve. The flour was further washed with *n*-hexane, and a flour containing less than 1% fat was obtained. The defatted flour was dried and used for extraction of total protein.

Isolation of α-Globulin. α-Globulin was isolated by a minor modification of the procedure of Nath and Giri (1957a). The total protein of the defatted flour was extracted in 0.02 M phosphate buffer of pH 7.5 containing 1 M NaCl using a flour to solvent ratio of 1:10. The supernatant obtained by centrifugation of the slurry at 4000 rpm for 20 min was diluted with distilled water 1:5.5 times when α-globulin precipitated. The solution containing precipitated α-globulin was centrifuged at 4000 rpm for 30 min, and the residue obtained was redissolved in the extraction solvent. The process of precipitation and centrifugation was repeated twice. α-Globulin thus obtained was dissolved in the extraction solvent and dialyzed against the same buffer for ~24 h.

Protein Concentration. The concentration of α-globulin was determined by macro-Kjeldhal procedure (AOAC, 1973). Nitrogen was estimated in a weighed amount of lyophilized α-globulin, and the nitrogen to protein conversion factor was determined as 6.25. A calibration curve relating the milligrams of nitrogen present in the protein sample with ultraviolet absorbance of the protein at 280 nm was obtained for routine determination of protein concentration. The absorption coefficient, E of 1% protein solution at 280 nm, i.e., $E_{1cm}^{1\%}$ gave a value of 10.8 for α-globulin. The corresponding value obtained for the total protein was 13.0.

Gel Filtration. Gel filtration was carried out with Sepharose 6B-100 gel in 0.02 M phosphate buffer of pH 7.5 containing 1 M NaCl; a 2 × 87 cm column of 265-mL bed volume was used. Nearly 50 mg of the protein in the buffer solution was loaded on the column and eluted with the same buffer. Two-milliliter fractions were collected.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was carried out using a Metrex gel electrophoresis unit, in 0.02 M phosphate buffer of pH 7.5, in a 7.5% gel. Protein samples (10 μg/μL) containing ~10% sucrose and 0.05% bromophenol blue (indicator dye) were loaded on the gel, and electrophoresis was performed at a constant current of 3 mA per gel for 70 min. The gels were stained in 0.5% amido black in 7.5% (v/v) acetic acid solution and destained in 7.5% acetic acid. The gels were scanned in a Joyce Loebel Chromoscan 200.

Determination of Subunits and Their Molecular Weights. The determination of the number of subunits and their molecular weight was carried out by the method of Weber and Osborn (1969) using standard proteins, e.g.,

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bovine serum albumin, egg albumin, β-lactoglobulin, carbonic anhydrase, chymotrypsinogen, lysozyme, and α-chymotrypsin as molecular weight markers. The relative mobilities of the bands were plotted against the logarithm of the molecular weight of the standard proteins from which the molecular weights of the subunits of α-globulin were determined.

Ion-Exchange Chromatography. DEAE–cellulose after regeneration (Peterson, 1970) was equilibrated with 0.01 M glycine–sodium hydroxide buffer of pH 9.0 and packed into a 2.5 × 30 cm column. Approximately 140 mg of the protein was loaded and was eluted with a linear gradient of 0–0.4 M NaCl. Sodium chloride was estimated in the fractions by Vohlard's method (Vogel, 1961).

Ultracentrifugation. Sedimentation velocity experiments were carried out at 27 °C in a Spinco Model E Analytical Ultracentrifuge equipped with phase plate schlieren optics and a rotor temperature indicator and control (RTIC) unit using a standard 12-mm duraluminum cell centerpiece and 1% protein solution at a speed of 59 780 rpm. The plates were read on a Gaertner microcomparator and $S_{20,w}$ values calculated by the standard procedure (Schachman, 1959). The molecular weight was estimated from $S_{20,w}$ values and also from approach to sedimentation equilibrium (Archibald method).

Viscosity. Viscosity measurements were made using 1–6% protein solutions with an Ostwald viscometer having a flow-time of 345 s with distilled water at 28 ± 0.1 °C. The intrinsic viscosity [η] was determined from extrapolation of viscosities determined at six different concentrations.

Optical Rotation. Optical rotation was measured at 578 nm in a Carl-Zeiss spectropolarimeter using 1 dm tube at 28 °C. Protein solution (0.75%) was used.

Ultraviolet Spectra. The spectrum of the protein was recorded in a Perkin-Elmer 124 double-beam spectrophotometer with a 0.86 OD/mL (0.08%) protein solution.

