ples. This in fact exists even among fruits carefully sorted according to their initial color and texture. These factors may contribute to the variation in the experimental results.

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Chickpea Seed Proteins: Conformational Changes in 10.3S Protein during Germination

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The changes that occur in the major 10.3S storage protein of chickpea during germination have been monitored by viscosity, heat coagulation, α-chymotryptic digestion, circular dichroism, and fluorescence spectral measurements. The hydrodynamic method suggested an alteration in the asymmetry, whereas from the CD spectral studies, it was inferred that the limited ordered structure of the 10.3S protein was considerably reduced during germination. The heat coagulation and free -SH group measurements indicated that changes in the quaternary structure occurred possibly through the scission of one of the three disulfide bonds present in 10.3S protein. Evidence of conformational changes in 10.3S protein during germination is also provided by the increased susceptibility of 6-day germination-modified (GM) protein to α-chymotryptic digestion as compared to 10.3S protein.

In our earlier study on chickpea seed germination (Ganesh Kumar and Venkataraman, 1978), we reported on the gross modification occurring in the storage proteins. Among the two major storage proteins, it was observed that 6.9S protein degraded faster in the initial stages, whereas 10.3S protein was found to do so in the later stages of germination. There are many structural constraints on the proteins which are operative and become important in the selective degradation of a protein fraction. In this work we have attempted to study the structural and conformational changes occurring in the major 10.3S storage protein of chickpea seed during germination. We have usually chosen a 6-day germination period since this represents the period of maximum degradation (Ganesh Kumar and Venkataraman, 1978). The germination-modified form of 10.3S has been isolated from the germinated cotyledon, which will be referred to as germination-modified (GM) protein. The structural and conformational characteristics of 10.3S and GM proteins were determined using the techniques of viscosity, heat coagulation, circular dichroism, in vitro enzymic hydrolysis, and fluorescence spectral measurements.

EXPERIMENTAL SECTION

Seeds and Germination. Seeds of chickpea (*Cicer Arietinum*) were obtained locally. They were surface sterilized with $HgCl_2$ (0.1% w/v) and thoroughly washed with distilled water. Germination was carried out in autoclaved moist vermiculite and grown at room temperature (28 ± 2 °C) in darkness. Only distilled water was provided during the germination period. Cotyledons of 3-, 6-, and 9-day germinated seeds alone were taken up for protein studies. The cotyledons were powdered in a hand mill passed through a 100-mesh sieve and were defatted with hexane. Finally they were dried at 40 °C and used as such for further study.

Preparation of 10.3S Protein. The defatted chickpea flour was extracted with 10% (w/v) NaCl in solute-to-solvent ratio of 1:10 for 2 h on a mechanical shaker. The slurry was centrifuged at 5000 rpm for 30 min. The supernatant was diluted with 10 volumes of distilled water. The resultant precipitate was collected by centrifugation dissolved in 10% (w/v) NaCl solution. Another dilution was performed with 20 volumes of distilled water. The precipitate obtained in this step was further purified by DEAE-cellulose chromatography.

DEAE-Cellulose Chromatography. The DEAE-cellulose column (2 × 20 cm) was equilibrated with 0.01 M borate buffer of pH 7.8 containing 0.25 M NaCl (buffer I). The protein solution (~30 mg/2.0 mL) in buffer I was applied on the column and eluted with the buffer containing 0.25 M NaCl (100 mL). The fractions collected were discarded. Then a continuous linear gradient was set up in buffer I from 0.25 to 0.40 M NaCl (500 mL). The fractions (4.0 mL each) were monitored at 280 nm and the protein eluting in the range of 0.30 to 0.33 M were collected, dialyzed against distilled water, and lyophilized.

Preparation of Germination-Modified (GM) Protein from Germinated Chickpea Cotyledons. The isolation of germination-modified proteins was similar to the preparation of 10.3S with the following modification in the DEAE-cellulose chromatographic step. The column was equilibrated with 0.01 M borate buffer of pH 7.8 containing 0.29 M NaCl; the protein also in the same buffer was applied on the column and a continuous linear gradient

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in the buffer was set up from 0.29 to 0.40 M NaCl. The protein fractions were monitored at 280 nm. The fractions eluting in the range 0.33 to 0.36 M NaCl were collected, dialyzed against distilled water, and lyophilized.

Concentration of Protein. The concentration of protein was determined by Lowry's method (1951) using bovine serum albumin as the standard.

Gel Filtration. Gel filtration was carried out on Sepharose-6B-100 gel in a 2 × 85 cm column. The flow rate was 25–30 mL/h, and 2.5-mL fractions were collected and monitored at 280 nm. The equilibration and elution were carried out in 0.03 M phosphate buffer of pH 7.8 containing 1 M NaCl.

