Studies on the Low Molecular Weight Proteins of Poppy Seed (*Papaver somniferum* L.)

H. Srinivas* and M. S. Narasinga Rao

The low molecular weight proteins of defatted poppy seed meal were separated from the high molecular weight fraction (10S) by chromatography on Sepharose 6B. Gel electrophoresis showed that the proteins were heterogeneous and consisted of at least five protein fractions. In sedimentation velocity a single diffuse peak was observed with $S_{20,w}$ value of 0.8S. The proteins contained 3.1% carbohydrate and no phosphorus. The amino acid composition of low molecular weight proteins was different from that of the 10S protein in having higher amounts of cysteine, glutamic acid, and arginine and lower amounts of aspartic acid, leucine, isoleucine, valine, histidine, tryptophan, and phenylalanine. The molecular weight of the proteins determined by gel filtration technique was 14 500. The low molecular weight proteins had higher helical content (35%), unlike the 10S protein which had very little helix (5%). Attempts to isolate the homogeneous fractions by DEAE- or CM-Sephadex chromatography did not succeed.

The occurrence of low molecular weight (MW) proteins has been reported from oilseed of diverse species (Youle and Huang, 1981). These proteins are water soluble and are termed albumins. Their sedimentation coefficient is approximately 2S. Their proportion varies from 20 to 60% of total proteins depending on the seed material. Other than the high amounts of arginine, glutamic acid and aspartic acid, these albumins are exceptionally high in cysteine. Schwenke et al. (1973) have isolated and characterized the albumins from sunflower seeds and rapeseeds. Madhusudhan and Singh (1985) have isolated 1.6S protein from defatted linseed meal in a homogeneous form and studied the amino acid composition and physicochemical properties.

It has also been shown that the albumins contribute significantly to the higher emulsification and foam capacities in food systems (Graham and Phillips, 1976; Satterlee et al., 1975).

The total proteins of poppy seed meal were made of 0.8, 6.3, 10.0, and 14.1S protein fraction as judged from their sedimentation velocities (Srinivas and Narasinga Rao, 1981). Recently we reported the isolation of high molecular weight, 10S protein and its physicochemical properties (Srinivas and Narasinga Rao, 1986). The present paper deals with low molecular weight proteins that represent about 15% of the total proteins of poppy seed. Electrophoresis in the presence and absence of SDS (sodium dodecyl sulfate) showed that low molecular weight proteins consisted of five fractions. Attempts to isolate them by gel filtration and ion-exchange chromatography were not successful.

MATERIALS AND METHODS

Poppy seed variety Dhawla chotta was obtained from the Government Opium Factory, Neemuch, Madhya Pradesh, India. Defatted poppy seed meal was prepared according to the procedure of Srinivas and Narasinga Rao (1981).

Sepharose 6B and Sephadex G-100 were from Pharmacia Fine Chemicals, Sweden. Molecular weight markers were from Sigma Chemical Co. Other chemicals were of reagent grade obtained locally.

Isolation of Low Molecular Weight Proteins. Poppy seed meal (4 g) was extracted with 40 mL of 1 M NaCl solution with stirring for 1 h. The suspension was centrifuged at 7000*g* for 20 min. The supernatant was dialyzed against 1 M NaCl solution for 18 h and centrifuged at 12000*g* for 20 min. An aliquot (10 mL, 350 mg of protein) of the supernatant was loaded on a column of Sepharose 6B (2.2 × 120 cm; bed 456 mL) equilibrated and eluted with 1 M NaCl. Protein eluted at 370–440 mL was pooled, dialyzed against water, and lyophilized.

Sedimentation Velocity. This was performed with a Spinco Model E analytical ultracentrifuge equipped with a rotor temperature indicator and control (RTIC) unit and phase plate Schlierne optics. The experiments were done at 28 °C on 1% protein solution in 0.5 M NaCl at 59 780 rpm. The $S_{20,w}$ value was calculated by the standard procedure (Schachman, 1959) from the photographs taken at different intervals of time.

