When NPN of the MeOH extracts was determined, the values averaged about 2% higher than those of the $Cl_3CCOOH$-extracted NPN, indicating that $Cl_3CCOOH$ was a more effective protein precipitant (data not tabulated). Samples of the MeOH extracts were also analyzed on the biological fluids column. Because the results were essentially the same as those for the $Cl_3CCOOH$ extracts, no further studies were conducted with these samples. The results suggested that essentially no hydrolysis occurred in the $Cl_3CCOOH$ samples.

The amounts of the amino acids were consistently greater after hydrolysis, suggesting that some of the NPN was due to nonprecipitable peptides. There were no seasonal changes in the differences between nonhydrolyzed and hydrolyzed samples, suggesting that the amounts of peptides were constant.

Amounts of the various amino acids changed during storage (Table III). Aspartic acid, threonine, and serine increased during the first part of storage and then decreased. Lysine appeared to increase slightly toward the end of the storage period. Glycine, cysteine, and ammonia did not change significantly during storage. The other amino acids decreased during the first part of storage and increased toward the end.

Recovery of NPN as amino acid N averaged 83.4% for the nonhydrolyzed samples and 83% for the hydrolyzed. Standard deviations were 15.4 and 12.2%, respectively.

These data suggest that the NPN is part of a metabolically active nitrogen pool (Sober, 1970), and that appreciable amounts of nitrogen are stored as asparagine and could be made available for synthesis of amino acids as demanded by the root. The relatively large amounts of aspartic and glutamic acids would be available for transamination reactions. The quadratic change previously reported for the NPN (Purcell et al., 1978a) suggests that this fraction is metabolically active. It may be that the minimum level of NPN represents a stable resting period for the root and the subsequent increase in NPN is caused by the root preparing itself for sprouting. When this nitrogen pool is at its minimum, modifying the storage conditions to maintain that minimum might increase the storage life of sweet potatoes.

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# Isolation and Characterization of the Major Fraction of Guar Proteins

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The major protein fraction of guar has been isolated in a homogeneous form. It does not contain any phosphorus and has 0.8% carbohydrate. Its sedimentation coefficient is 10.5 S, molecular weight 223 000, and intrinsic viscosity 0.047 dL/g. It consists of at least six nonidentical polypeptide chains. It exhibits an absorption maximum at 280 nm and fluorescence emission maximum at 325 nm and consists mainly of $\beta$ structure and random coil.

Guar (*Cyamopsis tetragonoloba*) is a commercially important crop. Guar meal, a byproduct of the guar gum industry, contains about 45% proteins. The meal is known to contain some toxic principles (Subramanian and Parpia, 1975; Ambegaokar et al., 1969; Couch et al., 1966), and it has been reported that the protein isolated from it is more toxic to rats than the original meal (Khopkar, 1976; D'Souza, 1972). It was therefore of interest to fractionate guar proteins and study their properties. This paper describes the isolation and characterization of the major globulin fraction of guar proteins.

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MATERIALS AND METHODS

**Materials.** Guar seeds of the variety FS-277 obtained from the Haryana Agricultural University, Hissar, India, were used. Defatted guar meal (60 mesh BSS) was prepared by the method reported earlier (Nath et al., 1978). The moisture and protein contents of the meal were 8 and 55%, respectively. Sephadex G-200 (40–120 $\mu$m) from Pharmacia Fine Chemicals (Sweden), DEAE-cellulose, bovine serum albumin, egg albumin, lysozyme, $\beta$-lactoglobulin, and pepsin from Sigma Chemicals (USA), Coomassie brilliant blue and amido black from Schwarz-Mann (Germany), acrylamide from CSIR Centre for Biochemicals

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(India), bisacrylamide and TEMED from Koch-Light Laboratories (England), and β-mercaptoethanol from Fluka (Switzerland) were used. All other chemicals were of reagent grade.

