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Association-Dissociation and Denaturation Behaviour of Conglutin α from Lupin Seed in Urea, Guanidine Hydrochloride and Sodium Dodecyl Sulfate Solutions

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루핀콩으로 부터 분리한 Conglutin α의 Urea, Guanidine Hydrochloride, Sodium Dodecyl Sulfate 용액에서의 회합-해리, 변성

김 찬 식

Summary

The major protein fraction of lupin seed, 'Conglutin α' is isolated to homogenity and characterized for various properties and the effect of sodium dodecyl sulphate (SDS), urea and guanidine hydrochloride (GdnHCl) on it is investigated. The protein sediments with an $S_{20.w}$ value of 11.0 and mostly consists of β and aperiodic regions in its secondary structure. The amino acids composition of the protein indicates it to be rich in acidic and hydrophobic amino acids. The protein in presence of SDS, urea and GdnHCl undergoes dissociation and denaturation. The schematics of dissociation appears to be different in SDS as compared to urea and GdnHCl. The genereal patteren of dissociation appears to follow the schematics 11S→7S→2S except in SDS solution. GdnHCl appears to be more effective than urea in bringing about dissociation and denaturation of the protein. The process of dissociation appears to basically arise from disruption of hydrophobic interactions of relevant subunits, which ultimately leads to a denatured molecule.

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Introduction

Lupin seed is one of the legumes with the highest content, which various from 30 to more than 40% on a dry matter basis (Hove, 1974). The total proteins from lupin seed is isolated and its properties studied (Joubert, 1955). The major protein of lupinseed called the 11S protein or 'Conglutin α' in the present study constitutes nearly 60 to 65% of the total proteins. Several attempts are there to obtain the protein in a homogeneous form (Blagrove and Gillespie, 1975; 1978). In this investigation in order to study the interaction of denaturants like urea, GdnHCl and SDS, the protein is isolated in a homogeneous form and its properties and subunit composition determined.

These data on the denaturation of conglutin α by various denaturants is primarily aimed at understanding the basic forces maintaining the native structure of protein and the mechanism of denaturation. Urea and GdnHCl are classical protein denaturants and their use are extensive (Lapanje, 1978). The anionic detergent sodium dodecyl sulfate is used extensively in the study of oligomeric proteins as it is capable of binding to the proteins leading to dissociation and denaturation (Parakash and Nandi, 1976). In most of the studies GdnHCl is shown to be more effective than urea in denaturing proteins. The mechanism of dissociation and denaturation of proteins in presence of these reagents and the thermodynamic aspects of this reaction is the subject matter of several extensive studies of physiologically important proteins as well as multimeric seed proteins. In this study I have isolated conglutin α, the 11S protein fraction from lupin seeds (*Lupinus angustifolius, L.*) and investigated the association-dissociation and denaturation behaviour in various denaturants in order to understand the forces stabilizing the native structure of the protein.

Materials and Methods

Materials

Lupin seeds (*Lupinus angustifolious, L*) were obtained from the Grain Pool of West Australia. Urea, GdnHCl, SDS were from Sigma Chemical Company, USA. Sepadex G-200 was from Pharmacia Fine Chemicals, Uppsala, Sweden. Coomassie Brilliant Blue R-250, amido black, bovine serum albumin, egg albumin, pepsin, β-lactoglobulin, ribonuclease, N, N'-methylene bis acrylamide were obtained from Sigma Chemical Company, USA. Acrylamide and N, N, N', N'-tetra methyl ethylene diamine were from Koch-Light

Laboratories, UK and β-mercaptoethanol was from Fluka, Switzerland. All other chemicals used were of analytical reagent grade.

Method

Preparation of lupin seed flour :

Dehulled lupin seeds were defatted by repeated extraction for six times with n-hexane. The defatted meal was dried and ground to a powder using a plate mill and passed through a 60 mesh.

Isolation and purification of conglutin α

Twenty grams of defatted lupin seed flour was extracted with 1000㎖ of water for 12 hr at 4℃, and the extract was discarded by centrifugation at 4500×g for 10min. The supernatant was set aside and the residue was again extracted twice with 500㎖ water for 6hr at 4℃ each and the water solubles were discarded. The residue was finally extracted with 200㎖ of 10 % NaCl containing 0.02% NaN_3 for 2hr and centrifuged at 4500×g for 20min. The supernatant was precipitated by adding ammonium sulphate to 85% saturation. The precipitate was dissolved in 50㎖ of 0.15M phosphate buffer, pH 7.0 and dialysed against 0.2M acetate buffer, pH 4.8 for 24 hr at ambient temperature. The proteins in solution were separated by centrifugation and the precipitate thus obtained was designated as crude 11S protein. This precipitate was dissolved in 0.15M phosphate buffer, pH 7.0 dialyzed against the same buffer and was further purified by gel filteration chromatography on Sephadex G-200.

Gel filtration :

Sephadex G-200 packed in a column(2.4 ×120㎝) was equilibrated with 0.15M phosphate buffer, pH 7.0 containing 0.02% NaN_3. The crude 11S protein was loaded on the column, and eluted with phosphate buffer. Fractions of 4㎖ were collected in an automatic fraction collector and the absorbance of the fractions were measured at 280nm. Fractions corresponding to the major peak were pooled and used for further measurements in 0.15M phosphate buffer, pH 7.0 and buffer salts removed by dialysis and lyophilized and stored.

Protein concentration :

This was determined spectrophotometrically. Initially the protein concentration was determined by nitrogen estimation by Kjeldahl method(AOAC, 1984) using a factor of 6.25 to convert nitrogen to protein. The stock solution was diluted serially and the absorbance at 280nm (wavelength of maximum absorption) was determined. The $E_{1cm}^{1\%}$ was detemined from a plot of absorbance vs protein concentration. The vlaue for the

11S protein was 8.2.

Determination of subunits and their molecular weights (SDS-PAGE) :

The determination of the number of subunits and their molecular weight was carried out by the method of Laemmli (1970) using bovine serum albumin, egg albumin, pepsin, β-lactoglobulin, and ribonuclease 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 weight of the subunits of the proteins were determined. The gels were scanned in a Joyce Loebl chromoscan 200.