DEAE-Cellulose Chromatography. DEAE-cellulose was adjusted to the initial pH of the borate buffer (pH 7.8) and packed into a 2 × 13 cm column. One hundred milligrams of protein was applied on the column. Desired linear gradient was set up in borate buffer, and the eluted fractions were monitored at 280 nm. The concentration of NaCl was determined by titration with standard 0.01 N $AgNO_3$ using K_2CrO_4 as indicator (Rieman et al., 1951).

Polyacrylamide Gel (PAG) and NaDodSO$_4$-PAG Electrophoresis. The electrophoretic conditions both for PAG and NaDodSO$_4$-PAG employed were essentially the same as in our earlier report (Ganesh Kumar and Venkataraman, 1978).

Circular Dichroism (CD) Spectra. CD spectra were recorded on a Cary Model 60 spectropolarimeter with Model 6001 circular dichroism attachment, fitted with a thermostatable cell holder calibrated with a thermistor probe. The instrument was modified in the field to eliminate possible artifactual signal on passing through intense absorbance bands. The slit width was programmed to yield 15 Å band width at each wave length. Quartz cells of 5- and 0.5-mm optical path were used for CD measurements in the UV region. The scale sensitivity was 0.004°/in. or 0.01°/in. Protein solution of 0.025% in appropriate buffer was used after obtaining a clear solution by centrifugation at 100000g for 1 h. All measurements were made at 25 °C. A value of 115 for the mean residue weight was used. CD values were converted to mean residue molecular ellipticity.

Viscosity Measurements. Viscosity measurements were made at 30 ± 0.1 °C using an Ostwald viscometer having a flow time of 180 s with distilled water. Viscosity was calculated from the flow time using the equation $\eta = \rho(At - B/t)$, where η is viscosity, ρ is density, the flow time t is in seconds, and A and B are constants. Five milliliters of the clear protein solution was introduced into the viscometer. After temperature equilibration, the flow time was recorded to ±0.1 s with a Rocar stopwatch. From a plot of $[(\eta/\eta_0) - 1]/C$, as a function of concentration (C), the intrinsic viscosity (η) was obtained from the intercept, where η is the viscosity of solution, η_0 is the viscosity of the solvent, and C is the concentration of the protein in g/100 mL (Bradbury, 1970).

Fluorescence Measurements. Perkin-Elmer fluorescence spectrophotometer Model 203 was used for fluorescence measurements. Both excitation and emission spectra were taken in the region where the fluorescence intensity was found to increase linearly with protein concentration (0.01% protein in buffer). The emission spectrum was measured between 300 and 400 nm. All the readings were taken at 25 °C. Measurements were also made with protein with and without added urea.

Heat Coagulation. Three milliliters of 1.0% protein solution in buffer was heated for 15 min. The solution was rapidly cooled to room temperature at the end of 15 min.

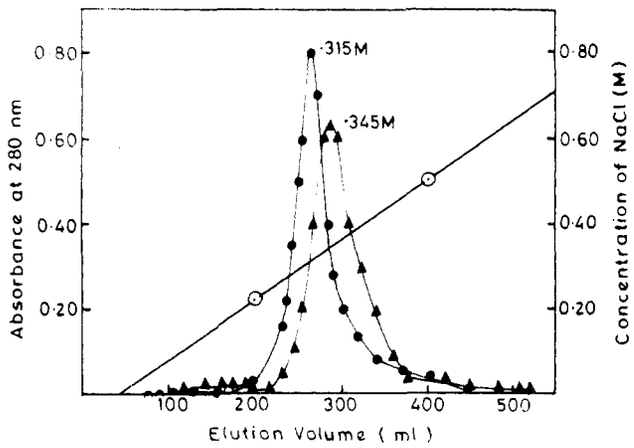

Figure 1. DEAE-cellulose ion-exchange chromatographic pattern of 10.3S and 6-day GM proteins of chickpea: (-●-) 10.3S and (-▲-) 6 day GM protein.

Turbidity was determined by measuring the transmittance of the solution at 540 nm. From the measurement, a quantity $(T_0 - T/T_0) \times 100$ was calculated, where T_0 is the transmittance of unheated solution, T is the transmittance of heated solution. The quantity $(T_0 - T/T_0) \times 100$ was taken as a measure of coagulation.

Rate of Enzymic Hydrolysis of Proteins. Fifteen milliliters of 10% protein solution in 0.025 M Tris-glycine buffer of pH 8.3 was incubated at 37 °C. Fifteen milliliters of α-chymotrypsin (5 μg/mL) was added, and the solutions were thoroughly mixed. Time intervals from 1 to 60 min were used. At regular intervals 2.0-mL aliquots of the reaction mixture were withdrawn, and reaction was arrested by the addition of 2.0 mL of 20.0% Cl_3CCOOH. After centrifugation, absorbance of the supernatant was read at 280 nm and plotted as a function of time (in minutes).

Free and Total -SH Groups. The procedure of Beveridge et al. (1974) was followed for the estimation of -SH and S-S groups of proteins.