Polyacrylamide Gel Electrophoresis. Electrophoresis was performed in 7.5% gels in 0.025 M Tris–glycine buffer at pH 8.3 and 0.05 M β-alanine–acetic acid buffer at pH 4.5 according to Davis (1964). Approximately 100 μg of protein was applied to the gel.

Carbohydrate and Phosphorus. Carbohydrate and phosphorus content of the protein was estimated by the procedure of Dubois et al. (1956) and Taussky and Shorr (1953), respectively.

Amino Acid Composition. A 30-mg portion of protein was hydrolyzed in vacuo with 6 mL of 6 N HCl containing 0.1% phenol and 0.01% β-mercaptoethanol at 110 ± 1 °C for 24 h, and amino acid analysis was done with an LKB model automatic α-amino acid analyzer. Tryptophan content was estimated according to Spande and Witkop (1967) using *N*-bromosuccinimide.

Circular Dichroism (CD). CD measurements were made at room temperature (~28 °C) on a Jasco J 20C automatic spectropolarimeter equipped with xenon arc lamp. The instrument was calibrated with camphor-d_{10}-sulfonic acid and was programmed to yield 1-mm band width at each wavelength. Far-UV-CD spectra were recorded in the range of 200–260 nm with a 1-mm path length cell using 0.03% protein solution in 0.5 M NaCl. Mean residue ellipticities $[\theta]_{MRW}$ values were calculated by the standard procedure (Adler et al., 1973), using a value of 115 for mean residue weight (MRW). The α-helix content was calculated by the procedure of Greenfield and Fasman (1969) and Chen and Yang (1971), and the β-structure, according to the method of Sarkar and Doty (1966).

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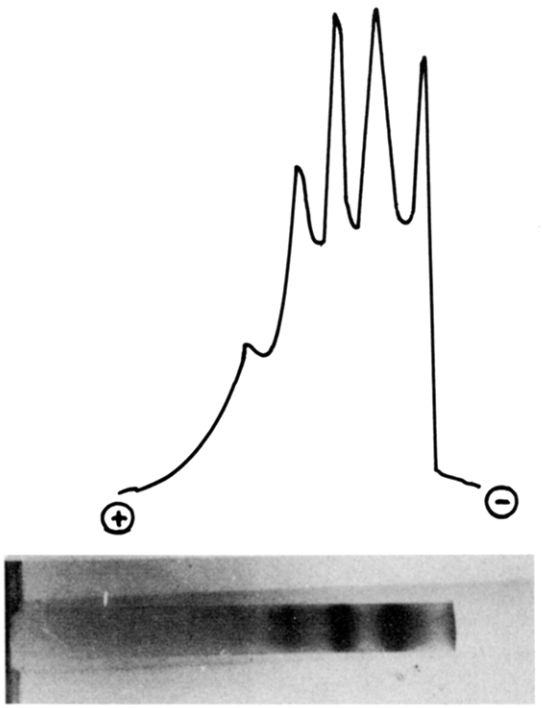

Figure 1. Polyacrylamide gel electrophoresis pattern of poppy seed low molecular weight proteins in 0.025 M Tris–glycine buffer, pH 8.3.

Molecular Weight Determination. (*a*) *Gel Filtration.* This was done according to Ackers (1975) using Sephadex G-100 (1.5 × 100 cm) and bovine serum albumin, ovalbumin, pepsin, chymotrypsin, and lysozyme as standards.

(*b*) *SDS-PAGE.* This was performed according to the method of Weber and Osborn (1969), using 10% gels and the same protein standards.

