Characterization of Glandless Cottonseed Storage Proteins by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis[1]

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ABSTRACT

Six fractions were obtained when a 10% NaCl extract of cottonseed storage protein was fractionated by gel-filtration chromatography. The polypeptide components from each fraction were characterized for size heterogeneity with sodium dodecyl sulfate-gel electrophoresis. The results showed that covalent bonding was present between the larger subunits of the individual globulin fractions. Hydrophobic interactions contributed substantially to the stability of the globulin fractions, as smaller subunits appeared as noncovalently linked aggregates. The 7S globulin, when obtained by gel-filtration chromatography, was similar to Acalin A prepared by cryoprecipitation in numbers and molecular weights of the polypeptides present in the fractions.

Cottonseed has the potential to be an important alternate food source capable of fulfilling protein nutritional requirements. Because cottonseed is deficient in some of the essential amino acids (EAA) (eg, lysine, isoleucine, and methionine), the nutritional quality of its protein is only moderate. It is therefore of interest to improve the quality of cottonseed protein through breeding programs and to identify important polypeptides having an improved EAA composition. One possible avenue for this improvement would be to develop cotton varieties that produce seed having proportions of the polypeptide components with this enriched EAA content. In particular, knowledge of some of the physicochemical characteristics of polypeptides that comprise the basic structure of the storage proteins can contribute to the goal of obtaining the desired nutritional quality.

Previous investigations have shown that disulfide bonding does not occur between all cottonseed polypeptide subunits. Martinez et al (1970) reported that ultracentrifugal, electrophoretic, and chromatographic characterization of the 12S and 7S globulins are not affected by the addition of dithiothreitol (DTT). Ovchinnikova et al (1975) and Redina et al (1976) found that the 7S globulin contained eight polypeptide subunits with no disulfide bonds. The globulin consists of two types of basic subunits having molecular weights of 18,500 and 16,000. A similar study (Ibragimov et al 1974) showed that the 7S globulin had a quaternary structure consisting of 10 polypeptides and no intermolecular covalent bonds.

Conversely, Wallace (1976) and Dieckert et al (1981) found that in the major cottonseed globulins, which they designated Acalin A and Acalin B, the principal subunits are covalently linked by disulfide bonds. The primary polypeptide subunit of Acalin A has a molecular weight of 98,000. Reduction of this subunit with 2-mercaptoethanol (2-ME) produces two subunits that both have molecular weights of 49,000 but may not be identical. Acalin B has two subunits with molecular weights of 35,000 and 42,000 that, on reduction, produce polypeptides with molecular weights of 20,000 and 15,000, and 20,000 and 22,000, respectively. Treating the 7S globulin with 2-ME results in the disappearance of a 100,000 mol wt polypeptide (Zarins and Cherry 1981). Cunningham et al (1978) reported that a mixture of the 7S and 12S globulins had reduced polypeptides with molecular weights that were similar to those determined by Wallace (1976) and by Dieckert et al (1981).

Cottonseed storage protein may be extracted and fractionated by any of several methods (Rossi-Fanelli 1964, Berardi et al 1969, Podgornov et al 1974, Wallace 1976, Dieckert et al 1981, Zarins and Cherry 1981). The characterization of the subunit composition and the interactions (covalent or noncovalent) between the polypeptide subunits as determined by sodium dodecyl sulfate-gel electrophoresis has previously focused on the complete storage protein fraction and the 7S or 12S globulins. In this article, we report the electrophoretic characterization of six individual fractions of storage protein obtained by gel filtration chromatography (Zarins and Cherry 1981). We have also used different combinations of denaturant and reducing agent to assess the subunit structural stability of storage protein.

MATERIALS AND METHODS

Storage Protein Extraction and Fractionation

Cottonseed storage protein was extracted from Liquid Cyclone processed glandless cottonseed flour (Southern Regional Research Center) and fractionated by gel-filtration chromatography (Sephadex G-200) as described by Zarins and Cherry (1981) (Fig. 1).

The effluent for individual peaks was pooled, concentrated by ultrafiltration, and purified by rechromatography with the G-200 column.