RESULTS

Purity of 10.3S and GM Protein. The homogeneity of 10.3S and GM protein, purified by DEAE-cellulose chromatography was tested by polyacrylamide gel (PAG) electrophoresis and rechromatography on DEAE-cellulose. The electrophoretic pattern of 10.3S showed a single band, whereas GM protein, in addition to the major band, showed the presence of fast moving components (~10%). However, on DEAE-cellulose chromatography, no contamination was seen (Figure 1). Thus both the 10.3S and GM proteins used in this study were fairly homogeneous.

Changes in the Size and Charge of the Protein. To determine the changes associated with 10.3S protein in size and charge as a result of germination, elution volume (in gel filtration), elution constant (in DEAE-cellulose), and relative electrophoretic mobility (in PAG electrophoresis) of 10.3S and GM proteins were determined and are shown in Table I. The observed difference in the elution volume of the two fractions, viz., 12.0 mL, was well above the range of experimental error and indicated a reduction in the size of the molecule (10.3S) possibly to a lower molecular weight protein (from 276000 to 213000).

On DEAE-cellulose chromatography the 10.3S protein eluted at 0.32 M and the GM protein at 0.35 M NaCl concentrations (Table I). This was in good agreement with the changes observed in the total protein pattern (Ganesh Kumar and Venkataraman, 1978). Further, the relative electrophoretic mobilities of the two proteins were dif-

Table I. Some Physicochemical Properties of 10.3S and 6-Day GM Proteins of Chickpea[a]

criteria	10.3S protein	6-day GM protein
elution volume (gel filtration, in mL)	162	174
elution constant (DEAE-cellulose, in M)	0.32	0.35
relative electrophoretic mobility	0.13	0.18
amide nitrogen (g/mg of protein)	12.1 ± 0.2	8.3 ± 0.6

[a] The values are means of three independent experiments.

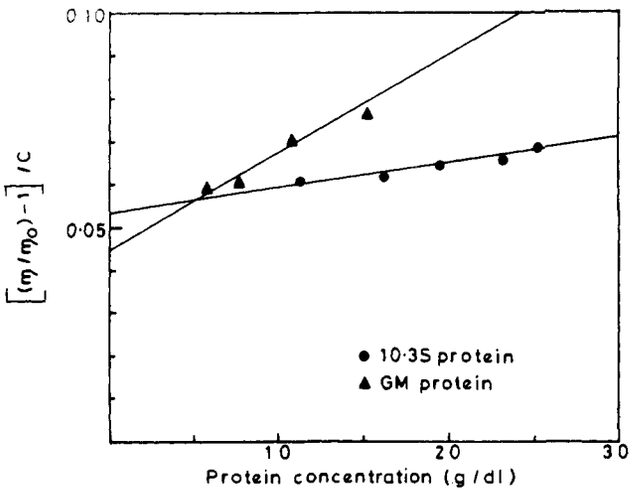

Figure 2. Plot of reduced viscosity as a function of protein concentration in buffer containing 1 M NaCl.

ferent. The R_f value of the 10.3S protein was 0.13 and of the GM protein 0.18. Thus, because of germination the elution constant and the relative electrophoretic mobility of 10.3S protein showed an increase. A similar increase has been reported in the legumin and vicilin fractions of vetch seed (Shutov and Vaintraub, 1972). It is apparent that, under the experimental conditions (pH 8.3), increase in the net negative charge of 10.3S protein should have occurred, resulting in the greater electrophoretic mobility and increase in the elution constant. Deamidation during germination has been shown to cause increase in the net negative charge of the protein molecule (Daussant et al., 1969; Catsimpoolas et al., 1968a). About 28% of the amide N content of 10.3S has been depleted during a 6-day germination period (Table I). Thus the changes detected in the DEAE-cellulose chromatographic and electrophoretic properties of 10.3S during germination were apparently due to deamidation of the protein.

Viscosity Measurements. Viscosity experiments have been extensively used to study the size and shape of protein molecules in solution (Yang, 1961). The intrinsic viscosity value is highly sensitive to conformational changes. The plot of reduced specific viscosity of the 10.3S and GM protein as a function of the concentration of protein is shown in Figure 2.. The intrinsic viscosity, the extrapolated reduced specific viscosity value at zero protein concentration, of the two fractions is 0.053 and 0.045 dL/g, respectively.