**Isolation and Purification of the Major Fraction.** Ten grams of guar meal was shaken with 100 mL of 1 M NaCl solution for 1 h and the centrifuged clear extract (~85 mL) was diluted to 850 mL with distilled water, when precipitation occurred. This was allowed to stand for 30 min. The precipitate was collected, dispersed in 50 mL of 1 M NaCl and centrifuged at 4500 rpm for 30 min. The supernatant was diluted to 500 mL with distilled water, allowed to stand for 30 min, and centrifuged for 10 min. The precipitate obtained was dissolved in 10 mL of 1.5 M NaCl and loaded on a preparative gel filtration column of Sephadex G-200 equilibrated with 1 M NaCl solution. Elution was carried out with 1 M NaCl solution, and the fractions from 200 to 240 mL were collected and dialyzed against three changes of distilled water. The precipitate was centrifuged and dissolved in 1.5 M NaCl and dialyzed against 1 M NaCl or 0.02 M phosphate buffer of pH 7.5.

**Polyacrylamide Gel Electrophoresis.** Electrophoresis was carried out in a Shandon electrophoresis apparatus using 7.5% gels in 0.02 M phosphate buffer of pH 7.5 and for 2.5 h at 3 mA/tube. Experiments were also conducted at pH 4.5 in β-alanine–acetic acid buffer.

**Sedimentation Velocity Experiments.** Using a 1% protein solution in 1 M NaCl, ultracentrifugation was performed at room temperature (~30 °C) at 59 780 rpm in a Spinco Model E analytical ultracentrifuge equipped with RTIC unit and phase plate schlieren optics. The $s_{20,w}$ value was calculated by the standard procedure (Schachman, 1959).

**Gel Filtration.** Sephadex G-200 equilibrated with 1 M NaCl was packed into a column 2 × 85 cm. Three milliliters of the solution containing 90 mg of the protein was loaded on the column and allowed to be absorbed. The protein was eluted with 1 M NaCl solution; 2.5-mL fractions were collected in an automatic fraction collector and the absorbance measured at 280 nm.

**DEAE-Cellulose Chromatography.** DEAE-cellulose was regenerated by the standard procedure (Peterson, 1970). It was equilibrated with 0.02 M phosphate buffer of pH 7.5 and packed into a column 2.5 × 15.0 cm. Five milliliters of the protein solution in the buffer containing 140 mg was loaded and allowed to be adsorbed. Elution was carried out using a continuous linear gradient of 0–0.8 M NaCl in the buffer. A flow rate of 25–30 mL/h was maintained, and 4-mL fractions were collected. NaCl was estimated in the fractions by Vohlard's method (Vogel, 1961).

**Protein Concentration.** The absorbance of 1% protein solution in 1-cm cell at 280 nm, $E_{1cm}^{1\%}$ was determined by plotting absorbance at 280 nm vs. protein concentration which was determined by microKjeldahl nitrogen estimation. A factor of 6.25 was used to convert nitrogen to protein value. The values for the total proteins and the major fraction were 8.2 and 7.0, respectively.

**Absorption Spectrum.** The absorption spectrum of the protein in 1 M NaCl solution was recorded in a Perkin-Elmer double-beam recording spectrophotometer 124 in the 240–330-nm range.

**Determination of Subunits and Their Molecular Weight.** The number of subunits and their molecular weight was determined by the method of Weber and Osborn (1969). The following crystalline proteins were used as molecular weight markers: bovine serum albumin (68 000), egg albumin (43 000), pepsin (35 000), β-lactoglobulin (18 400), and lysozyme (14 700). Using 10% polyacrylamide gel, electrophoresis was carried out for 7 h at 6 mA/tube; 200–300 μg of the protein was loaded. The gels were stained with Coomassie brilliant blue for 2 h.

**Viscosity Measurements.** Apparent viscosity measurements were made at 30 ± 0.1 °C in an Ostwald viscometer having a flow time of about 180 s with distilled water. Protein solutions in 1 M NaCl in the concentration range 0.5 to 2.0% were filtered in a sintered glass funnel before introduction into the viscometer. After temperature equilibration for 30 min, the flow time was measured to within ±0.1 s. Apparently viscosity measurements were also made with protein solutions in 0.02 M phosphate buffer of pH 7.5 in the concentration range 0.5 to 1.6%.

