Soluble Proteins of Wheat. III. Isolation and Characterization of Two Albumins, ALB 13A and ALB 13B, from Flour[1]

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ABSTRACT

Two albumins were purified from extracts of common wheat flour through consecutive fractions by preparative free-flow electrophoresis, gel-permeation chromatography (Sephadex G-50), and DEAE-cellulose chromatography. The isolated proteins, designated ALB 13A and ALB 13B by the Feillet system, were homogeneous in polyacrylamide and starch-gel electrophoresis at pH 3.1 and 8.4. They had the same mobility, -2.5×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}, in free electrophoresis in veronal buffer at pH 8.6. ALB 13A contained all the common amino acids, with alanine predominating, and no free sulfhydryl. ALB 13A had a relatively high valine content, no phenylalanine, histidine, or free sulfhydryl. Molecular-weight values for 13A were 24,800, 20,100, and 19,800 from sedimentation equilibrium, amino acid analysis, and gel-permeation chromatography, respectively. For 13B, corresponding values were 13,900, 12,900, and 11,200. $E_{1\,cm.}^{1\%}$ was 13.35 (278 mμ) and 20.50 (274 mμ) for 13A and 13B. Fluorescence spectra were typical of tryptophan-containing proteins. Circular dichroism measurements suggested 25 to 30% alpha-helix for 13A, about 40% for 13B. ALB 13A and ALB 13B are apparently absent from durum wheats.

The heterogeneity of wheat albumins has been demonstrated by several workers, as recently summarized (1). A classification based on their mobility in starch-gel electrophoresis in Tris buffer at pH 8.9 has been described (2), designating 21 detectable components. The component designated ALB 13 is one of the most abundant among the albumins in common wheat, but is apparently absent or in low concentration in durum wheats (constituents *a* of refs. 3 and 4). By DEAE-cellulose chromatography and polyacrylamide-gel preparative electrophoresis (5), Feillet and Bourdet succeeded in isolating ALB 13 as a single component, as judged by gel electrophoresis (6,7).

The work reported here, directed toward further characterization of the ALB 13, has demonstrated that it was a mixture of two components, now designated ALB 13A and ALB 13B, not differentiated by electrophoresis, but of different molecular weight as shown by separation by gel-permeation chromatography. Procedures for isolating these two proteins are described, and several of their properties are reported. The properties are compared with those of the wheat-soluble proteins separated and characterized by Kelley (8), Tkachuk and Tipples (9,10) and Fish (11).

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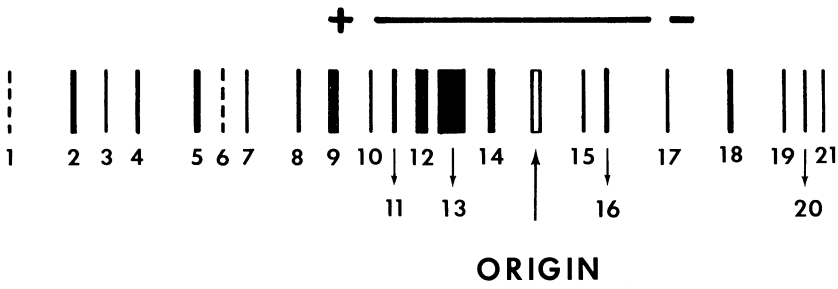

Fig. 1. Nomenclature of wheat albumins according to their electrophoretic mobility in starch gel in Tris buffer (2).

MATERIALS AND METHODS

Wheat

A commercial HRW baker's patent wheat flour, unbleached, unbrominated, and unmalted, was used for most of the preparations. For work comparing common and durum wheats, varietal samples were obtained from the North Dakota Agricultural Experiment Station.

Protein Nomenclature

The proteins are designated by number according to the starch-gel electrophoretic classification of Feillet and Bourdet (2), as given in Fig. 1.

Preparation of ALB 13A and ALB 13B

Protein mixtures were obtained from the salt-solution extract described below and, in some cases, as mixtures previously prepared as ALB A by salting-out (2), as ALB 13 from chromatography and electrophoresis (7), or from the ion-exchange procedure of Nimmo et al. (12).

Salt Solution Extract. Flour (100 g.) was dispersed by gentle stirring in 200 ml. of buffer (15.13 g. Tris, 1.50 g. EDTA, 1.25 g. boric acid per liter, pH 8.8), for 1 hr. at 4°C., and centrifuged 30 min. at 30,000 × g. The supernatant solution was dialyzed 24 hr. against 4 liters of the same buffer, then recentrifuged.

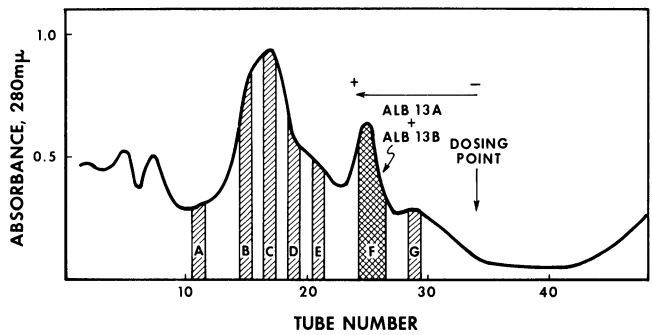

Fig. 2. Free-flow electrophoresis fractionation of buffer-soluble proteins of flour (Tris buffer, pH = 8.8, 1,850 v., 90 ma.).

