

ALBUMIN POOLS WITH DIFFERENT EXTRACTION BEHAVIORS IN WHEAT SEED

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ABSTRACT

Several consecutive extractions with water failed to extract wheat albumins completely from whole wheat flour; a significant amount of these albumins (about one-third the amount extracted by water) could be extracted only by salt solutions. A 0.26M (NH₄)₂SO₄ solution was the most suitable for extraction of this albumin fraction. Two albumin fractions were obtained by salting out the first water extract from flour with (NH₄)₂SO₄ between 0.4 and 1.8M (albumin I) and between 1.8 and 4.0M (albumin II). Only one albumin fraction (albumin III) was obtained from the 0.26M (NH₄)₂SO₄ extract of the flour residue remaining after five consecutive water extractions. Although different in their

extraction behaviors, albumin I and albumin III fractions were both soluble in distilled water and were precipitated from the wheat extract in the same concentration range of (NH₄)₂SO₄ (0.4 to 1.8M). When submitted to polyacrylamide-gel electrophoresis, these two albumin fractions were both heterogeneous with slightly different electrophoretic patterns. Moreover, they exhibited identical staining properties with aniline blue-black as well as similar gel filtration and amino acid patterns. These data are consistent with the presence in wheat kernel of two closely related albumin pools, one of the two being bound by salt linkages to some water-insoluble components of the seed.

In 1907 Osborne (1) introduced the term "wheat albumin" to indicate the water-soluble protein fraction extracted from wheat flour by means of water or dilute salt solutions. Further studies, extensively reviewed by Bailey (2) and Blish (3), showed the presence in such extracts of significant amounts of gliadin. In 1953 Pence and Elder (4) showed that an albumin preparation, free of gliadin and globulin, can be obtained by salting out wheat seed water extracts between 0.4 and 1.8M (NH₄)₂SO₄. These authors first showed that wheat albumin is a quite heterogeneous group of proteins with similar solubility properties. Since that time many studies carried out by means of gel electrophoresis or filtration have confirmed the heterogeneity of wheat albumin. As shown by typical blue-black bands (5) obtained by submitting wheat albumin samples prepared according to Pence and Elder (4) to polyacrylamide-gel electrophoresis and staining with aniline blue-black, the components of the albumin group are also related from a structural standpoint and, in this respect, clearly differ from globulin and gliadin components that gave blue-green and reddish-brown bands, respectively. Physicochemical characterization data concerning some purified albumin components suggest that wheat albumins might be constituted of a small number of isoprotein families possibly phylogenetically related (6,7). Recent studies from our laboratory (8) have shown the presence in wheat seed of two pools of a *bread* wheat-specific cathodic albumin different in their extraction behaviors, but identical in their solubility and immunological properties. This paper deals with experiments designed to establish whether significant amounts of albumins with anomalous extraction behaviors are present in wheat seed. We also evaluate the extraction procedures for wheat albumins with regard to their selectivity and completeness.

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MATERIALS AND METHODS

Materials

The hexaploid wheat, pure Mentana variety, was purchased from Istituto di Genetica per la Cerealicoltura (Rome, Italy) and milled in a laboratory-type mill (Bühler-Switzerland). Reagents for polyacrylamide electrophoresis were purchased from Canalco Industrial Corporation (Rockville, Md.). All other chemicals used were reagents of analytical grade provided by Carlo Erba (Milan, Italy).

Protein Extraction

Twenty grams of finely ground whole wheat flour (var. Mentana) was extracted at room temperature for 3 hr in a shaker with 300 ml water. After extraction, the suspension was centrifuged for 30 min at $45,000 \times g$. Further extractions of the residue from the first extraction were carried out, under identical experimental conditions, by mechanical mixing of the sediment in 300 ml water. These experimental conditions were also used for the extraction of the water-extracted residue with $(\text{NH}_4)_2\text{SO}_4$ solutions of increasing molarity (as described in Fig. 4).

Fractionation of Protein Extracts

Water and salt extracts were submitted to fractionation by salting out with $(\text{NH}_4)_2\text{SO}_4$. The first protein precipitate was obtained by adding salt up to a concentration of $0.4M$, keeping the suspension at 4°C for 3 hr, and centrifuging it for 30 min at $45,000 \times g$. The second protein precipitate was obtained from the clear supernatant by adding $(\text{NH}_4)_2\text{SO}_4$ up to a concentration of $1.8M$ under identical experimental conditions and the last one was obtained at $4.0M$. The amounts of proteins precipitated at each $(\text{NH}_4)_2\text{SO}_4$ concentration were calculated by measuring protein concentration of the clear supernatant, before and after each precipitation, according to the spectrophotometric method of Waddel (9). The three precipitates were separately resuspended in water, dialyzed against water, and freeze-dried to be further analyzed by gel filtration and electrophoresis.