**Fluorescence Spectrum.** A Perkin-Elmer fluorescence spectrophotometer, Model 203, was used. The emission spectrum was measured in the 300–400-nm range after excitation at 280 nm. Protein in 0.02 M phosphate buffer of pH 7.5 and with an absorbance of 0.06 at 280 nm was used.

**Circular Dichroism (CD).** CD measurements were made at room temperature in a JASCO-J-20 automatic spectropolarimeter equipped with xenon lamp.

A 0.2- or 0.5-mm cell and protein solution in 0.02 M phosphate buffer of pH 7.5 were used. Measurements were made at two protein concentrations, 0.04 and 0.09%, and the average values were taken. Molar residue ellipticity was calculated by the standard procedure (Adler et al., 1973). A value of 115 was used for mean residue weight.

**Phosphorus Content.** Phosphorus was estimated by the method of Taussky and Shorr (1953) using a 3% protein solution in 1 M NaCl.

**Carbohydrate Content.** Carbohydrate was estimated by the method of Montgomery (1961) using a 3% protein solution in 1 M NaCl.

**Tryptophan and Tyrosine Content.** Tryptophan was estimated by the method of Spande and Witkop (1967) using *N*-bromosuccinimide. Tyrosine was estimated by the method of spectrophotometric titration (Donovan, 1973).

## RESULTS AND DISCUSSIONS

The major fraction of guar protein is a globulin with a sedimentation coefficient of 10.5S (Nath et al., 1978). Of the various methods attempted to isolate the protein in a homogeneous form, $(NH_4)_2SO_4$ precipitation yielded a homogeneous fraction, but its solubility in salt or buffer solution was poor. On the other hand, the double dilution method described above gave a fraction which had good solubility. However, this fraction was still contaminated with impurities and further purification was achieved by gel chromatography on Sephadex G-200.

**Gel Electrophoresis.** The gel electrophoresis pattern of the preparation showed an intense band and another faint band near the origin (Figure 1). The gel electrophoresis pattern of guar total proteins, however, did not show the presence of the minor band (Nath et al., 1978). The pattern obtained at pH 7.5 and 4.5 was the same.

**Sedimentation Velocity Experiments.** The sedimentation velocity pattern of the fraction consisted of a symmetrical major peak with $s_{20,w}$ value of 10.5 and a faster moving component which was about 5% of the total (Figure 2). The faster moving protein component was not observed in the sedimentation velocity pattern of guar total proteins (Nath et al., 1978). Both gel electrophoresis and sedimentation velocity showed the presence of a higher molecular weight component in the isolated 10.5S protein fraction.

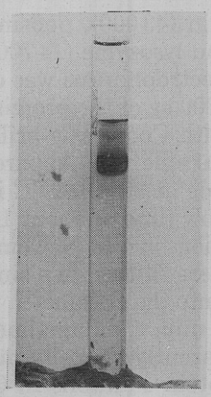

**Figure 1.** Gel electrophoresis pattern of 10.5S protein (0.02 M phosphate buffer of pH 7.5).

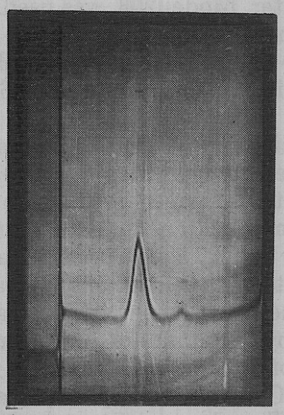

**Figure 2.** Sedimentation velocity pattern of 10.5S protein in 1 M NaCl solution. Photograph taken after 15 min at 59 780 rpm; bar angle, 70°. Sedimentation proceeds from left to right.

When the protein was isolated in the presence of 0.5% $\beta$-mercaptoethanol or 1.25% *N*-ethylmaleimide, the high-molecular-weight component was still observed, suggesting that this was not an aggregate formed by disulfide interaction. This component could be present in very small quantity in the total proteins and perhaps could not be seen in the ultracentrifuge or electrophoresis pattern of the total proteins. It could have been precipitated with 10.5S protein during its isolation.

**Gel Filtration.** The protein eluted as a single symmetrical peak in gel filtration (Figure 3). Its elution volume corresponded to that of the major fraction of guar total proteins.