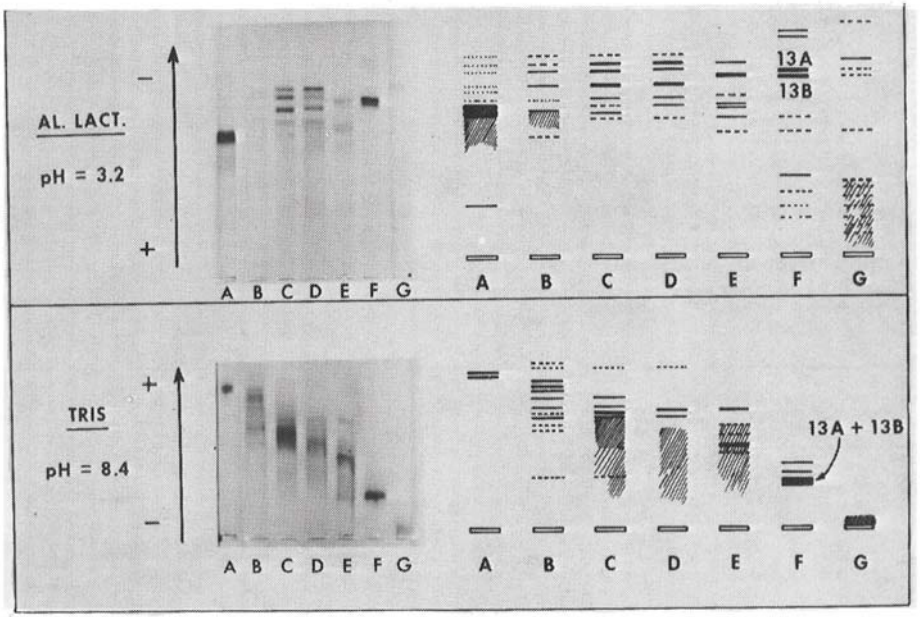

Fig. 3. Polyacrylamide-gel electrophoresis of wheat-soluble protein fractions obtained by free-flow electrophoresis fractionation (Fig. 2).

Preparative Electrophoresis. The extract was fractionated on a free-film continuous electrophoresis apparatus (Elphor FF-1) at about 37 volts per cm., 90 ma., 10°C., in the same buffer, with sample injection at 2.5 ml. per hr. and buffer flow at 80 ml. per hr. (Figs. 2 and 3).

Gel-Permeation Chromatography. Fraction F of Fig. 2 was dialyzed against deionized water, lyophilized, and fractionated on Sephadex G-50 in 0.1M ammonium acetate, pH 6.9, in a column 4 X 80 cm. Protein load was 200 mg. in 10 ml. Each effluent tube collected 10 ml. The separated fractions (Figs. 4 and 5) were

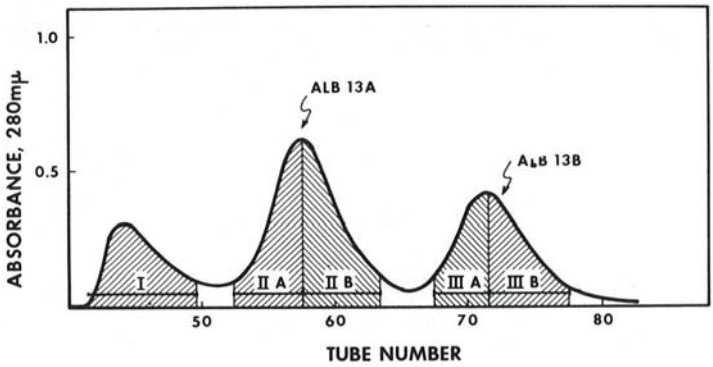

Fig. 4. Gel-permeation chromatography of fraction F (Fig. 2) (Sephadex G-50 0.1M ammonium acetate, pH 6.9).