In general, globular proteins have low values of intrinsic viscosity of 0.03–0.04 dL/g (Yang, 1961). The value of 0.053 dL/g for 10.3S protein is rather high. This probably suggests a structure approximating that of random coil (Jayaramashetty and Narasinga Rao, 1973). However, the GM protein showed a reduction in the intrinsic viscosity value to 0.045 dL/g. In globular proteins, dissociation and denaturation cause an increase in viscosity, whereas the

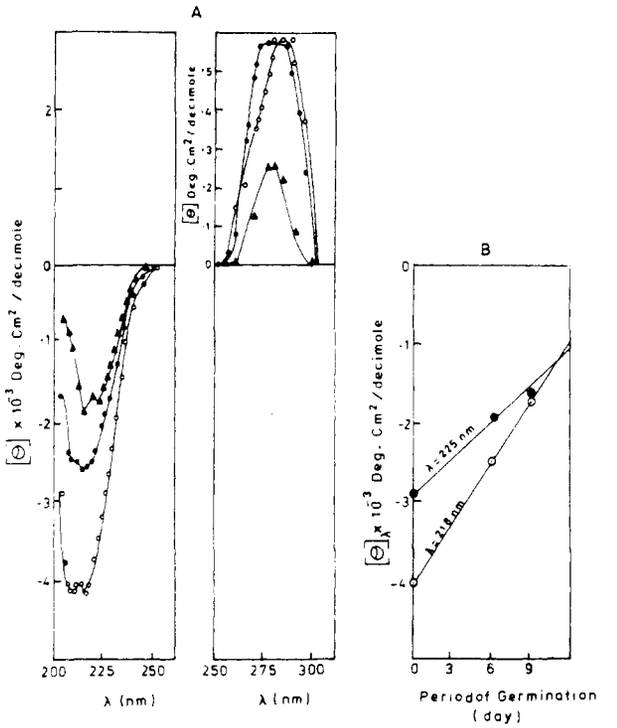

Figure 3. (A) Circular dichroism spectra of 10.3S (–O–), 3-day (–●–), and 6-day (–▲–) GM proteins of chickpea. (B) Effect of germination on θ_{218nm} and θ_{225nm} of 10.3S protein.

viscosity of rigid rodlike proteins decreases due to the decrease in the asymmetry of the molecule (Bradbury, 1970). The lower intrinsic viscosity of the GM protein may be attributed to alteration in the asymmetry of the 10.3S during a 6-day germination period.

Circular Dichroism Measurements. The CD spectra of 10.3S and GM proteins in the far and near ultraviolet region are shown in Figure 3A. Interpretation of the CD spectra in terms of conformation is possible on the basis of several criteria (Bush, 1971; Adler et al., 1973). One of these is to compare the CD spectra of proteins of unknown conformation with those of known conformation (Jirgensons, 1973). It has been shown that polypeptide chains could assume only a limited number of stable structures. Among them the α helix, the interchain hydrogen bonded β structure, and a fully extended parallel or antiparallel arrangement of peptide chains are of importance in protein. The CD band positions for various structures have been reported (Timasheff et al., 1967).

The CD curve of 10.3S exhibited a band with minima at 208 nm and 216–217 nm. The minimum at 216 nm represents the β structure in the protein. The corresponding mean residue ellipticity (θ) was −4150 deg·cm²/dmol. If it is assumed that the molecular ellipticity for 100% β structure is −23 000 deg·cm²/dmol at 217 nm (Sarkar and Doty, 1966), the proportion of β structure in 10.3S is estimated to be 18%. Similarly, the α-helical content was estimated, using eq 1 (Sears and Beychok, 1973), to be 0.5%. Thus only a limited ordered structure

$$\% \ \alpha \text{ helix} = \frac{-\theta_{208nm} - 4000}{33\,000 - 4000} \times 100 \qquad (1)$$

is present in 10.3S protein.

The CD curve of 6-day GM protein showed a minimum at 214–216 nm and a shoulder at 208 nm with the θ values of −2650 and −2000 deg·cm²/dmol, respectively. The calculated β-structure content of this protein is 12%. Thus reduction in the ordered structure of 10.3S protein appeared to have occurred during the 6-day germination

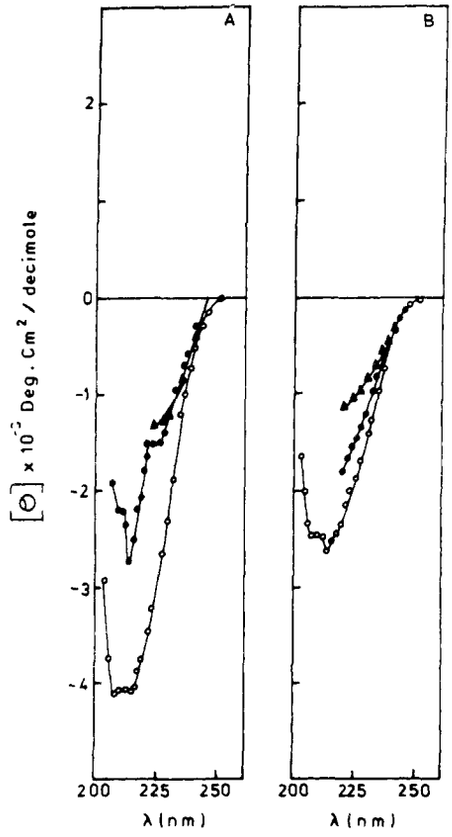

Figure 4. Effect of increasing concentration of urea on 10.3S (A) and 6-day GM protein (B) of chickpea: (-○-) untreated, (-●-) 4 M urea treated, and (-▲-) 8 M urea treated.

period. Further decrease in θ_{214nm} occurred in 9-day GM protein (from −2650 to −1900 deg·cm^2/dmol).