Fluorescence. Fluorescence measurements were made in a Perkin-Elmer Hitachi Fluorescence Spectrofluorometer at 28 °C with 0.004% protein solution. The excitation wavelength was 280 nm. The fluorescence emission was measured after 10 s when the fluorescence intensity attained constancy.

Amino Acid Analysis. The amino acid analysis was carried out in a Hitachi KLA-3B amino acid analyzer following the standard procedure of hydrolyzing the protein in 6 N HCl (Spackman et al., 1958). Since tryptophan is destroyed during acid hydrolysis (Blackburn, 1968), it was determined by NBS (Spande and Witkop, 1967) and Edelhoch's methods (Edelhoch, 1967), and the average of the values obtained from the two methods was calculated.

Carbohydrate Estimation. This was estimated following the procedure of Montgomery (1961) using 0.2 mL of 1% protein solution.

Phosphorus Estimation. An aliquot (0.5 mL) of 0.5% protein solution was digested successively with 10 N H_2SO_4 and perchloric acid. The phosphate content of the digested sample was estimated by the method of Taussky and Shorr (1953).

Proteolytic Activity. This was determined using a 2% solution of denatured hemoglobin and a 1% of α-globulin in 0.1 M borate buffer of pH 7.8 by the procedure of Greenberg (1955).

RESULTS AND DISCUSSION

The sedimentation velocity patterns of α-globulin isolated by different procedures have been shown in Figure 1, where the pattern of the total proteins has also been presented for comparison. The total protein shows four components with $S_{20,w}$ values of 2, 7, 11, and 16S constituting approximately 20, 5, 65, and 10%, respectively, of the protein (Figure 1a). It can be seen that the protein isolated by the method described here gives a fairly homogeneous preparation of α-globulin (Figure 1d) with a sedimentation value of 11 along with 5% of 16S component. In contrast, α-globulin isolated by following the procedures of Nath and Giri (1957a) and Ventura and Lima (1963) (Figure 1b and 1c) contains considerable amounts of 2, 7, and 16S components. In addition, the yield of α-globulin obtained by the method described here was ~20–22% on the basis of flour weight, whereas it was ~6 and ~15% by the methods of Nath and Giri (1957a) and Ventura and Lima (1963), respectively. α-Globulin isolated by us indicates a low content of phosphorus and carbohydrate (see Table I). The amide content is 2%.

Gel Filtration. Gel filtration of the total proteins shows three peaks (Figure 2a). α-Globulin isolated elutes as a single symmetrical peak, corresponding to the major peak of total protein at ~160 mL (Figure 2a and 2b).

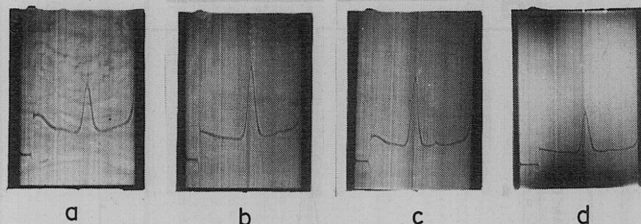

Figure 1. Sedimentation velocity patterns of total protein of sesame seed and α-globulin isolated by various methods in 0.02 M phosphate buffer, pH 7.5, containing 1 M NaCl: (a) total protein; (b) α-globulin isolated by the procedure of Nath and Giri (1957a) by dilution of 10% NaCl protein extract 1:10 and successive (twice) reprecipitation by dilution; (c) α-globulin isolated by Ventura and Lima (1963) by dialysis of one volume protein extract in a phosphate–NaCl buffer (0.03 M KH_2PO_4, 0.02 M K_2HPO_4, 10% NaCl (w/v), pH 7) against ten volumes of distilled water and redissolution of the precipitate in the same buffer followed by dialysis twice as above; and (d) α-globulin isolated in the present investigation.

Table I. Chemical and Physicochemical Properties of α-Globulin of Sesame Seed[a]

Property	Total protein	α-Globulin
Intrinsic viscosity,[b] [η]		0.03 dL/g
Isoelectric point	4.50	4.90
Sedimentation velocities,[b] $s_{20,w}$	2, 7, 11, 16	11
Molecular weight[b]		250 000 ± 15 000
Specific rotation $[\alpha]_{578}^{28}$		−40°
Absorption coefficient,[b] $E_{280nm}^{1\%,1cm}$	13.0	10.8
Absorption maximum,[b] nm	278–280	278–280
Fluorescence emission maximum,[b] nm		328
Nitrogen content,[b] %		15.9
Amide content, %		2.0
Phosphorus content, %	0.09	0.04
Carbohydrate content, %	4.3	0.8
Subunit number		12
Proteolytic activity		Nil

[a] A few values of total protein have been included for comparison. [b] Values are in 0.02 M phosphate buffer (pH 7.5) containing 1 M sodium chloride.