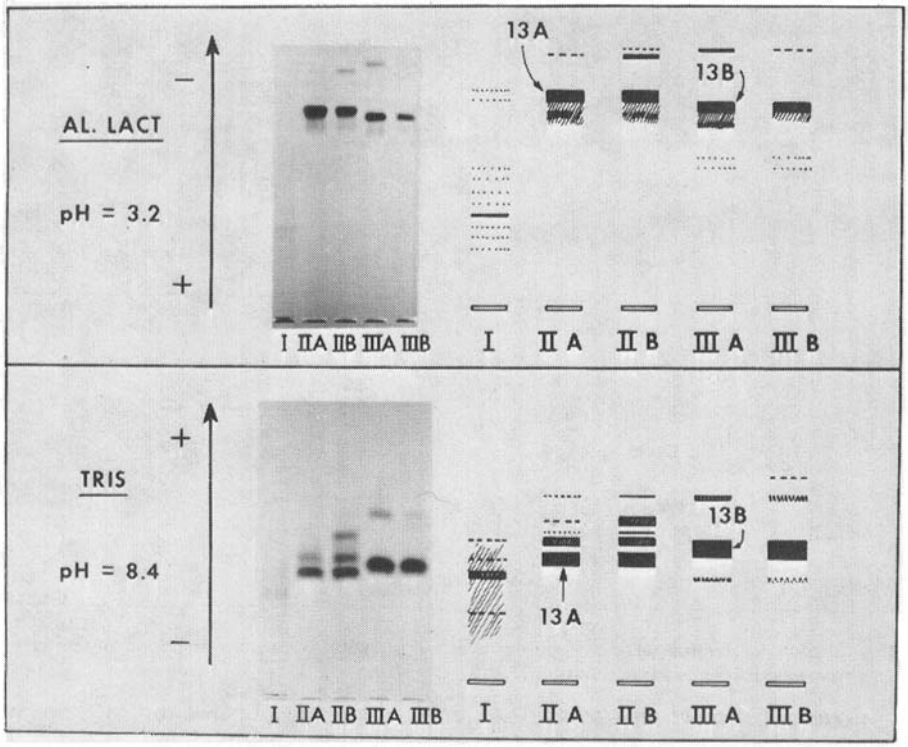

Fig. 5. Polyacrylamide-gel electrophoresis of the fractions obtained by gel-permeation chromatography (Fig. 4) of fraction F.

dialyzed for 1 hr. against water, lyophilized, and relyophilized from a solution in 3 to 4 ml. of water to eliminate the volatile salt.

Ion-Exchange Chromatography. The two fractions IIA and IIIB (Fig. 4), selected to avoid mutual contamination of 13A and 13B, were fractionated on DEAE-cellulose as previously described (13) with slight modification. Fifty milligrams of either fraction, dissolved in buffer A (24 mg. boric acid, 29 mg. KCl, 382 mg. Na_2CO_3 per liter, pH 10.4), was applied to a 1 X 30-cm. column of DEAE-cellulose (Whatman DE-32) equilibrated with the same buffer. Adsorbed proteins were eluted by a convex salt gradient, 0.00 to 0.04M NaCl; a mixing chamber of 100 ml. of buffer A, and a limit buffer containing 2.32 g. NaCl in 1 liter of buffer A were used. A second limit buffer (120 g. NaCl in 1 liter of buffer A) was started at the point marked "NaCl 2M" (Figs. 6,7,8,9). Each collection tube contained 10 ml.

Electrophoresis

Free-boundary electrophoresis was carried out in a Perkin-Elmer model 38A apparatus in sodium veronal buffer, pH 8.6, $\Gamma/2 = 0.1$, conductivity 0.00302 $ohm^{-1}\,cm.^{-1}$ (0°C.). Mobility was calculated by reference to bovine serum albumin ($\mu = 6.6$, Longsworth and Jacobsen (14)). Migration rates for the wheat-protein

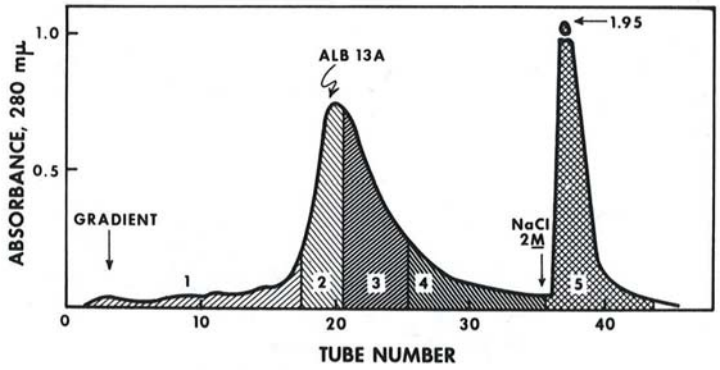

Fig. 6. DEAE-cellulose chromatography of fraction IIA (Fig. 4).