Figure 3B shows the plot of θ as a function of period of germination. The value of $\theta_{214-218nm}$ denoting the β structure decreased as germination progressed. This suggests a regular loss of β structure of the major chickpea 10.3S protein during a 9-day germination period.

The effects of 4 and 8 M urea on the θ of 10.3S and 6-day GM protein are shown in Figure 4. The limited ordered structure in the native 10.3S protein was found to be destroyed in the presence of urea. At 4 M urea concentration, the loss in the ordered structure was more for 10.3S protein than for GM protein. Further, the CD curve of 4 M urea-treated 10.3S protein compared favorably with the untreated 6-day GM protein. This indicates a possible mode of degradation of the native 10.3S protein during 6 days of germination, which may be similar to those of the dissociative effect of 4 M urea. Thus 10.3S and GM protein behave differently toward 4 M urea, indicating different conformational states of the protein.

Fluorescence Spectral Measurements. The fluorescence spectra of 10.3S and GM protein with and without added urea are shown in Figure 5. The fluorescence emission spectra of 10.3S and 6-day GM protein showed a maximum at 320 nm. The emission maxima of tryptophan, tyrosine, and phenylalanine are 348, 303, and 282 nm, respectively. Thus the observed maximum at 320 nm for the two proteins may have contributions both from tryptophan and tyrosine.

The relative fluorescence intensity of 6-day GM protein showed a significant reduction from 54 to 42. This decrease in general may be attributed to (a) change in the polarity and microenvironment around the aromatic amino acids and (b) conformational change and difference in the number of aromatic amino acids. However, there is no

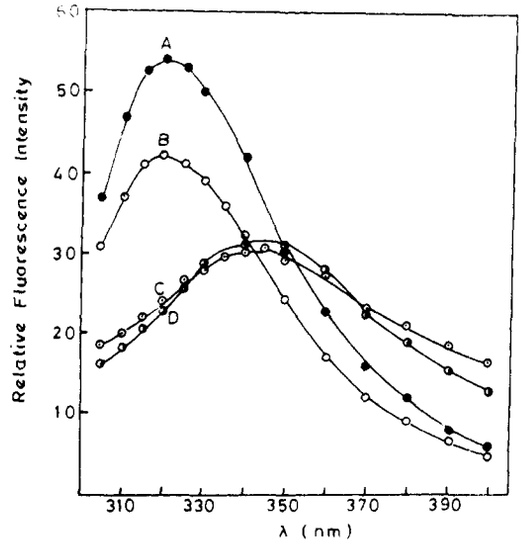

Figure 5. Fluorescence spectra of 10.3S (A, C) and 6-day GM protein (B, D) of chickpea: (A and B) without urea, (C and D) with 8 M urea.

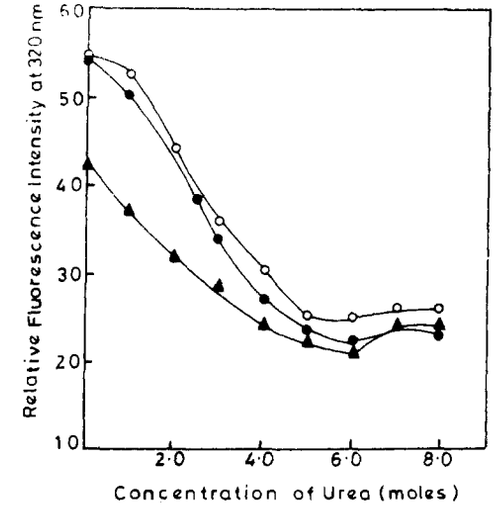

Figure 6. Relative fluorescence intensity of 10.3S (-○-), 3-day (-●-), and 6-day (-▲-) GM chickpea proteins as a function of urea concentration.

significant difference in the number of aromatic amino acids of 10.3S (~132 residues/mol of protein) and 6-day GM protein (~136 residues/mol of protein) (Ganesh Kumar, unpublished).

Fluorescence intensity of 10.3S, 3-day and 6-day GM proteins was also measured at 320 nm as a function of urea concentration in the range of 1 to 8 M. The plots of relative intensity at 320 nm against urea concentration for the three proteins are shown in Figure 6. In all the three fractions, the relative intensity decreased with increasing urea concentrations. However, this is not due to quenching of fluorescence by urea since there was a linear increase of tryptophan fluorescence with urea concentration in the model compound *N*-acetyltryptophanamide (Chen et al., 1969). The urea concentration corresponding to the midpoints in fluorescence intensity as a function of urea concentration plots (Figure 6) was 2.5 M (10.3S), 2.0 M (3-day GM), and 1.8 M (6-day GM), respectively. Thus, the relative stability of the three proteins in the decreasing order was 10.3S, 3-day GM, and 6-day GM.

