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Homology in oilseed proteins

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Abstract. The proteins of groundnut, soybean, sesame, cottonseed, rapeseed/mustard, sunflower, safflower, poppy seed, etc contain a high molecular weight fraction. The sedimentation coefficient of this protein is ~ 12S and its proportion varies from 20 to 65% among various oilseeds. Data on the amino acid composition, molecular weight and subunit composition, intrinsic viscosity, secondary structure and fluorescence spectra of HMW protein from different oilseeds are presented. This protein dissociates and denatures in the pH range 5 to 2; below this pH it reaggregates and renatures. The similarity in the properties of HMW protein from different oilseeds and the significance of the similarity are discussed.

Keywords. Oilseed; homology; globular; secondary structure; subunit; association-dissociation; denaturation; viscosity; oligomeric; hydrolysis.

1. Introduction

Oilseeds such as groundnut, soybean, sesame, cottonseed, sunflower, etc contain about 20–25% protein (Altschul 1958). After removal of the oil they contain 50–60% protein. The proteins from the defatted cake can be extracted in water or dilute salt solution. In our laboratory we have studied the proteins from groundnut, sesame, mustard/rapeseed, sunflower, safflower, cottonseed, soybean, poppy seed, and guar seed, and have observed homology in the physico-chemical properties of the high molecular weight protein fraction. The results are discussed below.

2. Total proteins

The protein solubility varies with pH, it being higher at acid and alkaline pH values. Minimum solubility is observed in the pH range 4 to 6 (figure 1). In some cases two minima are also observed (figure 1). Proteins from defatted groundnut, soybean and sunflower have a single solubility minimum in the pH range 4–6. On the other hand, proteins from safflower, cottonseed, mustard, rapeseed and sesame have two solubility minima, one around pH 8 to 9 and the second below pH 5. It is observed that extraction of the protein at alkaline pH values around 8–9, removal of the carbohydrate residue by filtration or centrifugation and adjustment of pH to 4 to 5 leads to precipitation of the protein. Such precipitates are referred to as protein isolates, which are heterogeneous and contain a number of protein fractions.

The usual method of designating the protein fractions in a protein isolate is their sedimentation coefficient ($S_{20,w}$) values. The protein components of various oilseeds studied are given in table 1 (Naismith 1955; Tombs and Lowe 1967; Shetty and

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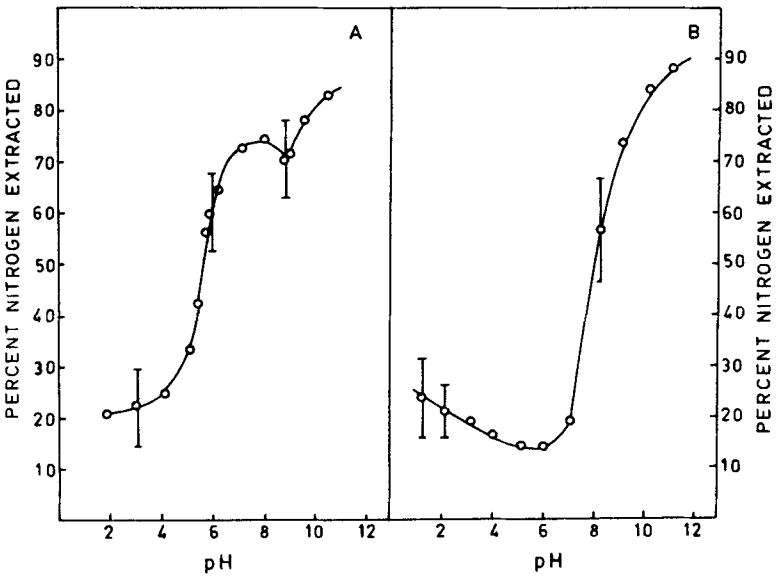

Figure 1. Percent nitrogen extracted from flour as a function of pH in 1 M sodium chloride solution. A—Safflower; cottonseed; mustard/rapeseed; and sesame. B—Groundnut; soybean; guarseed and sunflower. Vertical lines (I) indicates the range of variation of the nitrogen extracted from the average values plotted (O) for all the proteins mentioned above.

Table 1. Sedimentation coefficient ($S_{20,w}$) values of protein components from various oilseeds.

