

---

**SUMMARY AND CONCLUSIONS**

---

### SUMMARY AND CONCLUSIONS

The present investigation on "Studies on the Physico-Chemical Properties of Sesame (Sesamum indicum) Seed Proteins" has been carried out as follows. We have concentrated our study on the major protein fraction  $\alpha$ -globulin of sesame seed, its isolation, determination of some of its chemical and physico-chemical properties and studies on the association-dissociation and denaturation phenomena under various solution conditions, e.g. acid, alkali, electrolytes, urea,  $\text{CuCl}_2$  and SDS which have been presented here. These studies on the association-dissociation and denaturation phenomena under the above solution conditions have been helpful in understanding the structural aspects of the protein.

(1) The protein  $\alpha$ -globulin isolated by the procedures of Nath and Giri (1957b) and Ventura and Lima (1962) contains (contaminations both low, 2S component and high molecular weight, 16S component)  $\sim 15\%$  and  $\sim 25\%$  respectively as revealed

by sedimentation velocity experiments. The  $\alpha$ -globulin isolated in the present investigation was homogeneous, > 95% by the techniques of gel filtration, polyacrylamide gel electrophoresis, DEAE-cellulose chromatography and sedimentation velocity experiments. The yield of  $\alpha$ -globulin also increased by the procedure adopted here.

(2) The protein isolated was practically free from nucleic acid and carbohydrate contamination as revealed by the low values of phosphorus and carbohydrate.

(3) Amino acid analysis of the protein indicates that it is rich in acidic, sulphur containing and aromatic amino acid residues.

(4) The protein has a molecular weight of  $2,50,000 \pm 15,000$  daltons as determined from Archibald's approach to sedimentation equilibrium method.

(5) It is an oligomeric protein containing at least 11 subunits.

(6) The value of intrinsic viscosity of 0.03 dl/gm indicates the protein to be globular.

(7) In acid solution, in the pH range of 4.2-1.5, the results of gel filtration, fluorescence and viscosity measurements indicate that  $\alpha$ -globulin

dissociates and denatures upto pH  $\sim$  3.

(8) The difference spectrum in this range of pH is characterised by troughs at 260, 287 and 292 nm and arises from a combination of dissociation, denaturation and charge effect on the chromophore.

(9) In still stronger acid solution (pH < 3) reassociation of the dissociated fraction takes place as indicated by difference spectra, fluorescence spectra and sedimentation velocity measurements.

(10) The temperature effect on the association-dissociation phenomena of  $\alpha$ -globulin at pH 1.5 indicates that the reassociation of the dissociated components takes place probably by hydrophobic interaction.

(11) In alkaline solution, in the pH range 7-12,  $\alpha$ -globulin dissociates around pH 8, and above pH 10, dissociation and denaturation proceed simultaneously as have been evidenced by sedimentation velocity, fluorescence spectra, ultraviolet spectral changes, optical rotation and viscosity measurements.

(12) The change in the shape of the ultraviolet spectrum with pH in alkaline solution indicates that both tyrosyl ionisation and conformational changes are taking place simultaneously in the protein.

The constancy of absorbance at 242 nm after tyrosyl ionisation is complete, can be taken as an evidence that no other reaction which might result from alkaline hydrolysis of disulfide groups is taking place.

(13) The phenolic group in the protein is abnormal ( $pK_{Int} = 10.6$ ). In 6M GuHCl the  $pK_{Int}$  of tyrosyl groups is 9.6 which is the expected value for the  $pK_{Int}$  of tyrosyl groups. The denaturation in alkaline solution is irreversible.

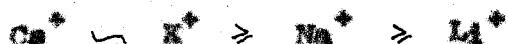
(14) The characteristic pH values of transition from 10.6-10.8 indicates that the transition of the protein involves a single step in alkaline solution.

(15) Effects of various sodium salts on the association-dissociation phenomena of  $\alpha$ -globulin show the following effectiveness towards dissociation of the protein:



the first two members reduce the extent of dissociation.  $CCl_3COONa$  has been found to be the most effective amongst the series in inducing dissociation in the protein.

(16) The cations  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cs}^+$  induce association, the effectiveness being



(17) Minor discrepancy from the Hofmeister series has been observed in the present investigation.