Rate of Hydrolysis with α-Chymotrypsin. The 10.3S and 6-day GM proteins were subjected to in vitro hydrolysis with α-chymotrypsin to determine if the protein

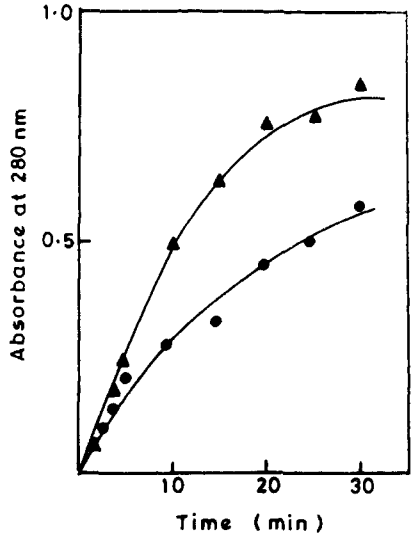

Figure 7. Proteolysis of chickpea proteins with α-chymotrypsin: (-●-) 10.3S protein, (-▲-) 6-day GM protein.

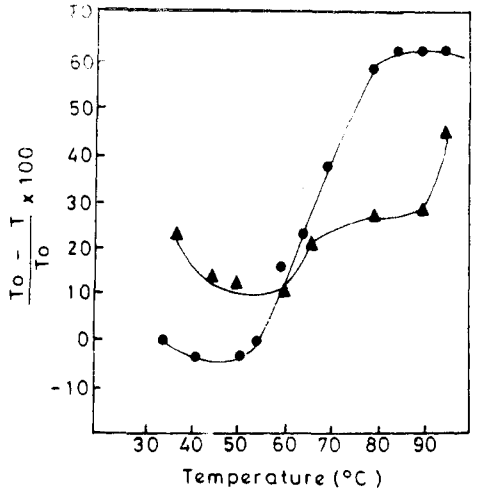

Figure 8. Heat coagulation pattern of 10.3S (-●-) and 6-day GM (-▲-) chickpea proteins in 0.025 M Tris-glycine buffer of pH 8.3.

degradation observed during germination involved any specific associative conformational changes. In this experiment, the rate of hydrolysis was followed for a period of 60 min, and the rate curves are shown in Figure 7.

The 10.3S and 6-day GM proteins were hydrolyzed at different rates. The rate of hydrolysis of GM fraction was high. The rates calculated from the linear portion of the curve showed that GM fraction is hydrolyzed nearly 1.6 times faster than 10.3S protein. The difference in rates of hydrolysis could be attributed to differences in the relative compactness of the protein molecule and/or difference in the number of accessible peptide bonds of the two proteins. Since α-chymotrypsin largely cleaves the peptide bonds adjacent to the aromatic amino acids, it is highly possible that during germination structural alteration of 10.3S protein may occur in the vicinity of these amino acids.

Heat Coagulation Studies. When 10.3S protein was heated it showed a regular increase in the turbidity of the solution up to 85 °C and a leveling off at higher temperature was observed (Figure 8). From the S-shaped curve, the transition point was determined to be 67 °C.

The 6-day GM protein showed a different pattern. It showed a two-stage transition curve. The first transition occurred at 62.5 °C, and a plateau region was observed between 65 and 90 °C. Above 90 °C, a linear rise in turbidity was noted. These observations suggested that the 6-day GM protein had a less stable structure.

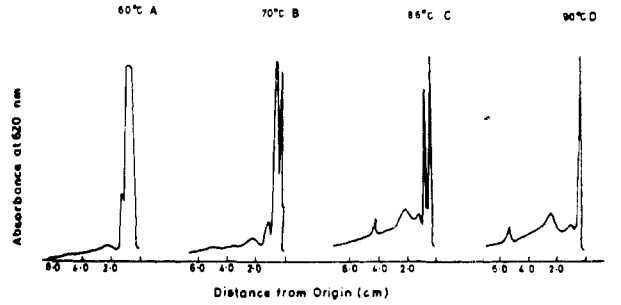

Figure 9. Microdensitometric tracings of PAG electrophoretic pattern of 10.3S protein subjected to different temperatures.

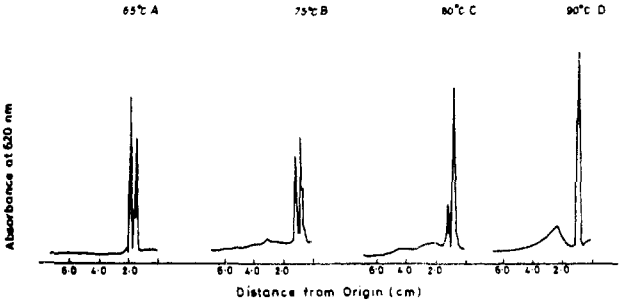

Figure 10. Microdensitometric tracings of PAG electrophoretic pattern of 6-day GM protein subjected to different temperatures.