Gel Filtration

About 100 mg of freeze-dried protein was dissolved in 5 ml of a pH 7.4 phosphate buffer (137 mM NaCl, 3 mM KCl, 1 mM KH_2PO_4 , 8 mM Na_2HPO_4 , and 15 mM NaN_3), applied on a column (110×0.8 cm) of Sephadex G-100, and eluted with the same phosphate buffer at a flow rate of 60 ml per hr. The absorbance of the eluate at 280 nm was monitored (Photochrome flow analyzer, Rastelli, Italy) and recorded continuously (Kompensograph, Siemens, Germany). Molecular weights of the protein peaks were determined according to Andrews (10) by comparing their elution volumes with those of the following proteins of known molecular weights: cytochrome C (mol wt 12,500) and chymotrypsinogen (mol wt 25,700) from Sigma Chemical Company (St. Louis, Mo.), bovine serum albumin (mol wt 69,000) from Armour Pharmaceutical Company (Chicago, Ill.), and blue dextran 2000 from Pharmacia (Uppsala, Sweden).

Polyacrylamide Gel Electrophoresis

Protein samples (about 400γ) were applied at the top of the gel in an 11% sucrose solution. All experimental conditions used for anodic electrophoretic

migrations and for detection of electrophoretic patterns were similar to those previously described by Minetti *et al.* (5).

Amino Acid Analysis

Amino acid compositions of the studied albumin and globulin fractions were determined according to the chromatographic technique of Spackman *et al.* (11). Duplicate samples (2 ml HCl 6*N* per 10 mg of protein) were hydrolyzed at 110° C under vacuum for 24, 48, and 72 hr. The analyses were performed automatically by means of a Technicon AA Analyzer (Technicon Instrument Corporation, Ardsley, N.Y.) using a 140 × 0.6-cm column filled with chromo-bead resins (type A), according to the Technicon standard method.

RESULTS

As shown in Fig. 1, the protein composition of five consecutive water extracts from whole wheat flour was strongly dependent on the extraction number. The amount of protein salted out at 0.4*M* (NH₄)₂SO₄ increased up to 100% in the fifth extract, whereas amounts of proteins precipitated at 1.8 and 4.0*M* (NH₄)₂SO₄ rapidly decreased with increase of the extraction number.

The gliadin nature of the protein fraction precipitated from the first water extract at 0.4*M* (NH₄)₂SO₄ was shown by submitting this fraction to polyacrylamide-gel electrophoresis in a *tris*-glycine buffer (pH 8.5) according to Minetti *et al.* (5). The electrophoretic pattern of this fraction was typical of gliadin with all the protein bands exhibiting mobilities lower than 0.2 (as compared to bromphenol-blue mobility taken as 1) and a reddish-brown color after staining with aniline blue-black (5,12,13). The extraction behavior of this

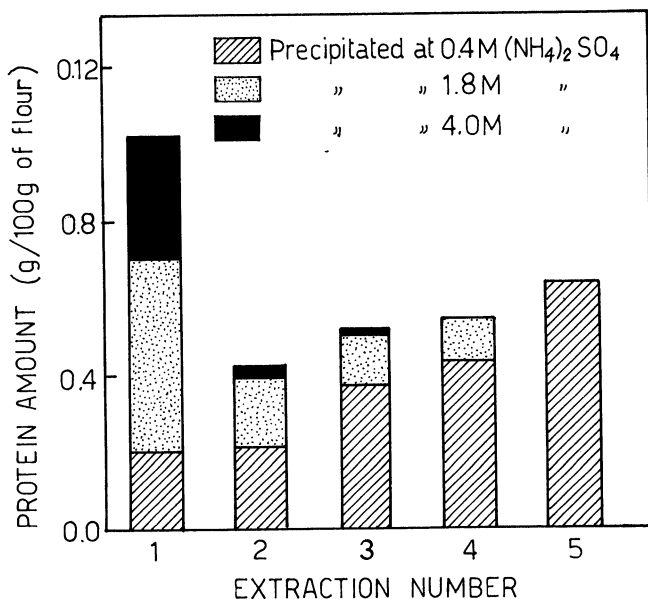


Fig. 1. Protein composition of five consecutive water extracts from wheat seed.

fraction (Fig. 1) was consistent with the well-known low water solubility of gliadin that is further reduced by the presence of natural electrolytes (14,15) and albumins.