RESULTS AND DISCUSSION

Rechromatography of low molecular weight proteins on Sepharose 6B (1.5 × 100 cm) gave a single peak eluting at 142 mL. This volume corresponded to that of peak 3 isolated when the total protein of the defatted poppy seed metal was subjected to gel filtration on the same column (Srinivas and Narasinga Rao, 1981). In sedimentation velocity, the low molecular weight proteins gave a broad single peak. The $S_{20,w}$ value was found to be 0.8S.

The gel electrophoretic pattern of the proteins at pH 8.3 and the densitometric tracing of the gel pattern shown in Figure 1 consisted of three major and two minor bands. Three sharp bands and two diffuse bands were also detected at pH 4.5 (data not shown), indicating the presence of five fractions.

The proteins contained 3.1% carbohydrate and therefore were richer than the major 10S protein (1.5%) (Srinivas and Narasinga Rao, 1986). They contained no phosphorus.

The amino acid composition showed (Table I) that these proteins are rich in glutamic acid, arginine, methionine, and cysteine. The contents of phenylalanine, tryptophan, isoleucine, and leucine were low. Thus, the amino acid composition of 0.8S proteins of poppy seed was different from that of the 10S protein (Srinivas and Narasinga Rao, 1986). The higher amount of glutamic acid, which might be present partly as its amide, and also arginine suggest that these proteins are basic in nature. The higher amounts of cysteine and methionine reported here are in good agreement with the published values of Youle and Huang (1981) for 2S proteins of various oilseeds. The 1.6S protein of linseed is reported to have higher amount of glutamic acid, lysine, arginine, cysteine, glycine, and tryptophan, but the methionine values are lower (Madhusudhan and Singh, 1985).

Table I. Amino Acid Composition of Low Molecular Weight Proteins (0.8S) of Poppy Seed (Number of Residues per 14 500 g of Proteins)[a]

aspartic acid[b]	6	methionine	4
threonine	4	isoleucine	2
serine	6	leucine	4
glutamic acid[b]	45	tyrosine	2
proline	6	phenylalanine	1
glycine	9	histidine	1
alanine	5	lysine	2
cysteine	8	arginine	15
valine	2	tryptophan	NS[c]

[a] Values are averages of two independent determinations. [b] Includes aspargine and glutamine. [c] NS = not significant.

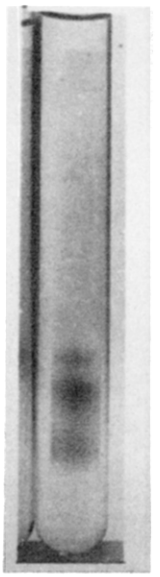

Figure 2. SDS-PAGE pattern of poppy seed low molecular weight proteins.

SDS-PAGE pattern showed three bands that are of high mobility (Figure 2). The molecular weights corresponding to the bands were 18 000, 16 000, and 13 000. The molecular weight of the 0.8S protein estimated by gel filtration was found to be approximately 14 500. Using the same technique, Schwenke et al. (1973) have reported values of 14 600 and 14 900 as molecular weights for sunflower and rapeseed albumins, respectively. Madhusudhan and Singh (1985) have reported a molecular weight of 15 000 for the 1.6S protein of linseed.

The value of 0.8S for a molecular weight of 14 500 appears to be small. Perhaps this could be due to the compactness of the molecule. The protein has a high amount of cysteine; disulfide bridges may make the protein more compact.

The far-UV–CD spectrum in the range of 200–260 nm showed two minima at 208 and 220 nm, with $[\theta]_{MRW}$ values of −14.268 and −12.971 deg·cm^2/dmol, respectively (Figure 3). The computed α-helical content obtained by the methods of Greenfield and Fasman (1969) and Chen and Yang (1971) was 35%. The β-structure calculated according to the method of Sarkar and Doty (1966) was 56%.