To determine whether different dissociated and aggregated states of GM protein caused this difference, the protein samples, subjected to heat treatment at various temperatures and immediately cooled were tested on PAG electrophoresis (Figures 9 and 10). With increasing temperature (from 50 to 90 °C), dissociation of 10.3S protein occurred. This was measured from the quantitative decrease of the slow-moving components and the concomitant increase in the fast-moving dissociation products.

However, in 6-day GM protein there was no significant dissociation up to 75 °C. From the R_f of the protein bands it could be inferred that up to 75 °C aggregation occurred. In other words, in 6-day GM protein heat favors larger aggregation from the already existing dissociated fragments at a relatively low temperature, viz., 63 °C. Thus heat favored aggregates of 6-day GM protein probably undergo later dissociation at high temperatures. Hence the observed difference in the mode of heat coagulation of 10.3S and 6-day GM protein can be attributed largely to the relatively stable and less stable quaternary structure of 10.3S and GM protein, respectively.

Subunits of 10.3S and 6-Day GM Protein. The Na-DodSO_4-PAG electrophoresis showed the presence of five subunits in 10.3S with molecular weights of 98 000, 52 000, 35 000, 32 000, and 23 000. By comparison with the subunit structures of 11S type proteins present in different species of legumes (Derbyshire et al., 1976), it is possible to conclude that the 10.3S protein may be composed only of the 23 000 and 32 000–35 000 type of subunits in multiple forms. Further, the 98 000 and 52 000 bands may not represent true subunits, but rather undissociated combinations of the other subunits. The 6-day GM protein was found to have at least three prominent subunits with molecular weights of 36 000, 32 000, and 23 000, respectively. Thus during a 6-day germination period, no significant alteration in the subunit structure of 10.3S occurred. However, it is interesting to note that 6-day GM protein contained three free -SH groups, whereas the 10.3S protein had only one, out of the total seven -SH groups.

This observation suggests the possibility of the participation of the -SH groups in the association of the subunits in 10.3S protein.

DISCUSSION

The 10.3S protein constitutes the major fraction of chickpea storage proteins. It is found to be present in the isolated protein body of chickpea, along with 6.9S protein (Ganesh Kumar, 1978). This major protein fraction is rather poorly utilized during the initial stages of germination (Ganesh Kumar and Venkataraman, 1978). However, after a 6-day germination period, it is found to degrade, resulting in the formation of 9.3S protein.

The resistance of 10.3S protein to in vivo enzymic degradation may be due to certain structural features like ordered structure, poor accessibility of the specific peptidyl bonds to proteolytic enzymes (Goldberg and St. John, 1976) or the presence of enzyme inhibitors (Richardson, 1977). In the presence of more than one storage protein, the cellular degradation may be determined by the size and charge state of the protein present (Dice et al., 1973).

It has been suggested that the rate-limiting step in degradation of most cellular proteins is spontaneous denaturation (Bellard et al., 1974). The actual free energy difference between the native conformation of most globular proteins and various unfolded states is found to be small (Pace, 1975). Thus it is possible that denaturation may be a relatively frequent event under in vivo conditions. It has been reported that urea accumulated in the germinating soybean seeds to a 1.5 M concentration on a dry weight basis (Tao and Komatsu, 1931). On the basis of this, Catsimpoolas et al. (1968b) suggested a possible initiation of protein degradation by the accumulated urea in the cotyledons.

In the in vitro conditions, 10.3S protein did not show any dissociation at 2.0 M urea concentration at pH 8.3, whereas extensive dissociation was noticed at 2.0 M urea at pH 4.0 (Ganesh Kumar, 1978). It is interesting to recall the similarity in the CD curves of 4 M urea treated 10.3S protein and the untreated 6-day GM protein (Figure 4), indicating a similar dissociative pattern in 10.3S storage protein during germination.

The $NaDodSO_4$–PAG electrophoresis pattern of 6-day GM protein requires some comment. When it has been examined for the number and molecular weight of subunits and the sedimentation coefficient value, 6-day GM protein found to have a sedimentation coefficient value of 9.3S and at least three subunits with molecular weights ranging from 23 000 to 40 000. Such a low subunit molecular weight figure suggests a subunit composition of $A_xB_yC_z$, where A, B, and C are the subunits of molecular weights 36 000, 32 000, and 23 000, respectively, and x, y, and z are integers. From the dye-binding ratio of the three bands, the relative proportion of the three subunits of 6-day GM was found to be 5 (23 000), 2 (32 000), 1 (36 000). On the basis of this, the molecular weight was found to be 215 000, which agreed fairly with the value (~213 000) obtained from sedimentation coefficient and viscosity of GM proteins.