Analytical ultracentrifugation :

Sedimentation velocity experiments were carried out at 25℃ in a Spinco Model E Analytical Ultracentrifuge equipped with a phase plate schlieren optics and a rotor temperature indicator and control (RTIC) unit using a standard 12㎜ Kel F cell centerpiece and 1% protein solution at a speed of 59780 rpm. The plates were read on a Gaertner microcomparator hooked to a digital display unit for x and y axis reading and $S_{20.w}$ values calculated by the standard procedure (Schachman, 1959). Enlarged tracings of the analytical ultracentrifuge patterns were obtained from which the percent fraction of the various components determined.

Circular Dichroism (CD) :

CD measurements were made at room temperature (28℃) in a JASCO J 20C automatic recording spectropolarimeter. The instrument was calibrated with D -10 -camphor-sulphonic acid and the slits were programmed to yield 10 A° band width at each wavelength. Near UV-CD measurements were made using cells of 1 ㎝ path length and protein (1.5 to 2.0㎎/㎖) in phosphate buffer in the range, 250 to 330nm. Far UV-CD measurements were made using cell of 1.0㎜ path length and protein (0.2 to 0.5㎎/㎖) in phosphate buffer in the wavelength range, 200 to 250nm. Mean residue ellipticity θ_{MRW} (deg. ㎠/d.mol) were calculated assuming a value of 110.

Ultraviolet absorption spectrum :

The ultraviolet absorption spectrum of the protein in 0.15M phosphate buffer of pH 7.0 solution was recorded in a Perkin Elmer double-beam recording spectrophotometer 124 in the range of 240-330nm.

Fluorescence emmision spectrum :

A Perkin-Elmer fluorescene spectrophotometer, Model 203, was used. The emmision spectrum was measured in the 300-400nm range after excitation at

280nm. Protein in 0.15M phosphate buffer of pH 7.0 and with an absorbance of 0.08 at 280nm was used in all the measurements.

Amino acid analysis :

The amino acid analysis was carried out in a LKB-amino acid analyzer equipped with a programmer and integrator following the standard procedure of hydrolyzing the protein in 6N HCl. Since tryptophan is destroyed during acid hydrolysis, it was determined by the method of Spande and Witkop (1967), using N-bromosuccinomide.

Carbohydrate and phosphorous estimatiation :

The carbohydrate and phosphorous contents of the protein was estimated by the methods of Dubois et al. (1956) and Taussky and Shorr (1953) respectively.

Results

The 11S protein isolated by the above procedure was tested for homogeneity using several techniques. The protein was found to be homogenous since it eluted as a single symmetrical peak in gel filtration at 210㎖ (Fig.1). Analytical ultracentrifuge studies with different concentrations of protein confirmed the homogeneity of the

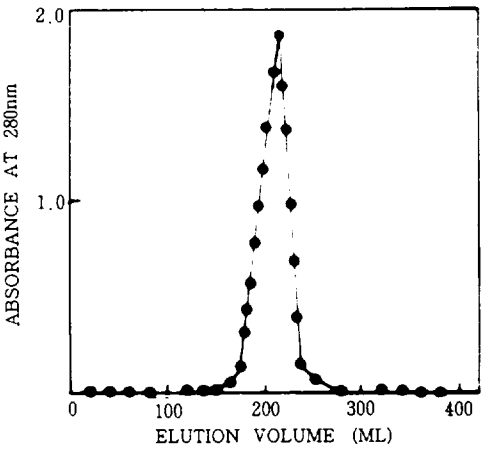

Fig. 1 Gel filteration pattern on conglutin α on Sepadex G-200.

sample (Fig.2). The protein was homogenous to 95% and was used for further work. Proper corrections were incorporated for the presence of 7S component and polymer in calculating the area under the curve for the sedimentation analysis. This is due to the fact that al-

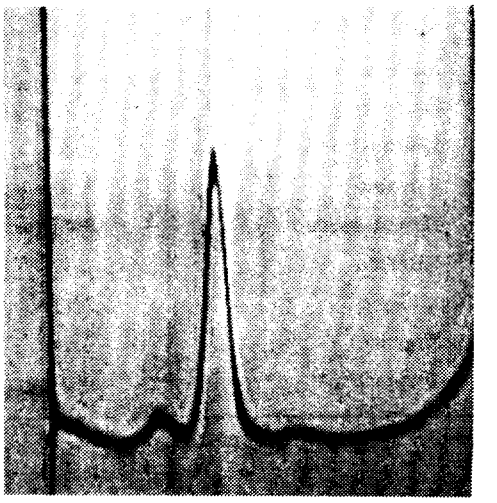

Fig. 2 Sedimentation velocity pattern of conglutin α at 20 mins after attaining maximum speed.

though the isolated 11S is homogeneous during freeze drying and storage it can rapidly associate-dissociate similar to other seed proteins.

The amino acid composition of the protein is shown in Table 1. The composition indicates that the protein is rich mainly in acidic amino acids, aspartic and glutamic acids. Also the protein appears to contain higher percentages of hydrophobic amino acids like tyrosine, leucine, phenylalanine and valine. Lysine and the sulfur amino acid content appears to be low. The amino acids profiles of other major protein fractions from several seed proteins like arachin, α-globulin, holianthin, etc. are very similar in comparison with conglutin α. The protein has an ultraviolet absorption maximum at 280nm indicating the presence of aromatic chromophores (Table 1 and Fig.3). The fluorescence emmission spectrum of conglutin α in the region 300-350nm is shown in Fig.3. The emission maximum at 324nm indicates that the fluorescence is dominated by tryptophan group; which are embedded in a nonpolar environment. The protein contains nearly 0.9% tryptophan (Table 1). The protein also contains 0.5% carbohydrate and 0.1% phosphorus.