**DEAE–Cellulose Chromatography.** The total proteins showed three peaks eluting at 0, 0.025, and 0.27 M NaCl concentration (Figure 4A). The isolated protein eluted as a single peak at 0.27 M NaCl concentration (Figure 4B) corresponding to the position of the major fraction. Thus the isolated protein was homogeneous by the above criteria.

**Absorption Spectrum.** The ultraviolet absorption spectrum of the 10.5S fraction was typical of a protein with its maximum absorption at 278 nm and minimum at 255 nm. The ratio of absorbance at 280/260 nm was 1.51.

The 10.5S fraction did not give a positive test for phosphorus. The carbohydrate content of the 10.5S fraction was 0.80%.

**Fluorescence Spectrum.** The maximum fluorescence emission occurred at 325 nm. The fluorescence is due to tyrosine and tryptophan residues. In most proteins containing both tryptophan and tyrosine residues, the fluorescence spectrum is more characteristic of tryptophan, and tyrosine fluorescence is not predominant (Teale, 1960). The protein contains 1.2% tryptophan and 2.6% tyrosine.

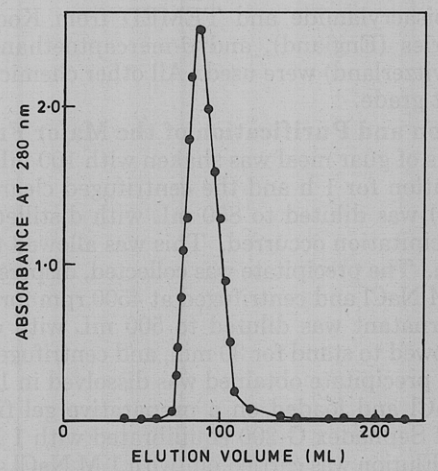


**Figure 3.** Gel filtration pattern of 10.5S protein in 1 M NaCl solution.

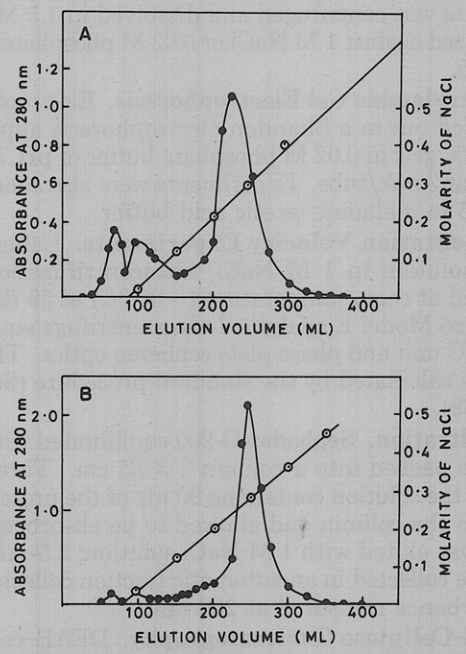


**Figure 4.** DEAE–cellulose chromatographic pattern in 0.02 M phosphate buffer, pH 7.5. (A) Total guar proteins and (B) 10.5S protein.

The observed maximum would suggest that the influence of tryptophan is considerable and these groups are embedded in a nonpolar environment of the protein (Lakshmi and Nandi, 1977).

**CD Spectrum.** The CD spectrum of the protein in the 200–300-nm region showed a maximum at 285 nm and a minimum at 208 nm with a shoulder at 220–225 nm (Figure 5). The maximum at 285 nm is characteristic of proteins rich in tryptophan and tyrosine (Adler et al., 1973). The protein contains 1.2% tryptophan and 2.6% tyrosine. The wavelengths of the minimum and shoulder correspond to those of the minima given by 100% $\alpha$ helix. However, the ellipticity values corresponding to the two minima (208 and 222 nm) of 100% helix are of equal magnitude (Greenfield and Fasman, 1969). The ellipticity value at 208 nm was −5700 deg cm$^2$ dmol$^{-1}$ and at 222 nm was −4100. One hundred percent $\beta$ structure also gives a minimum at 220 nm and the elipticity value is much lower than that of $\alpha$ helix (Greenfield and Fasman, 1969). Thus the 10.5S protein could consist of $\alpha$ helix and $\beta$ structure. Using the ellipticity values at 208 and 222 nm for 100% $\alpha$ helix, 100% $\beta$ structure, and 100% random coil structure given by Greenfield and Fasman (1969), the proportion of