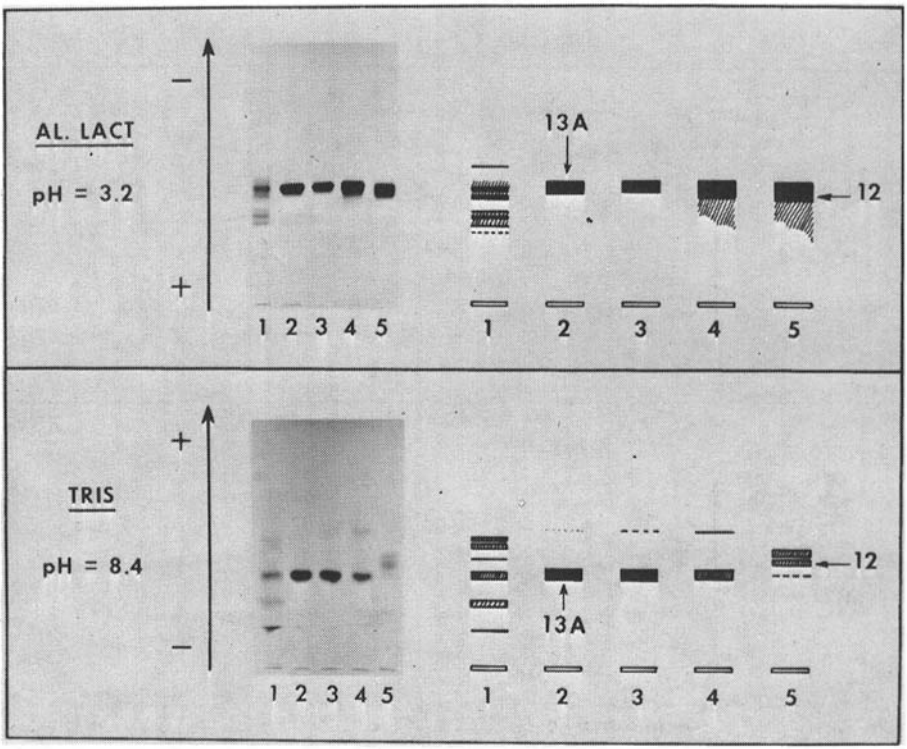

Fig. 7. Polyacrylamide-gel electrophoresis of the fractions obtained by DEAE-cellulose chromatography (Fig. 6) of fraction II A.

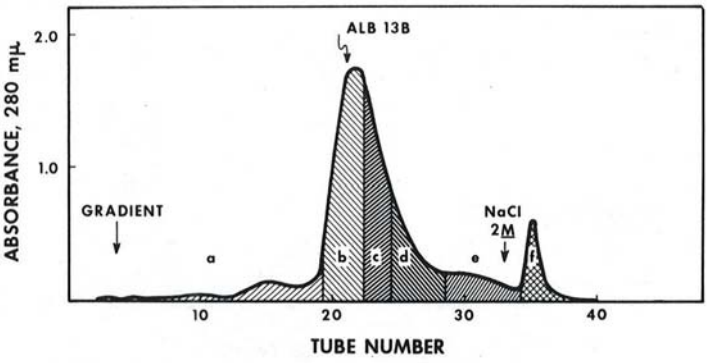

Fig. 8. DEAE-cellulose chromatography of fraction III B (Fig. 4).

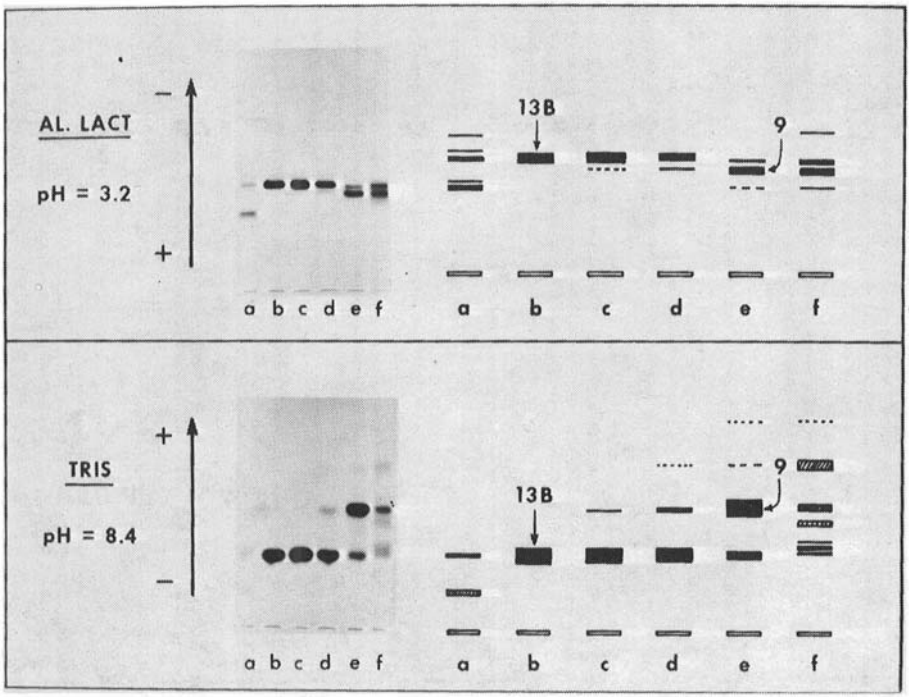

Fig. 9. Polyacrylamide-gel electrophoresis of the fractions obtained by DEAE-cellulose chromatography (Fig. 8) of fraction III B.

components were the same when measured in the presence of BSA or alone.

Starch- and polyacrylamide-gel electrophoresis were carried out in aluminum lactate or Tris buffers as described previously (3,12). No urea was used in polyacrylamide gels.