Source	$S_{20,w}$			
Groundnut	2	7	11	18
Sesame	2	7	11	16
Mustard	1	7	12	—
Rapeseed	1	7	12	—
Sunflower	2	7	12	16
Safflower	2	7	12	17
Cottonseed	2	8	11	18
Soybean	2	7	11	15
Poppy seed	1	6	10	14

Narasinga Rao 1974; Hanumantha Rao 1977; Prakash and Nandi 1978; Gururaj Rao *et al* 1978; Nath *et al* 1978; Rahma and Narasinga Rao 1979; Schwenke and Raab 1979; Nath 1980; Latha and Prakash 1984a; Srinivas and Narasinga Rao 1981). All of them contain a low molecular weight fraction ($\approx$ 2S), a 7S–8S fraction and a high molecular weight 10–12S fraction. Some of them also contain a small proportion of fast moving components (polymer) having sedimentation coefficients of 14–18S. It is not certain if the polymer is an aggregate of other protein components. Substances such as trypsin inhibitors and hemagglutinins are normally associated with the low molecular weight ($\approx$ 2S) fraction (Sosulski 1979). In some cases materials such as polyphenols,

glucosinolates, etc which are constituents of some oilseeds also elute along with the low molecular weight fractions in gel filtration (Sosulski 1979; Kishore Kumar Murthy 1982; Rahma and Narasinga Rao 1979; Latha and Prakash 1984a).

3. High molecular weight (HMW) protein

It may be seen from table 2 that the 10–12S protein fraction (high molecular weight component, HMW) forms the major protein fraction, the proportion varying from 20 to 65%.

3.1 *Amino acid composition*

The amino acid composition of the HMW protein of oilseeds is given in table 3 and is compared with that of bovine serum albumin (Tristram and Smith 1963; Shetty 1975; Appu Rao 1975; Prakash 1976; Gururaj Rao and Narasinga Rao 1981; Schwenke and Raab 1979). The amino acid composition does not show any unique features. The HMW protein of oilseeds appears to be rich in glutamic acid and low in lysine, and contains a fair amount of aromatic amino acids.

Table 2. HMW protein from various oilseeds.

Source	Protein	$S_{20,w}$	% of total proteins
Groundnut	Arachin	11	55
Sesame	α-globulin	11	65
Mustard/rapeseed	12S protein	12	25
Sunflower	12S protein	12	60
Safflower	Carmin	12	65
Cottonseed	11S protein	11	20
Soybean	Glycinin	11	30
Poppyseed	10S protein	10	60
Guarseed	12S protein	12	60

Table 3. Content of selected amino acids (number of residues per 100000 g) of the HMW protein of various oilseeds. Data on bovine serum albumin are also shown for comparison.

	Protein					
Amino acid	Bovine serum albumin	Arachin	Glycinin	Sesame α-globulin	Mustard/ rapeseed 12S	Sunflower 12S
Aspartic acid	64	95	106	72	53/71	93
Glutamic acid	91	133	169	136	134/160	172
Lysine	89	17	33	14	17/24	16
Tryptophan	3	3	7	10	9/9	9
Tyrosine	29	25	24	22	16/14	18
Phenylalanine	41	36	34	30	23/26	43

3.2 *Intrinsic viscosity*

The intrinsic viscosity of the HMW protein from various oilseeds is given in table 4 (Appu Rao 1975; Shetty 1975; Prakash and Nandi 1978; Rahma and Narasinga Rao 1979; Gururaj Rao and Narasinga Rao 1981). They all have a value characteristic of globular proteins, 3–5 ml/g (Tanford 1961). For comparison, the intrinsic viscosity of ribonuclease which is a globular protein and collagen which exists in a highly assymetric form are also given. The value obtained with the HMW protein of oilseeds is close to that of ribonuclease (3·3 ml/g).

3.3 *Secondary structure*

These proteins have characteristic Cotton effects in the ORD and far UV-CD spectra. A few typical examples are given in figure 2 (Sureshchandra *et al* 1982; Prakash *et al* 1980). The CD spectrum is characterised by a minimum around 208 nm with a shoulder around 228 nm.

A fully α-helical or a fully β-structured protein has varying characteristics in ORD and CD spectra. A comparison of the ORD and CD spectra of the HMW protein from various oilseeds with those of standard polypeptides (McKenzie 1970; Greenfield and Fasman 1969) suggests that these have predominantly aperiodic structures and a certain amount of β-structure (table 5) (Appu Rao 1975; Shetty 1975; Gururaj Rao *et al* 1978; Prakash *et al* 1980; Sureshchandra *et al* 1982; Latha and Prakash 1984b; Srinivas 1984). They have very little α-helical structure.