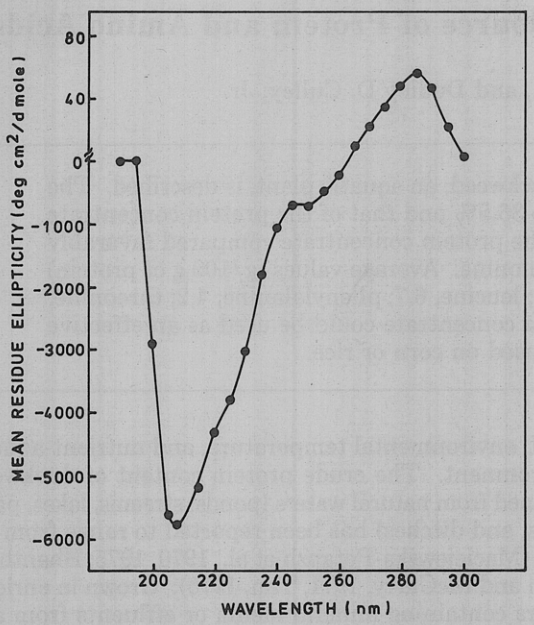


**Figure 5.** CD spectrum of 10.5S protein in 0.02 M phosphate buffer, pH 7.5.

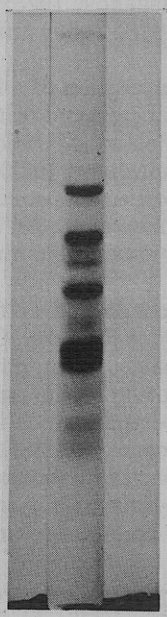

**Figure 6.** $NaDodSO_4$–polyacrylamide gel electrophoresis pattern of the 10.5S protein.

the three structures in the 10.5S protein was calculated and found to be 7% $\alpha$ helix, 30% $\beta$ structure, and 63% random coil. These values are in agreement with those for other seed proteins (Jacks et al., 1973).

**Viscosity Measurements.** The intrinsic viscosity of the 10.5S fraction in 1 M NaCl was found to be 0.047 dL/g, whereas in phosphate buffer it was 0.032 dL/g. Globular proteins generally have values in the range of 0.03 to 0.04 dL/g (Tanford, 1969). Although high in comparison with the intrinsic viscosity values of globular proteins, the value of 0.047 dL/g would still indicate a certain degree of compactness of the molecule. A value of 0.032 dL/g in phosphate buffer suggests that the protein assumed a more compact shape in the buffer. The reason for this is not obvious.

**Molecular Weight of the 10.5S Protein.** From the $s_{20,w}$ and intrinsic viscosity values, the molecular weight of the protein was estimated using following equation (Schachman, 1959):

$$M = \frac{4690(S)^{3/2}[\eta]^{1/2}}{(1 - \bar{\nu}\rho)^{3/2}}$$

where $S$ is the sedimentation coefficient; $[\eta]$, the intrinsic viscosity; $\bar{\nu}$, the partial specific volume of the protein; and $\rho$, the density of the solution. Most proteins have a partial specific volume in the range of 0.7 to 0.8 (Tanford, 1969). Therefore $\bar{\nu} = 0.75$ was used. The molecular weight thus calculated was 228 000.

Since the molecular weight was high, the protein was likely to be an oligomeric protein with subunit structure (Klotz et al., 1970). $NaDodSO_4$ *polyacrylamide* gel electrophoresis was performed to determine the number and molecular weight of the polypeptide chains. It consisted of at least six nonidentical polypeptide chains (Figure 6). The molecular weights were 52 000, 43 000, 37 000, 32 000, 28 000, and 25 000. In spite of the closeness of the molecular weights of some subunits, the resolution was good. The total of the subunit molecular weights was 217 000, which compared well with the value obtained from $s_{20,w}$ and $[\eta]$ values.

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