(18) The low concentration of salts (anions) necessary to induce dissociation does not involve any detectable change in protein conformation.

(19) The temperature effect on the association reaction  $7\text{S} \rightarrow 11\text{S}$ , indicates that the subunits of  $\alpha$ -globulin are associated predominantly by hydrophobic interaction.

(20) The dissociating effect of the electrolytes may be due to a combination of favourable energetics of the chaotropic ions with the amide dipole against a low positive unfavourable free energy of interaction of the nonpolar groups with the same ions.

(21)  $\alpha$ -globulin undergoes dissociation, aggregation and denaturation in urea and  $\text{CuCl}_2$  solutions. The protein precipitates at low concentration of these reagents.

(22) The aggregation phenomenon is maximum at a critical concentration of either of the denaturants.

(23) The protein precipitated at low concentrations of these reagents goes back into solution at higher reagent concentration.

(24) The soluble aggregate having a sedimentation coefficient of  $\sim 120S$  is the precursor of this insoluble aggregate.

(25) Viscosity results indicate that the denaturation of the protein is complete at 8M urea and 6M GuHCl.

(26) The difference spectrum of the protein is characterised by troughs at 280, 287 and 293 nm indicating that both tryptophan and tyrosine groups have been effected by the denaturants. A red shift at 300-305 nm is also observed in both the reagents and is attributed to the denaturation of the protein and anomalous tryptophan absorption change.

(27) Fluorescence measurements indicate a red shift of the spectrum from 325 to 345 nm with increasing concentration of the denaturant. This indicates that the tryptophan fluorophor is experiencing increasing polar environment with increasing concentration of urea and/or GuHCl. The fluorescence

transition is complete at a lesser concentration of both urea and  $\text{GuHCl}$  as compared to viscosity and difference spectral results.

(28) The simultaneous dissociation and aggregation reactions have been explained by considering two types of subunits present in the protein molecule, one leading to smaller sedimenting component (4S) and the other producing the aggregate ( $\sim 120\text{S}$ ).

(29) The amino acid analysis of the aggregated fraction indicates that it is rich in aliphatic amino acid residues.

(30) The endothermic nature of the aggregation process has been explained as probably arising from hydrophobic interaction of aliphatic side chains of the relevant subunits i.e. which are rich in aliphatic amino acid residues.

(31)  $\alpha$ -globulin exists in a more denatured state in  $\text{GuHCl}$  than in urea solution. The extent of denaturation in these solutions are reduced in the presence of  $0.5\text{M}$   $\text{KCl}$ .

(32)  $\alpha$ -globulin undergoes stepwise dissociation initially and then denaturation in SDS solution.



(33) Gel filtration, polyacrylamide gel electrophoresis and sedimentation velocity measurements indicate the dissociation of the protein. The dissociation is complete at  $5 \times 10^{-3} M$  SDS and four components are observed in the sedimentation velocity pattern with  $S_{20,w}$  values of 2S, 4S, 7S and 11S.

(34) The specific rotation measurements indicate a cooperative transition between  $3 \times 10^{-3} M$  to  $8 \times 10^{-3} M$  detergent concentration, suggesting conformational change.

(35) The difference spectra of the protein is characterised by blue shift with troughs at 280, 287 and 292 nm indicating that both tryptophan and tyrosine groups have been effected by the detergent.

(36) The appearance of a blue shift in the difference spectra of protein below  $3 \times 10^{-3} M$  arises from the binding of the detergent near the tyrosine and tryptophan chromophores. Above  $3 \times 10^{-3} M$  SDS, the conformational change contributes to the observed blue shift in the difference spectra.

(37) In the presence of detergent, the fluorescence intensity decreases and a red shift in the fluorescence maximum occurs resulting in an 'isoemissive point' at 355 nm. The red shift in the fluo-

rescence maximum at the highest concentration of the detergent used is 330-335 nm. This red shift is less as compared to urea and CaHCl solutions where it is 345 nm.

(38) The binding studies of SDS to protein indicates a steep binding curve above  $2.5 \times 10^{-3}$  M SDS. Analysis of the binding data in the range  $1 \times 10^{-5}$  to  $1 \times 10^{-3}$  M SDS indicates the presence of  $\sim 50$  binding sites in the protein and a binding constant of  $3 \times 10^3$ .