3.4 *Molecular weight and subunit composition*

These proteins are high molecular weight proteins, the molecular weight being in the range of 240000 to 350000 (table 6) (Tombs and Lowe 1967; Catsimpoolas *et al* 1971; Plietz *et al* 1978; Prakash and Nandi 1978; Gururaj Rao and Narasinga Rao 1981; Prakash 1984; Srinivas 1984). They are also oligomeric in nature having a number of subunits (Catsimpoolas *et al* 1971; Singh and Dieckert 1973; Shetty and Narasinga Rao 1974; Prakash and Nandi 1978; Reichelt *et al* 1980; Gururaj Rao and Narasinga Rao 1981). The number of subunits ranges from 7–14 (table 6). The number of subunits has generally been determined by SDS-PAGE, in the case of arachin by urea dissociation (Yamada *et al* 1979) and in the case of α-globulin by sedimentation equilibrium in GuHCl solution (Prakash 1984). There seems to be some discrepancy in the values

Table 4. Values of intrinsic viscosity $[\eta]$ of the HMW protein from various oilseeds.

Protein	$[\eta]$ ml/g
Arachin	4·7
Sesame α-globulin	3·0
Mustard 12S	3·6
Rapeseed 12S	3·7
Sunflower 12S	3·6
Carmin	3·7
Glycinin	4·9
Poppyseed 10S protein	3·5
Ribonuclease	3·3
Collagen	1150

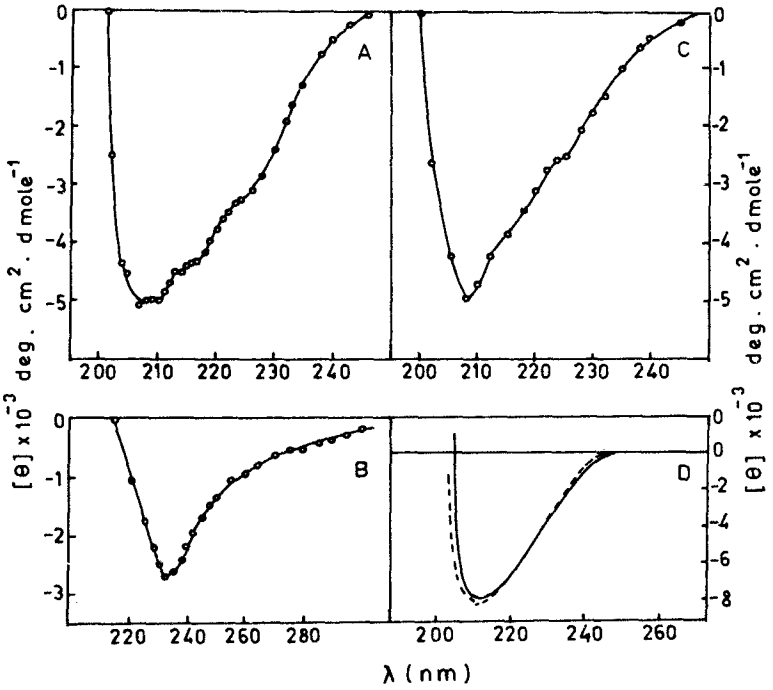

Figure 2. ORD (200–300 nm) and CD (200–250 nm) spectra of HMW proteins. A, CD spectrum of glycinin; B, ORD spectrum of glycinin; C, CD spectrum of sunflower protein; and D, CD spectrum of α-globulin (——experimental curve; - - - - computer fit curve for α-helix of 5%; β-structure of 25% and aperiodic structure of 70%).

Table 5. Secondary structure of HMW protein fraction from various oilseeds.

Protein	α-helix	Percentage β-structure	Aperiodic/ structure
Arachin	—	More of β and aperiodic	
Sesame α-globulin*	5	25	70
Mustard/rapeseed 12S protein	9	28	63
Sunflower 12S protein	2	28	70
Carmin	3	15	82
Glycinin	5	20	70
Poppyseed 10S protein	5	20	75

* Curve fitted.

obtained by the two methods. It is probable that SDS-PAGE is not the best method for determining the subunit structure of the oilseed proteins. However, the more interesting fact appears to be that these subunits all have more or less a similar molecular weight range 7000–80000 (Catsimpoolas *et al* 1971; Singh and Dieckert 1973; Shetty and Narasinga Rao 1974; Prakash and Nandi 1978; Reichelt *et al* 1980; Gururaj Rao and Narasinga Rao 1981; Prakash 1984).

Table 6. Molecular weight and number of subunits of the HMW protein from various oilseeds.