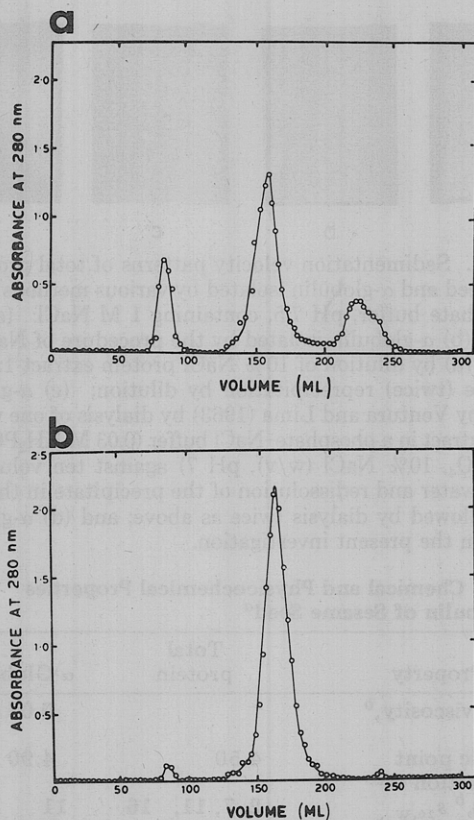

Figure 2. The gel filtration pattern of (a) total protein and (b) α-globulin in 0.02 M phosphate buffer, pH 7.5, containing 1 M NaCl in Sepharose 6B-100 gel.

DEAE–Cellulose Chromatography. DEAE–cellulose chromatography of the total proteins shows a sharp peak eluting at 0.04 M NaCl concentration along with a trailing edge eluting up to 0.3 M NaCl (Figure 3a). α-Globulin shows a single peak with the peak portion eluting at 0.04 M NaCl concentration (Figure 3b).

Polyacrylamide Gel Electrophoresis. Gel electrophoresis of the total protein in 0.02 M phosphate buffer of pH 7.5 shows a major band along with several slow and fast moving components (Figure 4a). α-Globulin shows a single band corresponding to the major band of the total protein (Figure 4b).

Effect of Storage. Lyophilized α-globulin (~1% moisture) stored at room temperature for 1 year on gel electrophoresis shows a single band at the position of the freshly isolated protein, indicating that the protein is stable during this period of storage (Figure 4c).

Proteolytic Activity. α-Globulin showed no proteolytic activity over a period of 24 h as determined by the absorbance of the trichloroacetic acid supernatant at 280 nm.

Physicochemical Properties. The different physicochemical properties of α-globulin are shown in Table I. The intrinsic viscosity of α-globulin is 0.03 dL/g, suggesting the globular nature of the protein (Yang, 1961; Bradbury, 1970). The molecular weight of the protein determined by the Archibald approach to sedimentation equilibrium method (Schachman, 1959) and sedimentation velocity experiments was $2.5 \times 10^5 \pm 15\,000$ dalton. This value is considerably lower than the value of $4.5 \times 10^5 \pm 30\,000$ dalton reported by Ventura and Lima (1963). Further, the sedimentation coefficient of 11S for α-globulin obtained in the present investigation is also lower than the value of 13S reported by Sinha and Sen (1962) and Ventura and Lima (1963). However, the sedimentation coefficient of α-globulin isolated by the methods of Sinha and Sen (1962) and Ventura and Lima (1963) using the sesame flour of the present study was also found to be 11S. The isolated α-globulin, however, sediments with a 13S value in Tris-HCl buffer (0.3 M) at pH 7.2. The fluorescence emission maximum of the protein at 328 nm indicates that the fluorescence is dominated by tryptophan groups which are embedded in a nonpolar environment of α-globulin (Lakshmi and Nandi, 1977).