Amino Acid Analysis

About 60 mg. of protein was dissolved in 20 ml. of deionized water and filtered through a pre-extracted (15) 0.45-μ Millipore filter. Two milliliters of the solution, mixed with 2 ml. of concentrated HCl, was evacuated and nitrogen-flushed, then evacuated and heated at 110 ± 1°C. for 24, 48, or 72 hr. according to the procedure of Moore and Stein (16). Cystine and methionine were separately determined as cysteic acid and methionine sulfone after performic acid oxidation (17). Nitroger and dry weight were determined on the same filtered protein solution respectively by micro-Kjeldahl and drying at 100°C. under vacuum to constant weight.

Tryptophan was determined by the method of Bencze and Schmid (18) in 0.1N NaOH, as modified by Lapuk and co-workers (19). Analysis for sulfhydryl groups (absent) was made with Ellman's reagent (20).

Molecular-Weight Determination

Molecular weight was estimated by gel-permeation chromatography, ultracentrifugation, and amino acid analysis.

Gel-permeation chromatography was performed on a 2 × 85-cm. column of Sephadex G-100 at pH 7.5 according to Andrews (21).

Sedimentation equilibrium measurements (22) on a Beckman Model E ultracentrifuge (ultraviolet absorption optics, photoelectric scanner, multiplex) were made at 23,750 r.p.m., 19.5°C., at pH 7.0 in 0.01M phosphate buffer, 0.1M NaCl, 1.5% sucrose. Solvent density at 20°C. was 1.0080. Concentration of each protein was adjusted to an absorbance (280 mμ, 1 cm.) of about 0.3. The two proteins were run in separate rotor holes in the same rotor. Molecular weights were calculated by the equation:

$$M = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \quad \frac{d \ln c}{d(x^2)}$$

where R = gas constant, T = absolute temperature, $\bar{v}$ = partial specific volume, ρ = density of solution, ω = angular velocity, c = protein concentration, and x = distance from axis of rotation. The slope of ln c vs. x^2 was calculated to give a measure of the molecular weight. Partial specific volume was calculated from the amino acid analysis. Protein concentration was represented by absorbance at 280 mμ.

Calculation of molecular weight from amino acid analysis was by the method of Delaage (23).

Spectroscopy

For ultraviolet spectra in 0.1M KCl, a Cary model 14 spectrophotometer was used, with cuvet light path of 0.1 cm.

Fluorescence spectra were obtained on a Turner model 210 spectrofluorometer, with water solutions of the proteins.

Circular dichroism measurements were made on a Cary model 60 spectropolarimeter with a C.D. attachment, as by Kasarda and co-workers (24). Protein solutions were 1.0 to 1.2 mg. per ml. in water.

RESULTS AND DISCUSSION

Isolation and Purification of ALB 13A and ALB 13B

The steps in the purification of the two proteins and the polyacrylamide-gel electrophoresis in aluminum lactate and Tris buffers are shown in Figs. 2 to 9. For electrophoretic evaluation of the fractionation, it was found advisable to use buffers of both high and low pH.

The preparative electrophoresis (Figs. 2 and 3) separated ALB 13A and 13B as fraction F, from other proteins, contaminated only by ALB 12 and some minor components.

Chromatography on Sephadex G-50 (Figs. 4 and 5) separated ALB 13A (fraction II) from ALB 13B (fraction III).

DEAE-Cellulose chromatography (Fig. 6) of fraction IIA of Fig. 4 further purified ALB 13A by removing ALB 12 and some other components (Fig. 7). From fraction IIIB, ALB 13B was similarly purified by removal of minor components including ALB 9 (Figs. 8 and 9).

The preparative electrophoresis step is not critical and can be replaced by other procedures, such as DEAE-cellulose chromatography of a salt extract, as described here (see "Methods") or the procedures of Nimmo et al. (12) or Feillet and Bourdet (13). Nevertheless, the preparative continuous electrophoresis was useful in separating larger amounts of the desired components in one step. It also gave another fraction (A of Fig. 2) whose two chief components showed beta-amylase activity on the gel (25), and could serve as a first step in separating wheat beta-amylases.

The Sephadex chromatography of fraction F showed three groups of proteins (Fig. 4). The first peak may contain gliadinlike components, judging from the mobility in aluminum lactate buffer. ALB 13A and ALB 12, as eluted in the second peak, must be very similar in molecular weight, as they occur in approximately the same proportions in IIA and IIB. In the third peak, containing ALB 13B and ALB 9, there is some difference in proportions of these two components between IIIA and IIIB.

The final purification by DEAE-cellulose, used to purify ALB 13A and ALB 13B, is particularly necessary to remove ALB 12 from ALB 13A in the fraction IIA.

All steps were necessary for purification of ALB 13A and ALB 13B, and are capable of being used on a larger scale. Low yields of the components were obtained, largely because small portions of each fraction were taken to avoid inclusion of impurities.