When submitted to electrophoresis under identical experimental conditions, the two fractions precipitated between 0.4 and 1.8M or between 1.8 and 4.0M $(\text{NH}_4)_2\text{SO}_4$ showed (Fig. 2) electrophoretic patterns typical of albumin with all the bands exhibiting mobilities in the range 0.2 to 0.6 and a blue-black color after staining with aniline blue-black (5,16). We will refer to these two albumin fractions as "albumin I" and "albumin II." Molecular weights of the components of albumins I and II, as calculated by gel filtration on Sephadex G-100 (Fig. 3), were consistent with those generally reported (17, 18, 19) for wheat albumins. Whereas peak A was excluded in the column void volume, peaks B,C, and D exhibited molecular weights of 60,000, 24,000, and 12,000 daltons, respectively. As shown in Fig. 3, low-molecular-weight components were present in both albumins I and II. Albumin I, as compared to albumin II, contained higher amounts of fractions with higher molecular weights. As shown in Table I, amino acid compositions of these two albumin fractions were distinctly different, but both of them were clearly related to the amino acid composition of wheat albumin reported by Feillet (20) that, but for some minor differences, is generally reported in the literature as typical of wheat albumin.

By using as extraction solvent $(\text{NH}_4)_2\text{SO}_4$ solutions of increasing molarity, we were able to further extract from the residue remaining after five water extractions significant amounts of a protein fraction that can be precipitated between 0.4 and 1.8M $(\text{NH}_4)_2\text{SO}_4$ (Fig. 4). This protein fraction with an albumin-like precipitation behavior that could not be extracted with distilled water was named "albumin III." As shown in Fig. 4, a 0.26M $(\text{NH}_4)_2\text{SO}_4$ solution was the most effective for the extraction of albumin III. The heterogeneity of albumin III was shown by polyacrylamide-gel electrophoresis and gel filtration on Sephadex G-100. Staining properties of albumin III bands with aniline blue-black were identical to those of albumin I, whereas electrophoretic mobilities were slightly

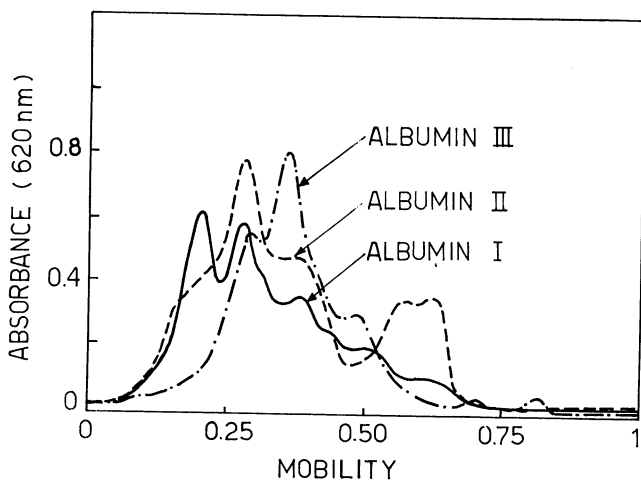


Fig. 2. Polyacrylamide-gel electrophoretic patterns of albumin fractions from wheat seed.

different (Fig. 2). Gel filtration profiles of albumin III and albumin I mainly differed from a quantitative standpoint (Fig. 3). In particular, albumin III did not contain peak A and contained a higher percentage of peak B. Albumins I and III exhibited very similar amino acid compositions (Table I), thus further confirming their close similarity.

The 0.26M (NH₄)₂SO₄ extract was almost free of gliadins, but contained a relevant amount of proteins that were precipitated between 1.8 and 4.0M

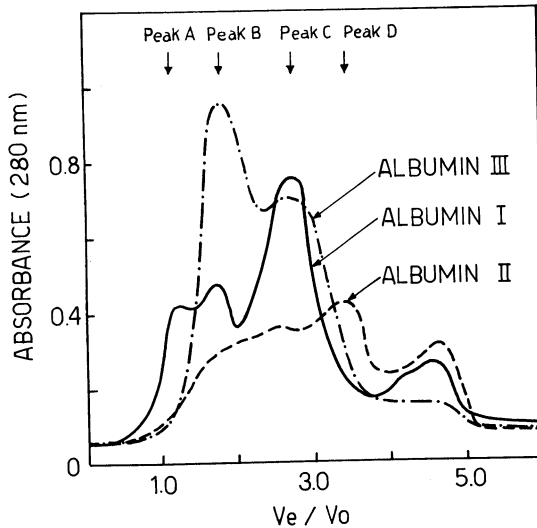


Fig. 3. Gel filtration patterns on Sephadex G-100 of albumin fractions from wheat seed. V_e = effluent volume; V_o = void volume.