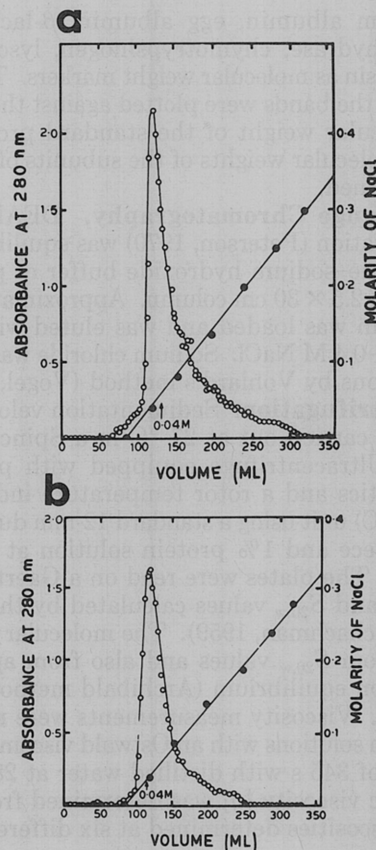

Figure 3. DEAE–cellulose chromatography pattern of (a) total protein and (b) α-globulin in 0.01 M glycine–NaOH buffer, pH 9.0.

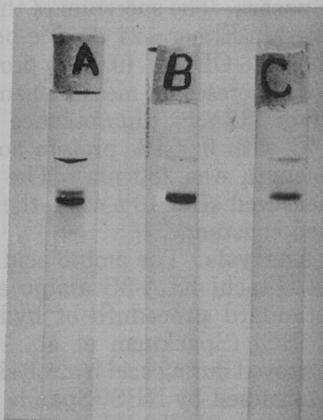

Figure 4. Polyacrylamide gel electrophoresis pattern of (a) total protein and (b) α-globulin in 0.02 M phosphate buffer pH 7.5, (c) lyophilized α-globulin after 1 year of storage. Top, cathode. In a 7-cm gel, the distance of the various bands moved from the top of the gel obtained from the scanning of the gels in centimeters are: (a) I, 0.7, II (corresponding to α-globulin), 0.95; III, 1.3; and IV, 1.7; (b) 0.9 cm; (c) 0.88 cm.

Subunits. The oligomeric nature of the protein is shown by the presence of 12 subunits in it (Figure 5). The peak 12 is not reproducible. The molecular weights of the subunits of α-globulin ranged from 8×10^3–8.5×10^4 dalton.

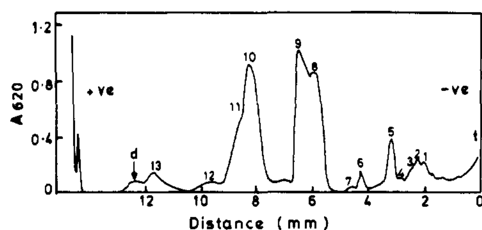

Figure 5. Scan of the SDS–polyacrylamide gel electrophoretic pattern of α-globulin. Molecular weight of the subunits are: 1, 85100; 2, 75100; 3, 66900; 4, 64000; 5, 55900; 6, 53100; 7, 40700; 8, 25600; 9, 23300; 10, 13900; 11, 13000; 12, 9900; 13, 8000.

Table II. Amino Acid Composition of α-Globulin

Amino acid	By Nath and Giri (1957b), g/100 g of protein	Present study, g/100 g of protein[a]
Lysine	2.90 ± 0.24	2.01
Histidine	3.60 ± 0.20	2.78
Ammonia		2.00
Arginine	8.40 ± 0.26	14.18
Aspartic acid	4.30 ± 0.22	9.63
Threonine	6.13 ± 0.2	4.14
Serine	3.17 ± 0.1	5.11
Glutamic acid	7.83 ± 0.1	19.98
Proline	1.05 ± 0.07	2.00
Glycine	3.45 ± 0.1	5.16
Alanine	3.50 ± 0.1	5.02
Half-cystine	3.50 ± 0.1	0.71
Valine	4.65 ± 0.2	4.54
Methionine	2.65 ± 0.2	2.59
Isoleucine	3.92 ± 0.34	3.57
Leucine	9.00 ± 0.14	7.09
Tyrosine	4.10 ± 0.1	3.95
Phenylalanine	2.65 ± 0.17	4.94
Tryptophan[b]	1.72 ± 0.17	2.00 ± 0.1

[a] Average of three analysis. [b] Average of NBS method (Spande and Witkop, 1967) and Edelhoch's method (Edelhoch, 1967).