Table 1. Amino acid composition of conglutin α from lupin seeds (mol %)

Amino acid	Conglutin α
Aspartic acid	12.90
Threonine	3.68
Serine	6.23
Glutamic acid	26.78
Proline	5.10
Glycine	8.00
Alanine	5.40
Cystine	0.09
Valine	2.86
Methionine	0.02
Isoleucine	3.21
Leucine	7.36
Tyrosine	3.02
Phenylalanine	3.09
Histidine	1.92
Lysine	2.73
Arginine	7.64
Tryptophan*	0.90

* by the spectrophotometric method

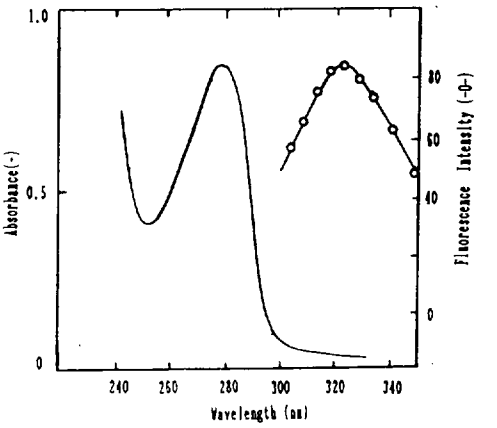

Fig. 3 Ultraviolet absorption spectrum and fluorescence emission spectrum of conglutin α.

The secondary structure of the protein is monitored by both near and far ultraviolet CD spectrum from 320 to 200nm (Fig. 8

and 9). The near UV-CD spectrum is characterized by a major peak at 285nm and 290nm with shoulder at 280nm a broad negative peak at 305nm (Fig.8). The above CD spectrum in characteristic of protein rich in tryptophan and tyrosine residues. Conglutin α contains nearly 0.9% tryptophan and 3% tyrosine (Table 1). Strickland (1974) indicates that the bands around 305nm and 290nm arise from an asymmetric environment around tryptophan moieties. However, the peak at 285nm originates from tryptophan residues alone. However, other contributions like dihedral angle of disulfide and vicinal interactions may also contribute to the various peak positions in the near UV-CD spectrum.

Fig.9 shows the far ultraviolet CD spectrum of conglutin α in the above mentioned buffer in the region 250-300nm. The structure is characterized by a major negative band around 205nm and a shoulder at 222nm. The spectrum appears to be dominated by aperiodic and β-pleated structures. However, an evaluation of α-helix content of the protein based on Greenfield and Fasman(1969) procedure indicates that at 5.0% α-helix is present in the protein. This low percentage of α-helical regions in the conformation of conglutin α is similar to the secondary structure profiles of other major seed proteins.

The subunit composition of the protein was analyzed in presence of SDS by polyacrylamide gel electrophoresis (PAGE) in presence of a disulfide reducing agent and indicates at least 6 non-identical subunits (Fig.4).

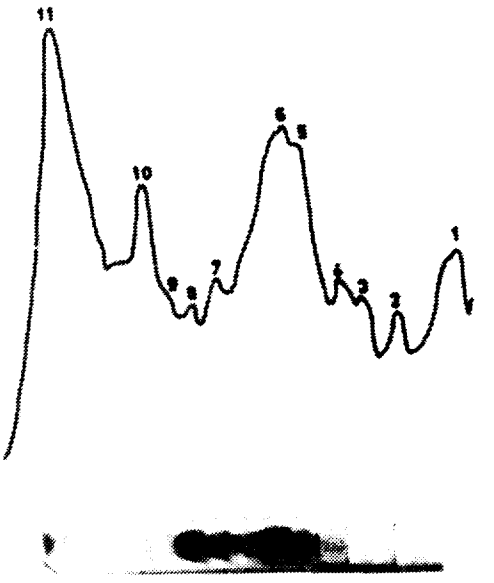

Fig. 4 Scan of SDS-polyacrylamide gel electrophoretic pattern of conglutin α.

The effect of SDS, urea and GdnHCl on the 11S protein (conglutin α) has been monitored mainly using the techniques of analytical ultracentrifuge, fluorescence and near and far UV-CD spectroscopy.

Effect of SDS :

Fig.5 shows the sedimentation velocity pattern of conglutin α in representative concentration of SDS at 0.35mM and 18mM. The 11S component apparently is getting dissociated to the 2S component.

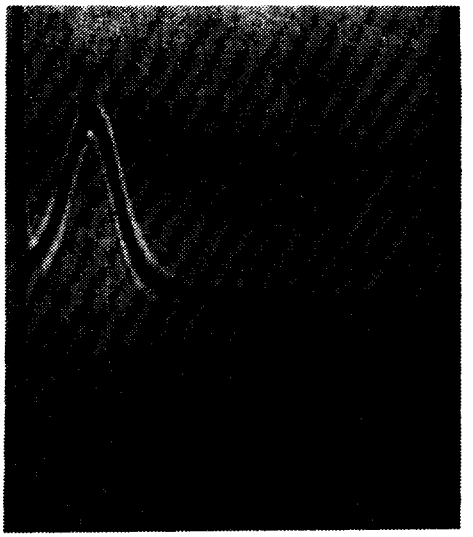

Fig. 5 Sedimentation velocity pattern of conglutin α (Top) at 18 mM SDS, 40 min. and (Bottom) at 0.35 mM SDS, 40 min. The time after each photograph indicates the time lapse after attaining maximum speed.

However, very small amount of other fractions were detectable but their concentration were low to form a schlieren peak. The data obtained from the area measurements were plotted as percent fraction vs. SDS concentration is shown in Fig.6. The results indicate that the 11S protein progressively dissociates to be 2S component. Around 1×10^{-2}M SDS, most of the protein is dissociated to the 2S component.