Homogeneity of the Isolated Proteins

The purity of ALB 13A and 13B were checked by starch- and polyacrylamide-gel electrophoresis in aluminum lactate and Tris buffer (Fig. 10) and by gel-permeation and ion-exchange chromatography (Fig. 11). By all these methods, in which resolution depends on charges and molecular dimensions, the two components

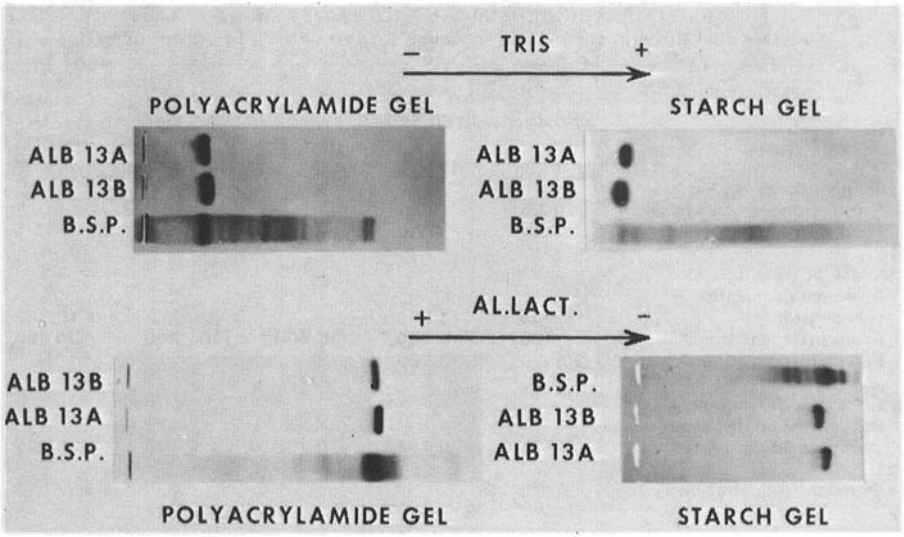

Fig. 10. Homogeneity of ALB 13A and ALB 13B in polyacrylamide-gel and starch-gel electrophoresis in aluminum lactate and Tris buffer (BSPF – buffer-soluble protein of flour).

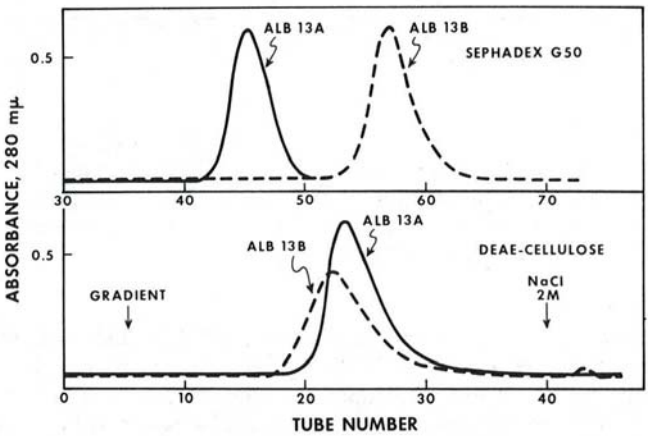

Fig. 11. Homogeneity of ALB 13A and ALB 13B in gel-permeation (Sephadex G-50) and ion-exchange (DEAE-cellulose) chromatography.

appear to be homogeneous. These results were confirmed in the ultracentrifuge and free-electrophoresis measurements.

It must be emphasized that no one of these criteria can be relied on to judge homogeneity of these protein preparations. The unresolved mixture of ALB 13A and 13B seems to be homogeneous in Tris buffer in either starch or polyacrylamide gel and also in DEAE-cellulose chromatography, but is separated into two components by Sephadex G-50 chromatography. Further, fractions IIA (Fig. 5)

TABLE I. PHYSICOCHEMICAL PROPERTIES OF ALB 13A AND ALB 13B (with comparison to Kelley's, Tkachuk's, and Fish's proteins)

	ALB 13A	ALB 13B	Kelley's Protein (Ref. 8)	Tkachuk's Protein[a] (Ref. 9)	Fish's Protein (Ref. 11)
Nitrogen content (% d.w.)					
From amino acid analysis	17.5	17.6		16.9	
From nitrogen analysis	16.4	17.0		16.6	15.5
Molecular weight					
From gel-permeation chromatog.	19,800	11,200			19,300
From ultracentrifugation	24,800	13,950	76,500	64,200	20,400
From amino acid analysis	20,100	12,900			
Partial specific volume $\bar{v}$ (ml./g.) calculated from amino acid content	0.727	0.733	0.722	0.733	0.727
Electrophoretic mobility[b]	−2.5 (pH = 8.6)	−2.5 (pH = 8.6)	−4.7 (pH = 8.6)	−6.97 (pH = 8.0)	
Specific absorbance $E_{1\ cm.}^{1\%}$	13.35 (0.1M KCl, 278 mμ)	20.50 (0.1M KCl, 279 mμ)		16.3 (0.1N NaOH, 290 mμ)	13.1 (278 mμ)

[a]Component A.
[b]10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} (veronal buffer, $\Gamma/2 = 0.1$).

and 5 (Fig. 7), both showing only one strong band in aluminum lactate buffer, are both resolved into two components in Tris buffer. This latter finding confirms a previous conclusion (2) that the Tris buffer is superior to the aluminum lactate for resolution of wheat-soluble proteins.