SDS-Gel Electrophoresis

Except where the variations are noted, the procedures of Weber and Osborn (1969) were used for the SDS-gel electrophoresis of the cottonseed storage proteins. 2-ME was used for the reduction of disulfide bonds, except where noted. The total acrylamide concentration was 10%, and the crosslinker (*N,N* methylenebisacrylamide) concentration (proportion of crosslinker to total acrylamide-crosslinker concentration) was 1.2%. Before electrophoresis, protein solutions were prepared by dissolving either protein or by mixing a protein solution with 1% SDS, 1% 2-ME in 0.01 *M* sodium phosphate buffer (pH 7.0) to result in a protein concentration of approximately 2.4 mg/ml. Protein solutions were then incubated at 37° C for 2 hr. After pretreatment, 200 μl of protein sample solution was mixed with 200 μl of glycerol and 12 μl of bromphenol blue. Fifty microliters of this solution was applied to each gel. The electrophoresis was a continuous system having a 0.01 *M* sodium phosphate buffer (pH 7.0) containing 0.1% SDS (w/v) and 0.1% 2-ME (v/v). Vertical tube gels (5 × 67 mm) were used, and the electrophoresis was conducted at 8 mA per tube for 5 hr. The gels were fixed with a 15% (w/v) trichloracetic acid solution containing 25% (v/v) isopropanol for 16 hr at 5°C. The gels were stained for 16 hr with a solution containing 0.02% Coomassie brilliant blue R250, 15% (v/v) methanol, and 6% (v/v) acetic acid. Destaining was achieved in 48 hr with a solution of 5% (v/v) methanol and 7.5% (v/v) acetic acid. Stained gels were scanned at 570 nm with a Schoeffel Spectrodensitometer, model SD 3000. The molecular weights of the polypeptides were estimated from a standard curve of molecular weight standard versus relative

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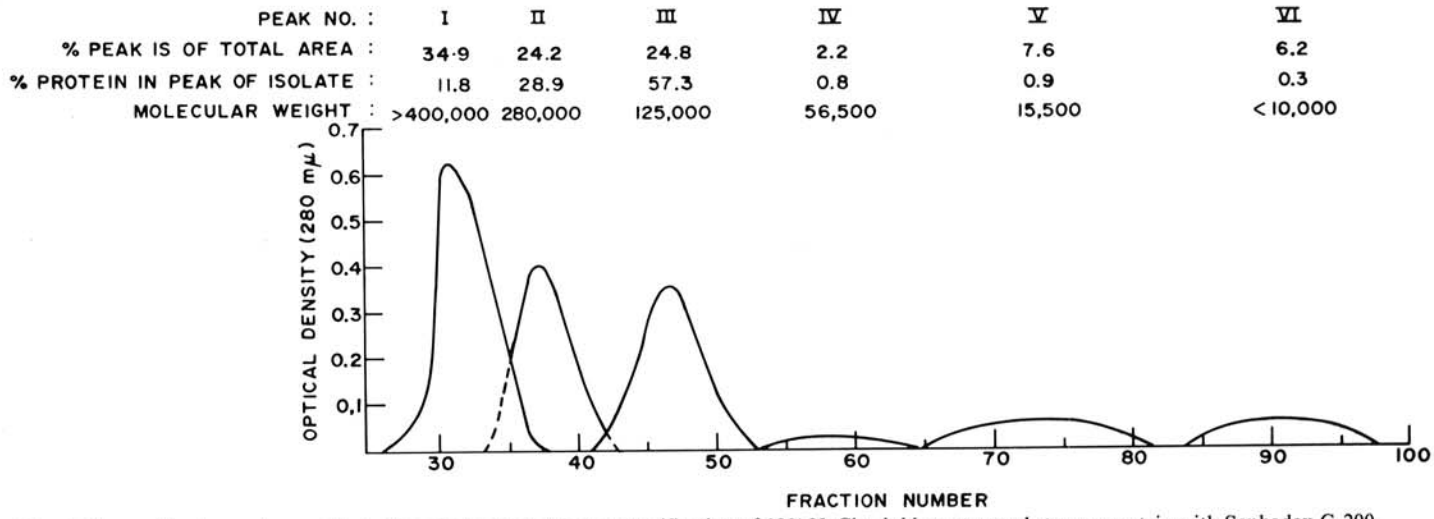

Fig. 1. Composite chromatogram illustrating the fractionation and purification of 10% NaCl soluble cottonseed storage protein with Sephadex G-200.

mobility. The proteins used to prepare the standard curve were thyroglobulin, collagenase, lipoxidase, bovine serum albumin, catalase, pyruvate kinase, ovalbumin, aldolase, lactic dehydrogenase, chymotrypsinogen A, trypsin, myoglobin, ribonuclease, hemoglobin, α-chymotrypsin, cytochrome C, and insulin.

The polypeptide subunit composition of the total storage protein fraction was evaluated with different concentration levels of common denaturants-reductants. For this series of trials, the SDS-gel electrophoresis methodologies were unchanged, with the exception of the variations specifically shown in Fig. 2.

RESULTS AND DISCUSSION

The extracted cottonseed storage proteins were fractionated by gel-filtration chromatography. Figure 1 is a composite chromatogram for the three-cycle purification of the isolated fractions obtained from the 10% NaCl extract.