The plot of relative fluorescence intensity versus SDS concentration (Fig.7) indicates that as the concentration of SDS is increased for e.g. 0.7mM, 1.8mM and higher two, things are observed (i) the

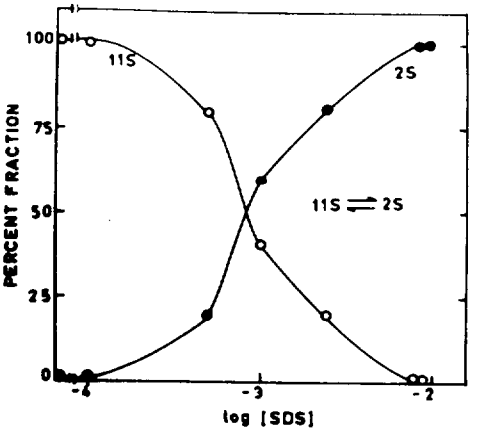

Fig. 6 Percent fraction of various fraction from sedimentation velocity experiments vs. SDS concentration.

fluorescence initially decreases and (ii) concomittantly the emmission maxima shifts towards red region (Fig.7). Above

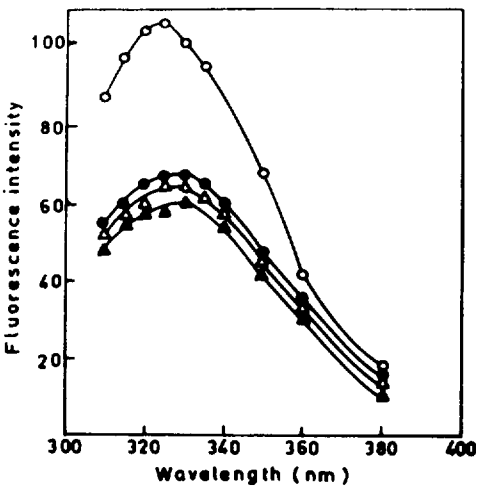

Fig. 7 Effect of SDS concentraftion on the fluorescence emission spectrum of conglutin α.
—○—Conglutin α
—●—Conglutin α with 0.7 mM SDS
—△—Conglutin α with 1.8 mM SDS
—▲—Conglutin α with 3.5 mM 9.0 mM and 18 mM SDs

3.5mM SDS no change in either of the above parameters is observed and reaches a plateau region. The decrease in fluorescence intensity in the detergent solution may arise from the binding of the detergent molecules at site(s) near the tryptophan moities which will increase the electronegativity in the microenvironment of the flurophor and quench the fluorescence. The shift in the emission maximum indicates that above 3.5mM SDS concentration, the protein does not change in its conformation.

The near UV-CD spectrum of conglutin α is shown in Fig 8, compared to the control spectra, upon increasing the concentration of SDS, initially the spectrum is characterized by a decrease in the amplitude with the fine structures remaining as in control at 0.35mM SDS (Fig.8). With further increase in concentration of SDS, at 0.7mM SDS along with the decrease in amplitude there is also observed disappearance of fine structures especially peak at 292nm. This indicates that the tryptophan and tyrosine chromophores are significantly perturbed at this concentration of SDS. At higher concentrations that these all the fine structure is absent and no rotations are observed positively indicating the total loss of tertiary structure.

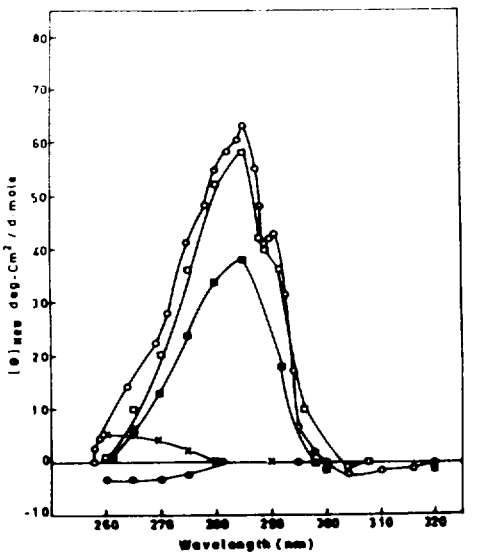

Fig. 8 Effect of SDS concentration on the near UV-CD spectrum of conglutin α.
- —○— Conglutin α
- —□— Conglutin α with 0.35 mM SDS
- —■— Conglutin α with 0.7 mM SDS
- —×— Conglutin α with 1.8 mM SDS
- —●— Conglutin α with 18 mM SDS

The far UV-CD spectra of conglutin α is shown in Fig.9 as a function of SDS concentration. At 0.7mM SDS there is dramatic decrease in the rotation as compared to the control. However, at higher concentration of SDS at 9.0mM and 18.0mM the rotations increase with more ordered structure on the system but still the extent of rotations are nearly half compared to the native structure. This is not very unusual in the sense other seed proteins like α-globulin, glycinin, etc. and other proteins also exhibit similar results of more ordered structures at higher concentrations of SDS. However in conglutin α even at 18mM SDS

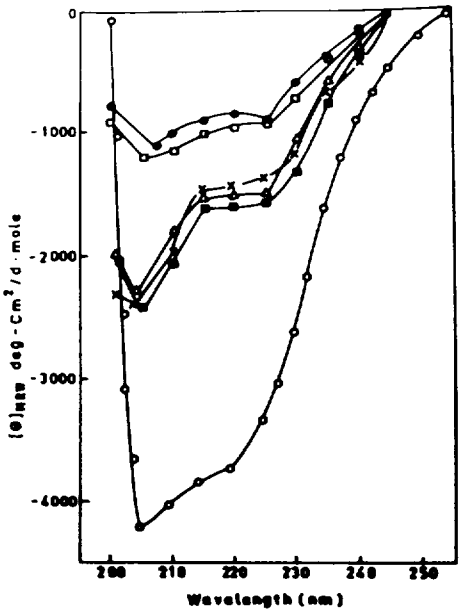

Fig. 9 Effect of SDS concentration on the far UV-CD spectrum of conglutin α.
—○—Conglutin α
—●—Conglutin α with 0.35 mM SDS
—□—Conglutin α with 0.7 mM SDS
—△—Conglutin α with 1.8 mM SDS
—■—Conglutin α with 9.0 mM SDS
—×—Conglutin α with 18.0 mM SDS

concentration the rotations are less as compared to the native protein.