Electrophoretic Mobility

The schlieren patterns (both boundaries) of ALB 13A and 13B, separately or mixed, or even the fraction F (Fig. 2), are sharp, symmetrical, and characteristic of a homogeneous protein. The two components have the same mobility in the buffer used (Table I), and are more basic than the proteins of Kelley (8) and Tkachuk and Tipples (9).

The mobilities of ALB 13A and 13B are very similar in gel electrophoresis (Fig. 10). However, in starch gel, in either of the buffers used, and in polyacrylamide in aluminum lactate, 13A is slightly faster than 13B; in polyacrylamide in Tris buffer, 13B is slightly the faster.

Amino Acid Composition

The amino acid analysis of ALB 13A and ALB 13B, compared to Kelley's (8), Tkachuk's (9), and Fish's (11) proteins, is listed in Table II.

ALB 13A and ALB 13B exhibit a very different amino acid analysis: ALB 13A contains all the common amino acids, with alanine predominating (13.5 residue

TABLE II. AMINO ACID COMPOSITION OF ALB 13A AND ALB 13B (with comparison to Kelley's, Tkachuk's, and Fish's proteins)

	Amino Acid (per mg. N)		Amino Acid (per M Protein)[a] Experimental Values		Integral Numbers		Amino Acid N (as % total N)[b]				
	13A μM	13B μM	13A M	13B M	13A M	13B M	13A %	13B %	Kelley's Protein (8) %	Tkachuk's Protein (9) %	Fish's Protein (11) %
Lys	1.29	2.69	4.64	6.17	5	6	3.70	7.65	2.99	5.38	4.29
His	0.81	0.04	2.91	0.09	3	0	3.49	0.17	3.36	7.76	3.26
Arg	3.24	2.98	11.65	6.84	12	7	18.61	16.94	16.80	15.42	17.80
Asp	3.03	3.93	10.90	9.02	11	9	4.35	5.58	4.70	7.85	4.45
Thr[c]	1.40	1.34	5.03	3.07	5	3	2.01	1.91	3.78	2.22	2.36
Ser[c]	3.15	3.73	11.33	8.56	11	8 or 9	4.52	5.30	4.04	2.81	4.31
Glu	5.95	5.33	21.40	12.23	21 or 22	12	8.54	7.58	8.30	7.88	8.20
Pro	3.83	4.92	13.77	11.29	14	11	5.50	6.99	7.89	4.35	5.63
Gly	4.18	4.21	15.03	9.66	15	10	6.00	5.98	5.23	6.27	6.49
Ala	7.04	4.26	25.32	9.78	25	10	10.11	6.05	3.44	5.71	9.91
1/2-Cys[d]	3.66	3.06	13.17	7.02	12 or 14	6 or 8	5.25	4.35	3.05	1.12	5.49
Val	4.01	5.68	14.42	13.03	14 or 15	13	5.76	8.07	4.00	5.24	6.11
Met[d]	1.17	0.94	4.20	2.16	4	2	1.69	1.34	1.59	1.65	1.87
Ileu	1.26	0.89	4.53	2.04	4 or 5	2	1.81	1.26	2.37	2.76	1.81
Leu	4.14	3.82	14.89	8.77	15	9	5.95	5.43	5.97	6.24	5.91
Tyr	2.02	1.64	7.26	3.76	7	4	2.90	2.33	2.73	3.17	2.95
Phe	0.88	trace	3.16	trace	3	0	1.26	0.00	1.54	3.13	1.24
Trp[e]	0.88	1.95	3.16	4.48	3	5	2.52	5.54	...	3.72	2.96
NH_3[c]	4.20	5.30	15.10	12.16	15	12	6.03	7.53	11.0	7.32	4.96
N recovered (%)	97.5	98.5					100.00	100.00	92.78	100.00	100.00

[a]Protein molecular weight estimated according to Delaage (23).

[b]Recalculated, with total N = amino acid N + ammonia N.

[c]Extrapolation to zero time.

[d]As cysteic acid and methionine sulfone.

[e]UV spectrophotometric method (18).

percent), whereas ALB 13B has no histidine, no phenylalanine, and a relatively high valine count (11 residue percent).

The tyrosine-tryptophan ratios (18,19) are respectively 2.30 in ALB 13A and 0.84 in ALB 13B. No sulfhydryl group was found in either protein by Ellman's procedure (20), even in the presence of 6M guanidine hydrochloride as a denaturing agent to detect the possible "unreactive" groups (26). Therefore, all the cysteine residues must be combined as cystine. On this basis, there are 12 or 14 disulfide bonds per mol. in ALB 13A and 6 or 8 in ALB 13B.