Protein	Mol wt (× 100000)	No. of subunits
Arachin	3·3	7/12
Sesame α-globulin	2·74	12 14†
Mustard 12 S	2·4	8
Rapeseed 12 S	2·4	8
Sunflower 12 S	3·5	10/6
Carmin	2·5	12
Glycinin	3·3	12
Poppyseed 10S protein	2·15	6

* By SDS-PAGE; †By Sed. Eq. in GuHCl.

3.5 *Fluorescence*

The fluorescence emission spectrum of the HMW protein of sesame is given in figure 3. It is characterized by an emission maximum around 320–330 nm. This is true of the HMW protein of all oilseeds. The emission maximum is characteristic of tryptophan emission embedded in the interior of the protein although the protein contains a considerable amount of tyrosine. This is compatible with literature reports that proteins having both tryptophan and tyrosine give characteristic tryptophan emission spectra (Shifrin *et al* 1971; Teale 1960).

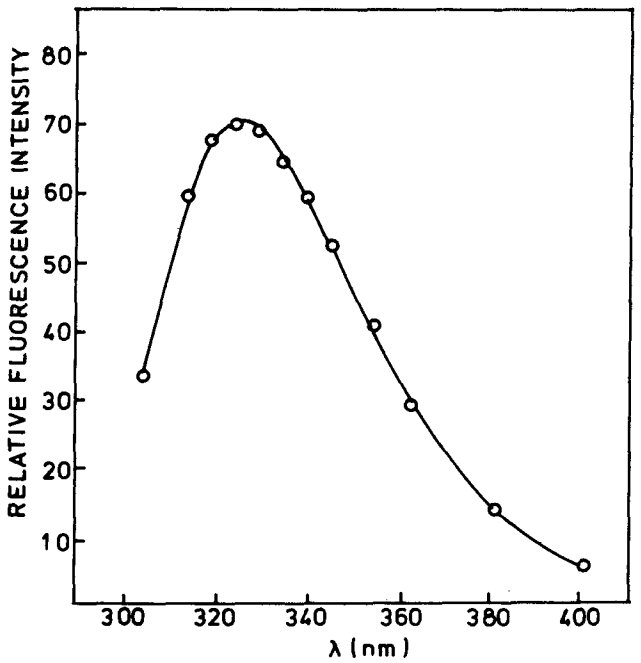

Figure 3. Fluorescence emission spectrum of α-globulin, excitation at 280 nm.

3.6 *Association-dissociation*

Another interesting property of the HMW protein from various oilseeds is its unusual behaviour at low pH values. We have followed the effect of low pH in the range 5–1 on the oligomeric structure, spectral properties and conformation of the protein by the techniques of UV difference spectrophotometry, fluorescence spectrophotometry, viscometry, sedimentation velocity and CD spectroscopy (Prakash and Nandi 1977; Kishore Kumar Murthy 1982; Navin Kumar 1982). A few data are given in figure 4. In the pH range 5–3 the protein dissociates and below pH 3 there is evidence of reaggregation. Similarly difference spectrophotometric data indicate the denaturation of the protein in the range 5–3 and refolding below pH 3. Similar trends are seen in fluorescence spectra and intrinsic viscosity.

It has been proposed that the subunits making up the oligomeric structure of the HMW protein consist of two classes namely acidic and basic (Okubo *et al* 1979; Yamada *et al* 1979). At highly acid pH values below pH 3, the acidic and basic units may associate because of charge effects. It may also be due to entropically driven hydrophobic interaction (Prakash and Nandi 1977).

3.7 *Hydrolysis*

The HMW proteins is not easily hydrolysed by proteolytic enzyme, such as papain, trypsin and chymotrypsin (Shetty 1976; Appu Rao 1975; Gururaj Rao and Narasinga Rao 1981). A few representative data of α-chymotrypsin hydrolysis are given in figure 5. Compared to casein the HMW protein has hydrolysed to a lower extent. It can be argued that the lower digestibility of the protein could be due to the presence of