Effect of urea :

Analytical ultracentrifuge measurements were carried out upto 8.0M urea concentration (Fig.10). Depending upon the concentration of urea three components sedimenting with 2.7 and 11S are observed (Fig.10). In Fig.10 is shown representative patterns of the sedimentation velocity runs. In Fig.11 is shown the percent fraction vs. urea

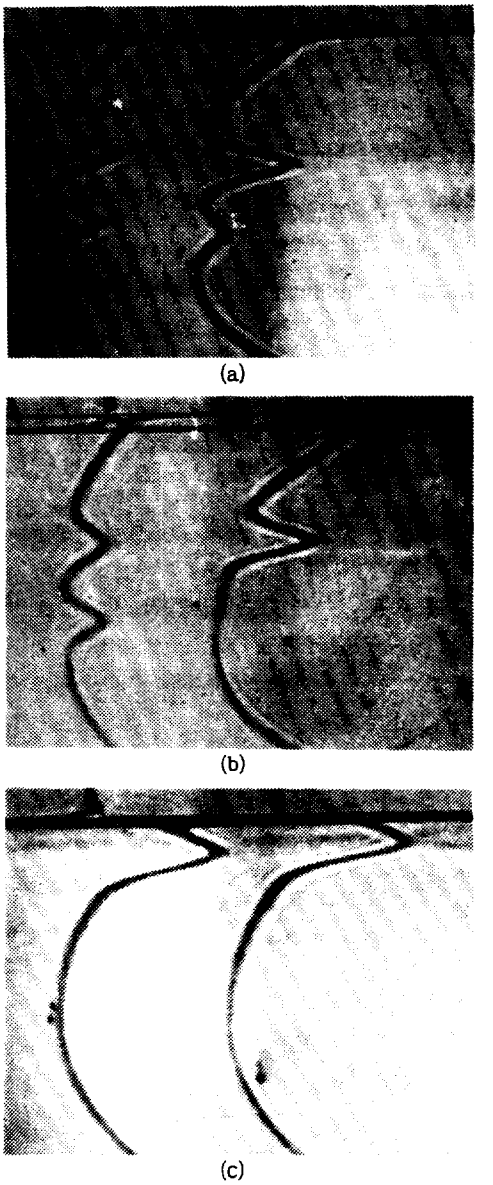

Fig. 10 Effect of urea and GdnHCl concentrations on the velocity sedimentation pattern of conglutin α.
(a) Top : Conglutin α with 1M GdnHCl
Bottom : Conglutin α with 2M urea
(b) Top : Conglutin α with 2M GdnHCl
Bottom : Conglutin α with 3.5M urea
(c) Top : Conglutin α with 6M GdnHCl
Bottom : Conglutin α with 8M urea

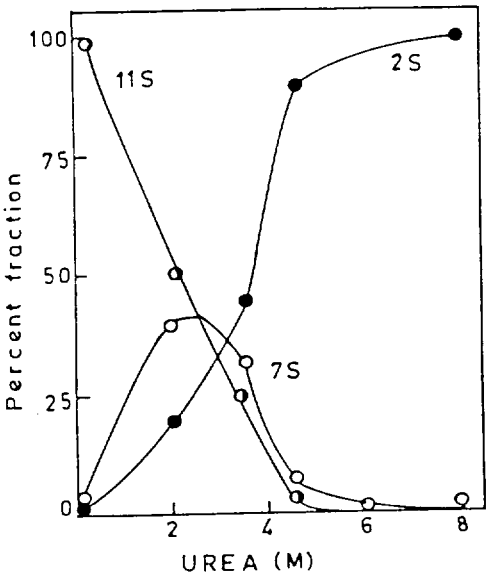

Fig. 11 Percent fraction of various fraction from sedimentation velocity experiments vs. urea concentration.

concentration. The concentration of 7S fraction increases at the cost of 11S component below 2.5M urea. At the same time there is generation of the 2S component also. Above 2.5M urea concentration the 7S component decreases along with the 11S component with a concomittant increase in 2S fraction. Above 6M urea the entire pattern is represented by 2S fraction. These results indicate that the 11S molecule progressively dissociates to the 7S and 2S molecules either sequentially or simultaneously.

Flourescence emission spectra of the protein as a function of urea concentration indicates that with progressive increase in urea concentration there is decrease in amplitude as well as shift in emission maximum as shown in Fig.12. Initially at 2.0 and 2.5M urea there is observed only a decrease in the fluorescence intensity and no shift in emission maximum (325nm) indicating that upto 2.5M no major conformational change is there and only perturbation of the tryptophan groups as the fluorescence spectra is dominated by tryptophan fluorescence emission. At 3.0M and 4.0M urea the emission maximum shifts to 340nm indicating conformational change and also that the tryptophanyl fluorophor experience increasing polar environment with increase in urea concentration. At 6.0M and 8.0M urea the emission maximum is around 343nm with

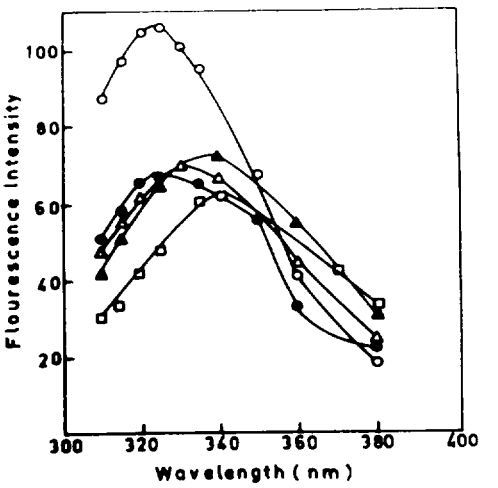

Fig. 12 Effect of urea concentration on the fluorescence emission spectum of conglutin α.

—○—Conglutin α
—●—Conglutin α with 2.0 and 2.5M urea
—△—Conglutin α with 3.0M urea
—▲—Conglutin α with 4.0M urea
—□—Conglutin α with 6.0M and 8.0M urea

further decrease in amplitude. Tryptophan or its amide has its emission maximum around 345-350nm in aquous phase.