Amino Acid Composition. The amino acid composition of α-globulin is shown in Table II. The values obtained by Nath and Giri (1957b) from paper chromatographic experiments are given for comparison. From the table it can be seen that the values obtained by Nath and Giri (1957b) for lysine, threonine, half-cystine, and leucine are significantly higher than the present values. Also, their values for arginine, aspartic acid, serine, glutamic acid, proline, and phenylalanine are considerably lower than the present value. We are not sure whether the methods of estimation used by us and Nath and Giri (1957b) are responsible for the observed discrepancy. The protein contains high amounts of aspartic acid, glutamic acid, and arginine.

LITERATURE CITED

Adolph, W. H., Lin, I., *Ind. Eng. Chem.* **28**, 734 (1936).
Association of Official Analytical Chemists, "Official Methods of Analysis", Washington, D.C., 1973.
Blackburn, S., in "Amino Acid Determination, Methods and Techniques", Marcel Dekker, New York, N.Y., 1968, p 18.
Block, R. J., Bolling, D., in "The Amino Acid Composition of Proteins and Foods", Thomas, L. C., Ed., Springfield, Ill., 1951, p 253.
Bradbury, J. H., in "Physical Principles and Techniques of Protein Chemistry", Leach, S. J., Ed., Academic Press, New York, N.Y., Part B, 1970, p 99.
Edelhoch, H., *Biochemistry* **6**, 1948 (1967).
Greenberg, D. M., in "Methods in Enzymology", Colowick, S. P., Kaplan, N. O., Ed., Academic Press, New York, N.Y., 1955, p 54.
Guerra, M. J., Park, Y. J., *J. Am. Oil Chem. Soc.* **52**, 73, 1975.
Jones, D. B., Gersdorff, C. E. F., *J. Biol. Chem.* **75**, 213 (1927).
Joseph, A. A., Tasker, P. K., Joseph, K., Rao, M. N., Swaminathan, M., Sankaran, A. N., Sreenivasan, A., Subrahmanyan, V., *Ann. Biochem. Exp. Med.* **22**, 13, 1962.
Lakshmi, T. S., Nandi, P. K., *Int. J. Pept. Protein Res.*, **10**, 120 (1977).
Montgomery, R., *Biochim. Biophys. Acta* **48**, 591, 1961.
Nath, R., Giri, K. V., *J. Sci. Ind. Res., Sect. C* **16**, 5 (1957a).
Nath, R., Rao, K. H., Giri, K. V., *J. Sci. Ind. Res.*, **16**, 228 (1957).
Nath, R., Giri, K. V., *J. Sci. Ind. Res. Sect. C* **16**, 51 (1957b).
Peterson, E. A., in "Laboratory Techniques in Biochemistry and Molecular Biology", Vol. 2, Work, T. S., Work, E., Ed., North Holland Publishing Co., Amsterdam, 1970, p 223.
Prakash, V., Ph.D. Thesis, Mysore University, 1976.
Prakash, V., Nandi, P. K., *Int. J. Pept. Protein Res.* **8**, 385 (1976).
Prakash, V., Nandi, P. K., *J. Biol. Chem.* **252**, 240 (1977a).
Prakash, V., Nandi, P. K., *Int. J. Pept. Protein Res.* **9**, 97 (1977b).
Prakash, V., Nandi, P. K., *Int. J. Pept. Protein Res.* **9**, 319 (1977).
Salem, H. M., Beckheit, M., *Pak. J. Sci.* **16**, 105 (1964).
Sastry, M. C. S., Subramanian, N., Parpia, H. A. B., *J. Am. Oil Chem. Soc.* **51**, 115 (1974).
Schachman, H. K., "Ultracentrifugation in Biochemistry", Academic Press, New York, N.Y., 1959.
Sinha, N. K., Sen, A., *Trans. Bose Res. Inst., Calcutta* **25**, 37 (1962).
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30**, 1190, (1958).
Spande, T. F., Witkop, B., in "Methods in Enzymology", Hirs, C. H. W., Ed., Academic Press, New York, N.Y., 1967, p 498.
Taussky, H. H., Shorr, E., *J. Biol. Chem.* **202**, 675 (1953).
Ventura, M. M., Lima, H. T., *An. Acad. Bras. Cienc.* **35**, 55 (1963).
Vogel, A. I., in "A Test Book of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis", The English Language Book Society and Longmans Green and Co. Ltd., 1961, p 266.
Weber, K., Osborn, M., *J. Biol. Chem.* **244**, 4406 (1969).
Yang, J. T., in *Adv. Protein Chem.* **16**, 323 (1961).

Received for review April 21, 1977. Accepted September 9, 1977.