By using different combinations and concentrations of SDS, urea, 2-ME, and DTT, we examined the electrophoretic behavior of a 10% NaCl extract of storage protein. The results are shown in Fig. 2. Protein samples treated with SDS or with combinations of SDS and urea have the same multibanded electrophoretic profiles. Disruption of the cottonseed globulins into individual components after treatment with the amphiphile SDS or with the denaturant urea shows that hydrophobic interactions have been disrupted; this contributes heavily to maintenance of the storage protein's native quaternary structure. Additionally, we found that treating the storage protein with 2-ME or with DTT results in three new protein bands of lower molecular weight, as seen in the electrophoresis gels of Fig. 2. This is clear evidence of a subunit composition stabilized by the presence of intermolecular disulfide bonds.

Each storage protein fraction obtained from the Sephadex G-200 gel purification was analyzed for subunit composition and size heterogeneity by SDS-gel electrophoresis in the presence of the reducing agent 2-ME. A representative gel and densitometer scan for each fraction are given in Fig. 3. Table I lists the molecular weights of the polypeptides as determined by SDS-gel electrophoresis (Weber and Osborn 1969). Fractions I and II had similar polypeptide profiles (Fig. 3). Fractions III and IV had similar profiles, especially in the molecular weight range of 24,000–63,000. Because of its large molecular weight (>400,000) and low protein content (Zarins and Cherry 1981), fraction I may consist of fragments of membrane-bound polypeptides, ie, portions of protein bodies or aleurone grains bound to polypeptides of the storage globulins (Dieckert and Dieckert 1976). The polypeptides may be those of fraction II. The electrophoretic profiles of the subunits of fractions III and IV may be similar because of association-dissociation between the polypeptides of the 7S

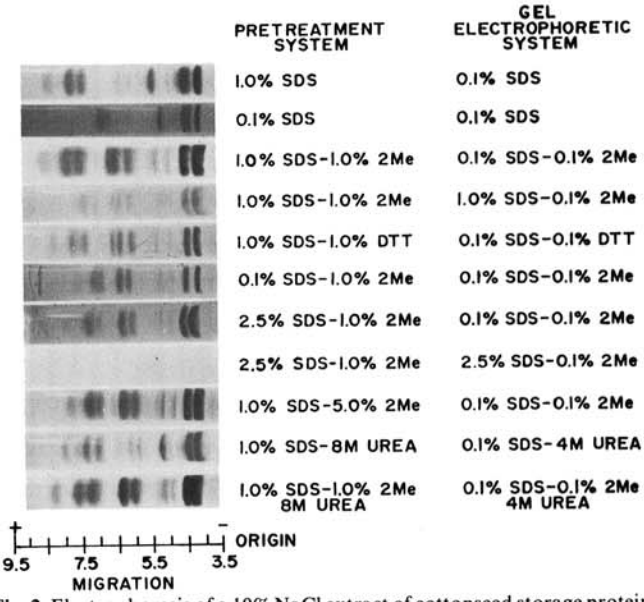

Fig. 2. Electrophoresis of a 10% NaCl extract of cottonseed storage protein, as affected by concentration variations of common denaturants-reducing agents.

globulin (peak III), which produces in the separation profile observed in Fig. 1. In the isolate and in fraction III (7S globulin), a few polypeptides have molecular weights of 70,000 daltons or more. These appear to be subunits that did not react with 2-ME. Because the storage protein globulins are so compact, the pretreatment conditions (37°C, 2 hr) could not totally reduce all disulfide bonds within the storage protein. Zarins and Cherry (1981) postulated that fractions II and III, as isolated by gel-filtration chromatography, are equivalent to Acalin B and Acalin A, respectively (Wallace 1976, Dieckert et al 1981). The subunit profile composition and the corresponding molecular weights we determined for these two fractions support this proposal.

Reduced Acalin A contains seven polypeptides that have molecular weights from 18,000 to 53,000 (Dieckert et al 1981). Similarly, if the unreduced subunits were omitted, fraction III would contain six polypeptides with molecular weights similar to those of Acalin A. The differences between the polypeptides of the two fractions are not significant. Wallace (1976) proposed that some of the lower-molecular-weight subunits in Acalin A are products of proteolytic digestion. According to Wallace, proteolysis is likely to occur with longer extraction times, and to give rise to isolates with different electrophoretic patterns in numbers of polypeptides and molecular weights. This is one