The near UV-CD spectra as a function of urea concentration are shown in Fig.13. With progressive increase in urea concentration the amplitude decreases and above 3M urea the fine structure is also absent, positively indicating major conformational change. However, the near UV spectra at 4M urea (Fig.13) has almost no fine structure and the spectrum at 6.0 and 8.0M urea have no rotations indicating a gross change in the tertiary structure of the protein similar to higher concentrations of SDS. These results confirm the fluorescence results as described above in presence of varying concentration of urea.

The far UV-CD spectra of conglutin α in presence of varying concentration of urea is shown in Fig.14. With increase in concentration of urea there is observed a decrease in rotation. Because of high refractive index at high concentraiton of urea measurents could not be made below 210nm. At 6 and 8M urea most of the

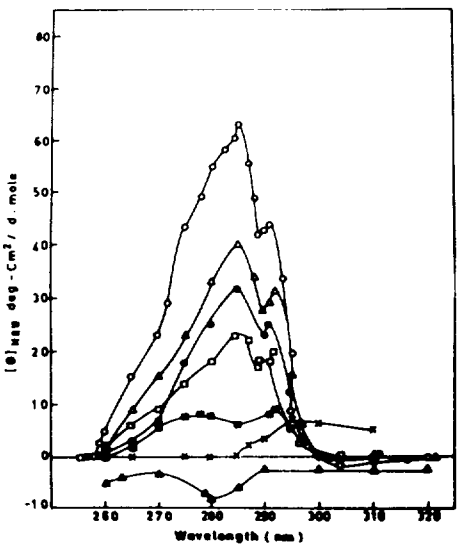

Fig. 13 Effect of urea concentration on the near UV-CD spectrum of conglutin α.
—○—Conglutin α
—△—Conglutin α with 2M urea
—●—Conglutin α with 2.5M urea
—□—Conglutin α with 3M urea
—■—Conglutin α with 4M urea
—×—Conglutin α with 6M urea
—▲—Conglutin α with 8M urea

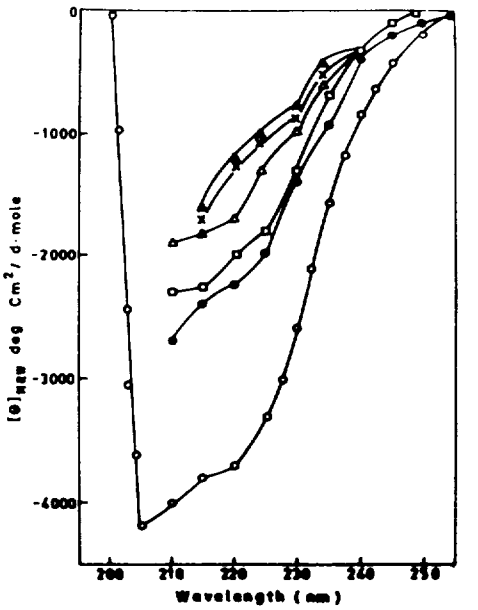

Fig. 14 Effect of urea concentration on the far UV-CD spectrum of conglutin α.
—○—Conglutin α
—●—Conglutin α with 2M urea
—□—Conglutin α with 2.5M urea
—△—Conglutin α with 4M urea
—×—Conglutin α with 6M urea
—▲—Conglutin α with 8M urea

secondary structure appears to be absent in the system with rotations to the order of -1200 deg cm/d-mole. These data were analysed by plotting $[\theta]_{MRW}$ vs. urea concentration at two specific wavelengths 225nm and 218nm and is shown in Fig.15. From the data it is apparent that the secondary structural changes reaches a plateau region around 6M urea. These results are in conformity with the fluorescence results and near UV-CD spectra.

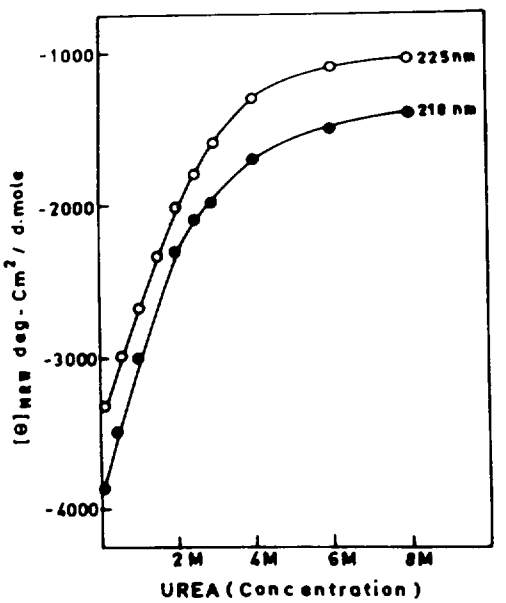

Fig. 15 Mean residue ellipticity values of conglutin α as a function of urea concentration at 225 nm and 218 nm.

Effect of GdnHCl :

The effect of increasing concentration of GdnHCl on the analytical ultracentrifuge pattern is shown in Fig.10. Depending upon GdnHCl concentration as in urea three components are observed only representative patterns of the sedimentation velocity runs. In Fig.16 is shown the percent fraction vs. GdnHCl concentration. The concentration of 7S fraction increase at the cost of 11S component with a concomittant increase in 2S component also. At 1.5M GdnHCl these is maximum of 7S component and the 11S component decreases rapidly. Avove 2M GdnHCl the pattern is represented mostly by 2S and very little of 7S fraction. At 6M GdnHCl the whole pattern is represented by 2S component as shown in Fig.10.