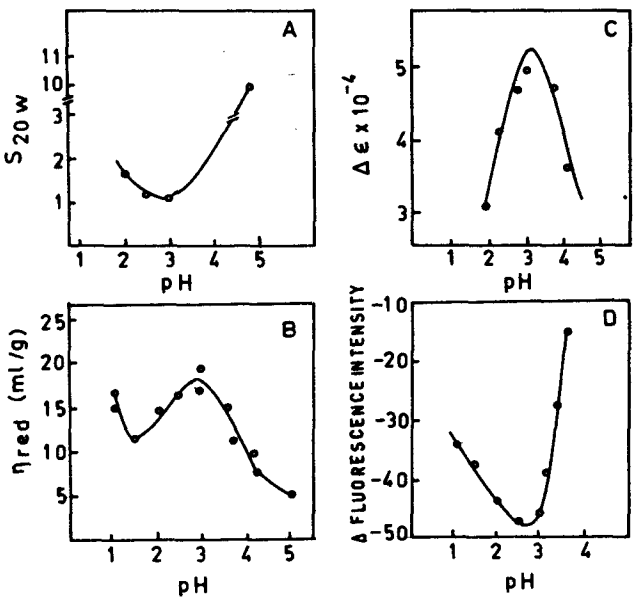

Figure 4. Effect of low pH on the sedimentation coefficient of mustard 12S protein (A); viscosity of mustard 12S protein (B); difference spectrum of α-globulin (C) fluorescence spectrum of mustard 12S protein.

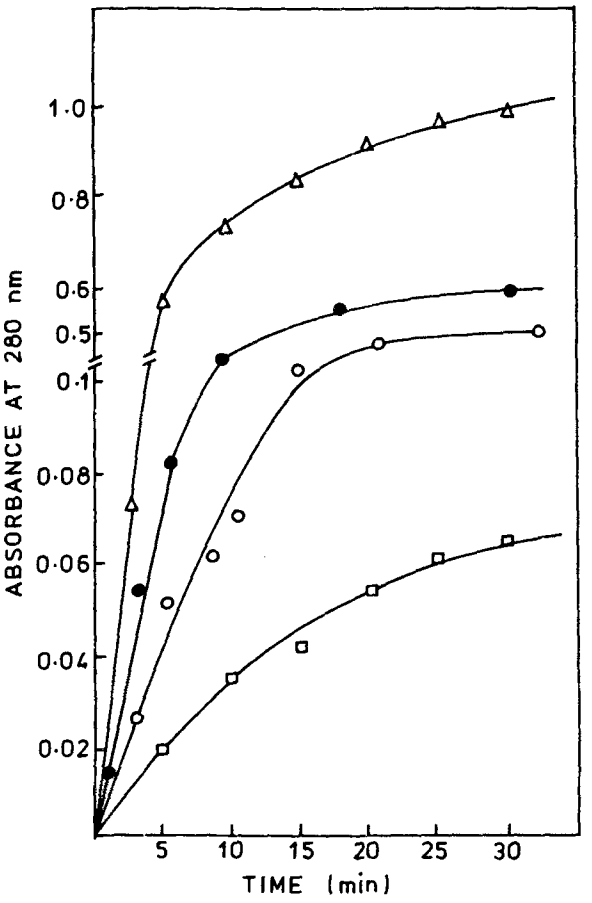

Figure 5. Rate of hydrolysis of HMW protein by α-chymotrypsin from (-□-□-) mustard; (-○-○-) groundnut; (-●-●-) soybean and (-Δ-Δ-) casein.

protease inhibitors in the HMW protein. Even in cases where the presence of protease inhibitors could be detected the rate of hydrolysis was much lower than that with casein. Perhaps these proteins have a 'hard core' of amino acid sequences which the proteases are unable to hydrolyse.

4. Discussion

Thus in many properties there seems to be considerable homology between the HMW protein from various oilseeds. It is probable that such homology may exist in the case of the 7S protein and also the lower molecular weight protein fraction. However, no data are available at present to support this view.

It would be interesting to speculate if there is a biological significance in the observed facts that the HMW protein has (a) globular shape; (b) predominantly aperiodic structure and (c) poor digestibility by proteases. The function of the HMW protein in the oilseeds

is to act as a storage protein. These are broken down during germination for resynthesis into new proteins. The subunits are held together by weak forces (ΔF = 0·2 kcal/mol at 27°C) and this facilitates the breakdown of the oligomeric structure (Prakash 1976). Globular shape and high mol wt would facilitate dense packing of these proteins in the cells. In other words, for the same volume of the cell a larger amount of protein can be packed in case of a globular and high mol wt protein than with an asymmetric and low molecular weight protein. Since these proteins do not perform any known specific biological function such as that of enzyme/hormone/regulator it is not necessary to have a well-defined secondary structure. Poor digestibility by proteases would provide protection against insects which may otherwise easily attack the seed material and digest it. The digestibility of the proteins may also be affected by low molecular weight ligands such as trypsin inhibitors present in the oilseeds. The other undesirable constituents such as glucosinolates in rapeseed/mustard and gossypol in cottonseed may also act as protectants and protect the oilseed materials against infestation. Temperature seems to have very little effect on the conformation of the proteins (Sureshchandra 1984) and this may be an advantage because high temperatures are usually developed during storage of the seed materials.

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