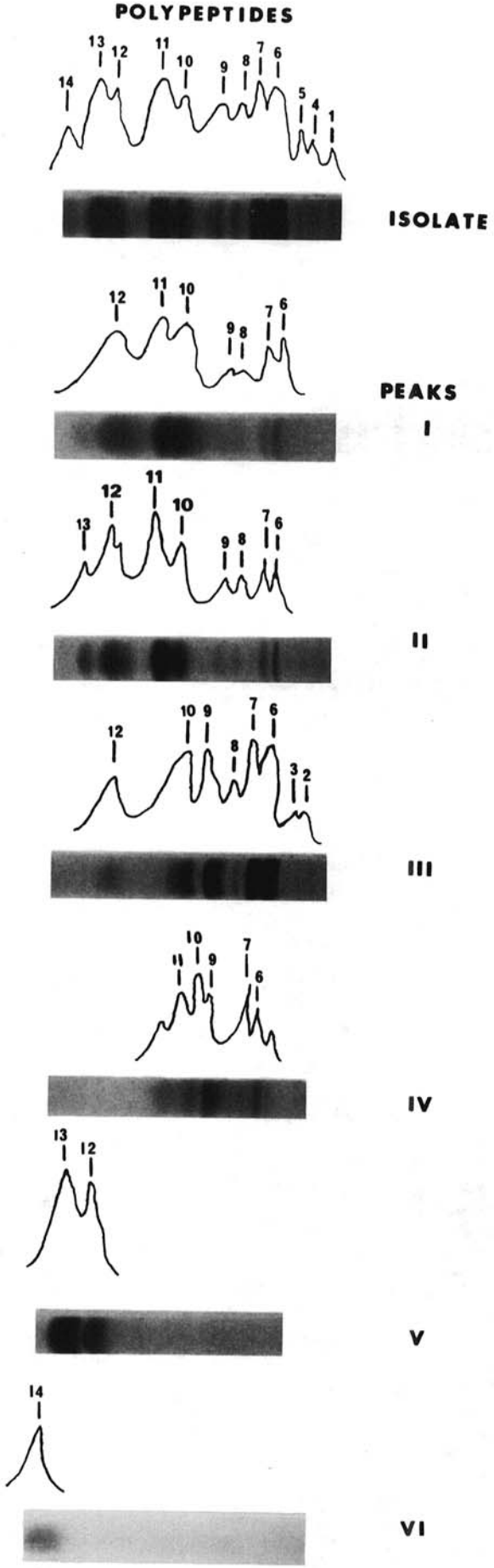

Fig. 3. Densitometer scans of SDS,2-ME-gel electrophoresis of purified individual fractions of a 10% NaCl extract of cottonseed storage protein.

TABLE I
Subunit Molecular Weights[a] as Estimated by Sodium Dodecyl Sulfate-Electrophoresis

Peak[b]	Fraction	Molecular Weight
1	Isolate	82,300
2	III	80,200
3	III	79,000
4	Isolate	75,000
5	Isolate	70,000
6	Isolate, I, II, III, IV	63,000
7	Isolate, I, II, III, IV	56,000
8	Isolate, I, II, III	41,000
9	Isolate, I, II, III, IV	35,000
10	Isolate, I, II, III, IV	26,000
11	Isolate, I, II, IV	21,200
12	Isolate, I, II, III, V	15,800
13	Isolate, II, V	11,700
14	Isolate, VI	10,000

[a]Present in the 10% NaCl isolate and in the individual fractions I-VI.
[b]Corresponds to number in Fig. 4.

possible explanation for why the smaller subunits in the two studies are different. Moreover, the variability that results will be greater when the molecular weight of small polypeptides is determined with a gel of 10% total acrylamide content. The results obtained do not show any significant deviations in number of polypeptides or size heterogeneity in similar fractions obtained by different isolation techniques (Wallace 1976, Dieckert et al 1981, Zarins and Cherry 1981). Differences in variety of cottonseed, in maturity, and in proteolysis during extraction, either singly or in combination, could account for the differences found. Intermolecular covalent linkages, the disulfide bonds, are more important to the stability of the native subunit composition of cottonseed storage protein than earlier studies indicated (Ovchinnikova et al 1975, Redina et al 1976).

CONCLUSIONS

Heterogeneity in charge and in molecular weight are characteristic of the polypeptides of the storage protein of seeds. High-resolution gel electrophoresis can be useful in characterizing the components of storage protein. The subunit composition of fractions isolated from a 10% NaCl extract of cottonseed storage protein and purified by Sephadex G-200 gel-filtration chromatography is similar to that of other investigations. Intermolecular covalent bonds (disulfide bonds) are important to the integrity of the structure of cottonseed storage globulins. Hydrophobic interactions contribute considerably to the quaternary structure of the cottonseed globulins by holding together the polypeptides of lower molecular weight.

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