Schwenke et al. (1973) have reported 40–45% α-helix and 11–16% β-structure for low molecular weight protein fractions of rapeseed. The 1.6S fraction of linseed proteins also contained 32% α-helix and 51% β-structure (Madhusudhan and Singh, 1985). Thus, the data on poppy seed low molecular weight proteins are similar to those of

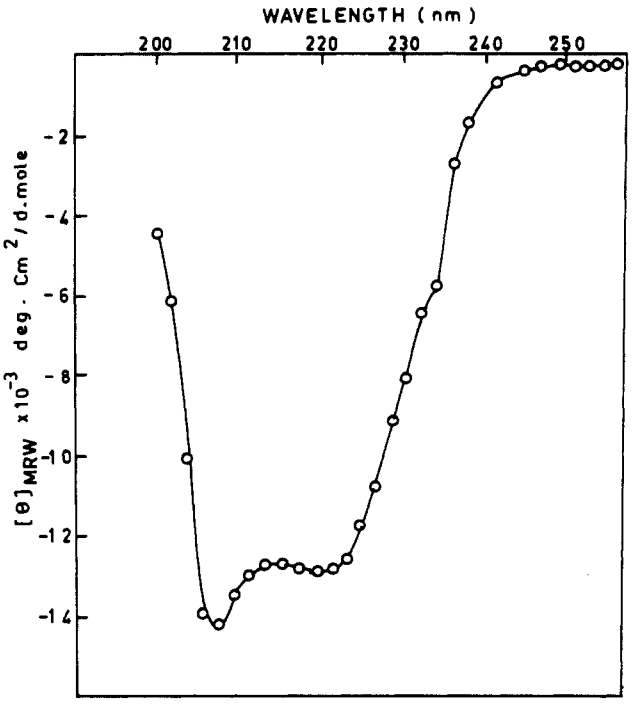

Figure 3. Far-UV-CD spectrum of poppy seed low molecular weight proteins in 0.5 M NaCl solution. Values are averages of two independent determinations.

linseed low molecular weight proteins but different from those of rapeseed proteins. This may be related to the composition and physicochemical properties of these proteins.

The data show that unlike the 10S protein that contains very little α-helix (Srinivas and Narasinga Rao, 1986), the low molecular weight protein fraction has a more ordered structure as evidenced by higher helical content.

The results indicated that the low molecular weight proteins consisted of three to five fractions. Apparently their molecular weights are very close to each other; SDS-PAGE indicated values ranging from 13000 to 18000. Because of this, it was difficult to resolve into homogeneous fractions in gel filtration. Attempts to isolate the homogeneous fractions by DEAE- or CM-Sephadex chromatography did not succeed. Since the proteins contained carbohydrate, attempts are being made to fractionate them to homogeneous protein by affinity chromatography.

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Analysis of Headspace Volatiles from Overheated Beef Fat

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Headspace volatiles from overheated beef fat were purged into the water of the gas-washing bottle and simultaneously were continuously extracted with dichloromethane. Gas chromatography/mass spectrometry analysis of the dichloromethane extract resulted in identification of 87 compounds. The compounds identified included 7 alkanes, 31 alkenes, 18 aldehydes, and 6 ketones. When the water of the gas-washing bottle was replaced with an aqueous cysteamine solution, trace quantities of six branched aldehydes not previously found in the extract from the water were recovered and identified as thiazolidine derivatives. The apparatus prepared in the present study may be used to collect large quantities of headspace volatiles under mild temperatures unlikely to cause chemical alteration of the volatiles.

The volatile components of cooked meats have been investigated by many researchers. Several reviews on beef flavors have been appeared in the last decade (Dwivedi, 1975; Chang and Peterson, 1977; Shibamoto, 1980). Volatile chemicals formed from animal fat alone have, however, not been thoroughly studied. Buttery et al. (1977) reported basic volatile components of roasted lamb fat. Ohnishi and Shibamoto (1984) investigated volatile chemicals formed from heated beef fat. They used simulated cooking temperatures of 150 and 200 °C. Many studies of heated fats have been done with what were called "laboratory-heated fats" in order to determine

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