The fluorescence emission spectra of the protein as a function of GdnHCl

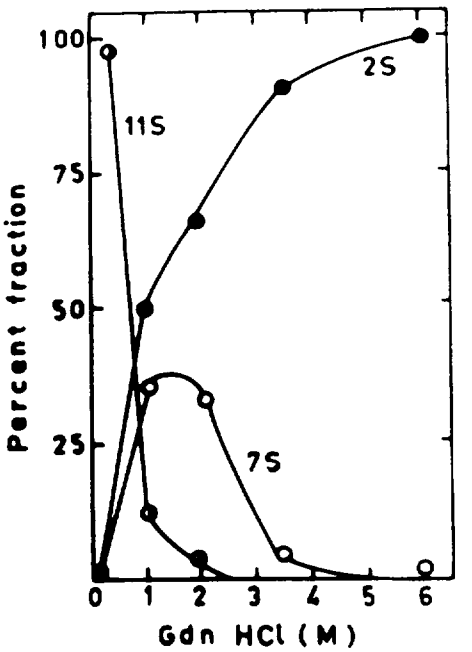

Fig. 16 Percent fraction of various fractions from sedimentation velocity experiments vs. GdnHCl concentration.

concentration is shown in Fig.17. Similar

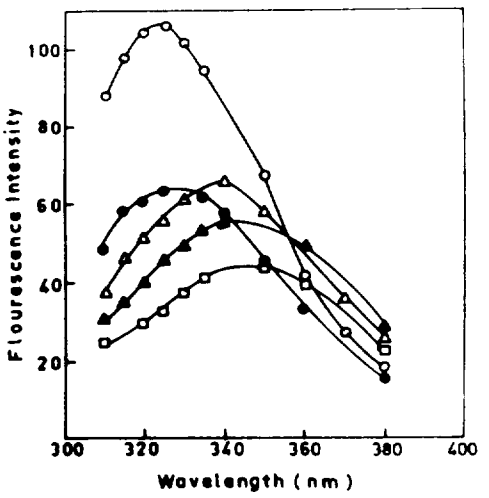

Fig. 17 Effect of GdnHCl concentration on the fluorescence emission spectrum of conglutin α.
—○—Conglutin α
—●—Conglutin α with 1.0M GdnHCl
—△—Conglutin α with 2.0M GdnHCl
—▲—Conglutin α with 3.0M GdnHCl
—□—Conglutin α with 4.0M and 6.0M GdnHCl

to urea in GdnHCl also there is obsereved decrease in amplitude at lower concentration (<2M) and above this concentration along with the decrease in amplitude, the fluoroscence emission maximum is also red shifted. At 4 and 6.0M GdnHCl the emission maxima is around 345nm indicating as in urea the tryptophanyl groups are experiencing more polar environment. However, the same effect is observed at much lesser concentration of GdnHCl as compared to in urea.

The near UV-CD spectra of the protein as a function of GdnHCl concentration is shown in Fig.18. The data indicates that at 1M GdnHCl the spectrum is very similar to that of control conglutin α except that the amplitude is decreased indicating that the chromophores are perturbed at this concentration of the denaturant. At higher concentration like 2M, the fine structure is lost at the same time the amplitude is decreased further indicating the initial loss of some tertiary structure in the protein supporting the fluorescence results indicated earlier. At higher concentrations than this, i.e. 3M and above essentially

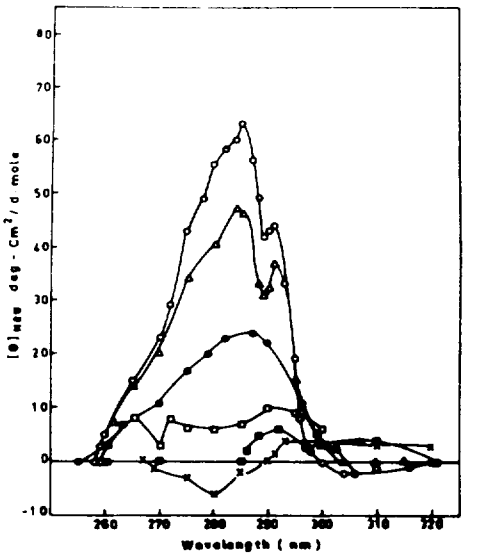

Fig. 18 Effect of GdnHCl concentration on the near UV-CD spectrum of conglutin α.
—○—Conglutin α
—△—Conglutin α with 1.0M GdnHCl
—●—Conglutin α with 2.0M GdnHCl
—□—Conglutin α with 3.0M GdnHCl
—■—Conglutin α with 4.0M GdnHCl
—×—Conglutin α with 6.0M GdnHCl

the spectra is close to baseline within experimental error indicating a gross change in tertiary structure. In fluoroscence measurement also the emission maximum is around 345nm (Fig.17) at this concentration indicating simillar results. As a comparsion the same effect is observed in 6M urea whereas here the effect is pronounced at nearly half the concentration of the denaturant (i.e. 3M GdnHCl).

The far UV-CD spectra of conglutin α as a function of increasing concentration of GdnHCl is shown in Fig.19. The results indicate that the specific rotation drastically decreases at 1M GdnHCl (Fig.19). Above this concentration there is observed further decrease in rotation and above 4M the protein is devoid of any fine structure. However, due to experimental limitations at high concentrations of GdnHCl, spectra could not be recorded beyond 215nm above 4M GdnHCl. In order to analyze the data a derivative plot was made from the above data at 218nm and 225nm and is shown in Fig.18. The data

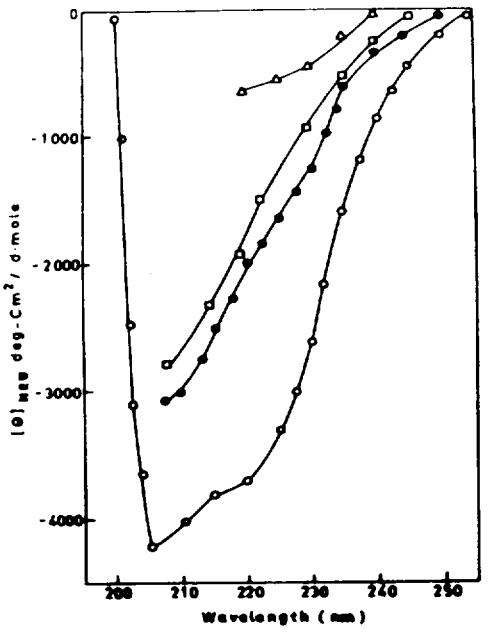

Fig. 19 Effect of GdnHCl concentration on the far UV-CD spectrum of conglutin α.
—○—Conglutin α
—●—Conglutin α with 1.0M GdnHCl
—□—Conglutin α with 2.0M GdnHCl
—△—Conglutin α with 4.0, 5.0 and 6.0M GdnHCl