It is interesting to see the various structural alterations brought about in 10.3S during seed germination. Prior to the extensive protein degradation, the higher ordered structures of storage proteins have to be destroyed or altered. The higher ordered structure of proteins arises out of the folding of the protein through hydrogen bonds, hydrophobic interaction, and disulfide bonds. In 6-day GM protein, appearance of two more additional free -SH groups out of the total seven -SH groups of 10.3S was noticed in which one was in the free form. This could mean a possible scission of one S–S bond. Moreover, the ordered structure estimated in terms of structure was found to be less in the GM proteins compared to 10.3S protein. The H-bonded structure was also found to be changed as indicated by the increased susceptibility to urea dissociation, as evidenced both by CD and fluorescence spectral measurements.

The degradation of 10.3S during germination caused perturbation of the microenvironment of the aromatic amino acids of the polypeptide chain. This is inferred from the observation of increase in susceptibility of GM protein to α-chymotryptic digestion. Differential reactivity of side chains such as tyrosine and tryptophan have also been observed in some proteins (Klotz et al., 1970) and these too reflect conformational adaptation to the specific need of the ensuing proteolytic enzyme machinery of the germinating chickpea seed.

The degradative utilization of 10.3S during germination could occur in two possible ways, viz., randon chopping of the macromolecules largely to simpler peptides and then amino acids or selective ordered cleavage via the formation of few defined intermediates, could occur in germinating seeds. The random cleavage may be accomplished by the simultaneous operation of many proteolytic enzymes. But many reports on the proteolytic enzymes of germinating seeds (Ryan, 1973) suggest largely the occurrence of maximum activity of a specific protease coinciding with the period of maximum degradation of the storage proteins (Ryan, 1973). This observation precludes the first possibility, favoring the second one. Perhaps a detailed study on the proteases involved at different degradation stages of the storage proteins may give additional information on the finer aspects of structure–function relationship involved in reserve protein mobilisation of germinating chickpea seed. However it is evident that structural rearrangements at the quaternary level, as well as within constituent subunits, offer a very general molecular mechanism for the coupling of metabolic transformations with the synthetic processes in the germinating seed.

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Chickpea Seed Proteins: Isolation and Characterization of 10.3S Protein

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The chickpea proteins were fractionated by gel filtration and DEAE-cellulose chromatography. The major protein fraction, with a sedimentation coefficient value of 10.3S, has been isolated by water dilution of the protein extracted by 10% (w/v) NaCl solution, followed by DEAE-cellulose chromatography. The protein preparation was found to be homogeneous by ultracentrifugation and polyacrylamide gel electrophoresis. The chemical composition, subunits, molecular weights of the protein, and subunit and spectral characteristics were determined. The storage protein role of 10.3S has been confirmed by its isolation in the protein bodies of chickpea.

Chickpea (*Cicer arietinum*), also known as Bengal gram, is a major food legume crop of India. It contains 17–21% protein on dry weight basis (FAO/UN, 1973). The overall composition, nutritional properties (Krishnamurthy, 1975; Jaya, 1978), and a few fractionation studies of the chickpea proteins (Radionova, 1957; Esh and De, 1960; Grigorcha and Klimenko, 1970; Alekseeva and Grigorcha, 1973) have been reported. The major water insoluble globulins of chickpea were reported to be complex with four or six fractions (Leonov, 1968). The trypsin inhibitors constituting less than 1.0% of the total protein have been well characterized (Belew et al., 1975; Belew and Eaker, 1976).

No detailed study of the various protein fractions of chickpea in terms of physicochemical characteristics appears to have been made. While working on the germination modification of storage proteins of legumes (Ganesh Kumar and Venkataraman, 1975, 1978), we isolated one of the major storage proteins of chickpea, employing dilution of the protein extracted with 10% (w/v) NaCl solution. This protein fraction differed from the major chickpea seed globulin, isolated by a different method of Alekseeva and Grigorcha (1973), in its sedimentation behavior.

In this investigation, an attempt has been made to study the fractionation characteristics of the total proteins of chickpea. Further, some of the physicochemical properties of the major storage protein, isolated by water dilution method, are presented.

EXPERIMENTAL SECTION

Materials. Seeds of chickpea (*Cicer arietinum*) were obtained locally. They were decorticated, powdered to 100-mesh size, and defatted with hexane. The defatted flour was used for protein extraction. The chemicals and reagents used in this study were of reagent grade.

Methods. *Extraction of Protein for Solubility Studies.* The defatted powdered flour was extracted with water adjusted to different pH's with either concentrated HCl or 10% NaOH for 1 h at 4 °C with continuous stirring. The slurry was centrifuged at 5000 rpm for 30 min. The N content of the clear supernatant was determined by the micro-Kjeldhal method.

Extraction of Protein for Fractionation Studies. The defatted flour was extracted with 0.01 M borate buffer of pH 7.8 for 2 h at 4 °C. The extract was centrifuged and

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