TABLE I
Amino Acid Composition of Albumin Fractions from Wheat Seed

Amino Acid	g Amino Acid per 100 g Amino Acid Recovered			
	Albumin I	Albumin II	Albumin III	Albumin Feillet (20)
Lysine	2.5	5.8	2.5	4.7
Histidine	1.8	2.5	2.7	2.7
Arginine	5.0	7.1	5.1	6.6
Aspartic acid	5.2	9.6	5.4	7.7
Threonine	3.4	4.7	3.5	4.1
Serine	4.1	4.7	4.0	5.2
Glutamic acid	28.3	14.5	28.8	21.0
Proline	10.5	6.6	11.0	7.9
Glycine	3.7	5.6	3.4	4.7
Alanine	4.7	6.5	3.3	5.7
Valine	5.6	6.5	5.3	6.4
Methionine	2.2	1.8	2.1	2.3
Isoleucine	3.3	3.5	3.8	4.0
Leucine	6.8	7.4	7.4	8.0
Tyrosine	4.2	4.7	3.9	4.4
Phenylalanine	4.0	3.8	3.6	4.2

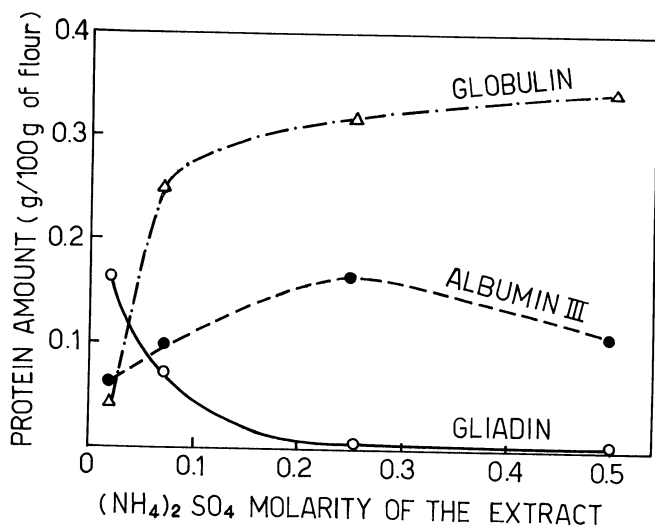


Fig. 4. Protein composition of $(\text{NH}_4)_2\text{SO}_4$ extracts of flour residue remaining after five consecutive water extractions.

$(\text{NH}_4)_2\text{SO}_4$ (Fig. 4). Amino acid composition of this fraction was typical of a wheat globulin preparation (20).

DISCUSSION

The data show that the anomalous extraction properties of a bread wheat-specific cathodic albumin shown by Minetti *et al.* (8) are also common to other albumin components that represent about one-fourth of the total albumin content of the seed. The extraction of a clearly defined albumin fraction of wheat seed (albumin III), in fact, cannot be achieved using distilled water and requires salt solutions. Although different in their extraction behaviors, albumin I and albumin III pools showed similar solubility and physicochemical properties. They both were soluble in water and in a $0.4M$ $(\text{NH}_4)_2\text{SO}_4$ solution, but were not soluble in a $1.8M$ $(\text{NH}_4)_2\text{SO}_4$ solution. No significant difference was evident in the color reaction of these two protein pools with aniline blue-black as well as in their amino acid compositions. Gel filtration and gel electrophoresis, however, showed in the albumin III pool a higher percentage of components with higher molecular weight and more acidic character. At this moment we have no explanation for the presence of these two albumin pools in the seed, although we suggest that a part of the albumin components might be bound to unidentified water-insoluble components of the seed by salt linkages that can be broken at high ionic strength. In this respect, it is quite suggestive that albumins with more acidic character are present in higher amount in the albumin pool that cannot be extracted with water. In conclusion our data clearly show that the salt concentration of the extract is not only critical for the extraction of globulins (20) and gluten proteins (21), but for the extraction of albumins as well. We have confirmed data by Pence and Elder (4) concerning the absence of gliadin and

globulin in the protein fraction precipitated between 0.4 and 1.8M (NH₄)₂SO₄, and have also shown that no quantitative recovery of the albumins extracted can be obtained with this procedure. In the adopted experimental conditions, in fact, about one-third of the extracted albumins (mainly those with lower molecular weight) remained in the 1.8M (NH₄)₂SO₄ supernatant and required higher salt concentrations to be precipitated. Since the amount of albumins that do not precipitate at 1.8M (NH₄)₂SO₄ depends upon the total protein concentration of the extract, it appears that no reproducible albumin preparation can be obtained without accurately controlling not only the ionic strength of the extraction solvent but also the ratio of flour to solvent used for the protein extraction.

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