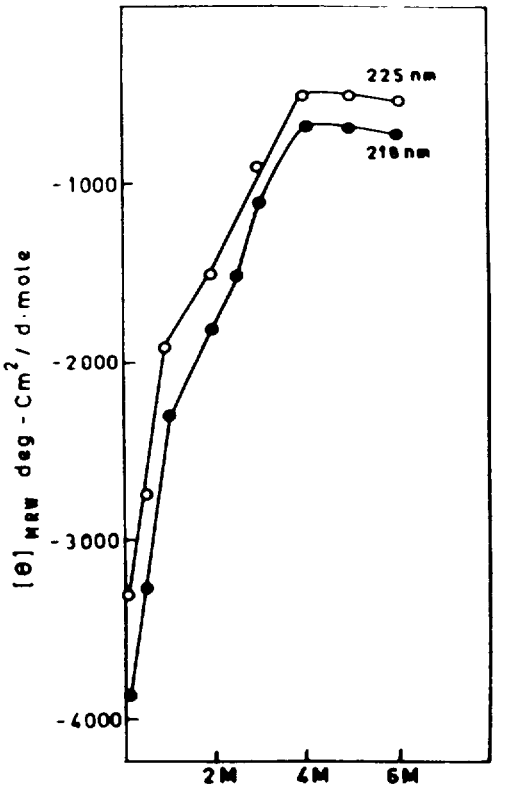

Fig. 20 Mean residue ellipticity values of conglutin α as a function of GdnHCl concentration at 225 nm and 218 nm.

indicates that at 4M GdnHCl concentration the decrease in rotations attains a constant vlaue indicating the completeness of the process of conformational transition both at 218nm and 225nm. As a comparison at 6M urea the rotation was around -750deg cm^2/d mole at 218nm (Fig.15).

Discussion

If one compares the sequence of the various intermediates in the dissociation process of the protein in SDS, urea and GdnHCl, the schematics can be represented as

11S ——————→ 2S (SDS)

11S ——→ 7S ——→ 2S (urea)

11S ——→ 7S ——→ 2S (GdnHCl)

The process of production of 2S the ultimate component in any of the above reagent appears to be denaturant concentration dependent. In fact in bringing about dissociation on a mole to mole basis SDS is more effective than GdnHCl which in turn is more effective than urea, i.e.

SDS〉GdnHCl〉urea (Dissociation)

Now with this hereby in mind if one looks at the denaturation profile mainly from fluoroscence data it appears that

SDS〉GdnHCl〉urea (Denaturation)

This strong dissociative and denaturation action of the detergent SDS is mainly due to its greater ability to break hydrophobic bonds and ionic linkages. However, both GdnHCl and urea compete for peptide bonds and GdnHCl being a salt is probably a more powerful denaturing agent. Parakash et al.(1986) and Sureshchandra et al.(1987) have reported similar results in their study of the effect of denaturants on the proteins, α-globulin and glycinin respectively. Also by applying the prediction mechanism and Parakash-Timasheff(1986) equation for estimating the moles of urea and GdnHCl bound, one ends up with the same conclusion.

Since conglutin α is a multimeric protein the question of whether dissociation and denaturation reaction occur sequentially or simultaneously occurs. Looking at the analytical ultracentrifuge data, it is clear that even at the lowest concentration of the denaturants the 2S molecule is already present. At these concentrations of the denaturants however, there is no shift of the fluoroscence emission maxima and the near UV and far UV-CD spectra do not indicate any drastic changes. These results go to show that initially the 2S molecule that is produced at low concentraton of the denaturants is not denatured to that extent or extended as compared to the 2S molecule at 18mM SDS or 6M GdnHCl or 8M urea. This is confirmed by the reduction of sedimentation coefficient (1.7S) of the 2S species in the analytical

ultracentrifuge (Fig.5, 10) at higher concentration of the denaturants, (wherein the 2S molecule is in extended configuration). This scheme fits into the model of the denaturation of 2S molecule proposed by Parakash and Narasinga Rao (1986). Based on this one can conclude that initially the disscoiation reaction occurs and as the dissociation reaction is progressing the concentration of the denaturant induces the 2S molecule to open up leading to the denaturation of the molecule. Hence the initial dissociation and denaturation reactions are sequential upto a certain concentration of denaturant and after which the two processes appear to be simultaneous.

These data helps us to visualise the possible forces stabilizing the native structure of conglutin α. Since according to Meyer and Kauzmann(1962) detergents do not compete for peptide hydrogen bonds but instead weaken the hydrophobic interactions it suggests the predominance of hydrophobic interactions and ionic linkages over hydrogen bonds in the stabilization of the native structure of conglutin α. Also, this confirms Parakash and Narasinga Rao's(1986) model for seed proteins in that the inter and intra hydrophobic interactions in 7S and 11S molecules in seed protein are different.

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國 文 抄 錄

루핀콩의 주요단백질인 conglutin α를 순수하게 분리하여 여러가지 특성을 조사하였다. 그리고 SDS, Urea, GdnHCl에 대한 영향들을 조사하였다. 단백질의 침강계수, $S_{20,w}$ 값은 11이었으며, 2차구조는 대부분의 β와 비규칙적인 구조로 구성되어 있었다. 단백질의 아미노산 조성은 산성 그리고 소수성 아미노산의 풍부하였다. SDS, Urea, GdnHCl의 존재하에서 단백질은 해리, 변성되었다. SDS 용액에서의 해리 매카니즘은 Urea, GdnHCl 용액에서와는 다른것을 보여주었다. 일반적인 해리 패턴은 SDS용액에서를 제외하고 11S→7S→2S의 패턴을 보여주었다. GdnHCl은 Urea보다 단백질의 해리 변성에 아주 효과적이었다. 해리공정은 궁극적으로 변성된 분자를 생성하는 관련 Subunit의 소수성 상호작용의 파괴에 기인한 것이다.