Despite some small differences, most of which are within limits of the experimental errors, ALB 13A and Fish's protein have a very similar composition, and there is a possibility of identity between these two proteins. We will discuss this point later.

Nitrogen Content

The micro-Kjeldahl analysis of ALB 13A showed it to contain 16.4% nitrogen, a lower value than the 17.5 obtained by direct calculation from the amino acid composition; likewise, the ALB 13B content is 17.0 (micro-Kjeldahl) and 17.6 (amino acid).

These small differences could be explained by experimental errors or by the presence of nonprotein material in the samples, or both, bound or not to the proteins. This point has not been investigated, except that we found no carbohydrate by the method of Dubois et al. (27) as modified by Drapron and Guilbot (28), in ALB 13A or ALB 13B.

Molecular Weight

From gel-permeation chromatography (21), ALB 13A and ALB 13B had apparent molecular weights of 19,800 and 11,200, respectively (Fig. 12).

From sedimentation equilibrium, with concentration distribution at equilibrium,

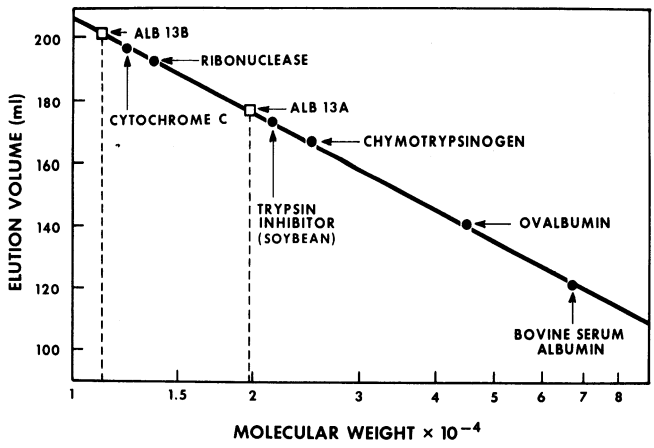

Fig. 12. Calibration curve for Sephadex G-100 column with reference proteins and for molecular-weight determination of ALB 13A and ALB 13B (Tris buffer, pH 7.5).

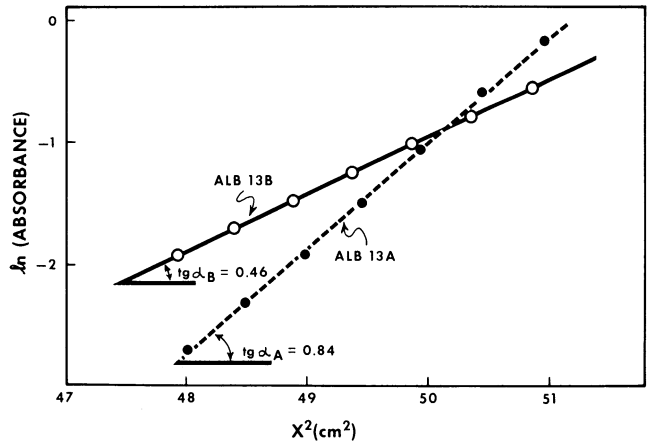

Fig. 13. Sedimentation equilibrium of ALB 13A and ALB 13B (X = distance of the proteins from the axis of rotation).

d ln c/d (x^2) was determined from the plot of Fig. 13. Molecular weights of ALB 13A and ALB 13B were calculated (20) as 24,800 and 13,950, respectively.

From amino acid analysis, and considering the values from the two above methods, the calculation method of Delaage (23) gave values of 20,100 and 12,900 for the two proteins.

The results are summarized in Table I. We have at present no explanation for the approximately 15% greater values from the ultracentrifugation. Again, the values for 13A and the protein of Fish (11) are similar.

Spectroscopic Properties

UV Spectra. The two isolated albumins exhibit an ultraviolet absorption spectrum similar to that of most proteins (Fig. 14). ALB 13A has maximum absorption at 278 mμ and ALB 13B at 279 mμ. Specific absorbance values, corrected for light-scattering, are listed in Table I. The high value found for ALB 13B (20.50) can be explained by the high tryptophan content of this molecule.

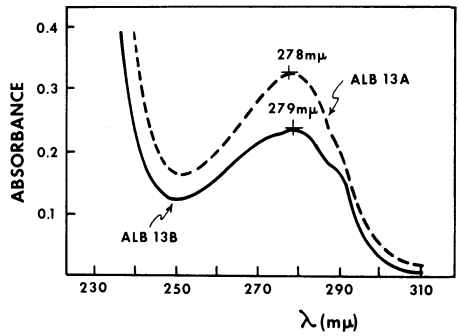

Fig. 14. UV spectra of ALB 13A ($E^{1\%}_{1\ cm.}$ = 13.35) and ALB 13B ($E^{1\%}_{1\ cm.}$ = 20.50) (in 0.1M KCl solution). 13A = 2.5 mg. per ml.; 13B = 1.2 mg. per ml.

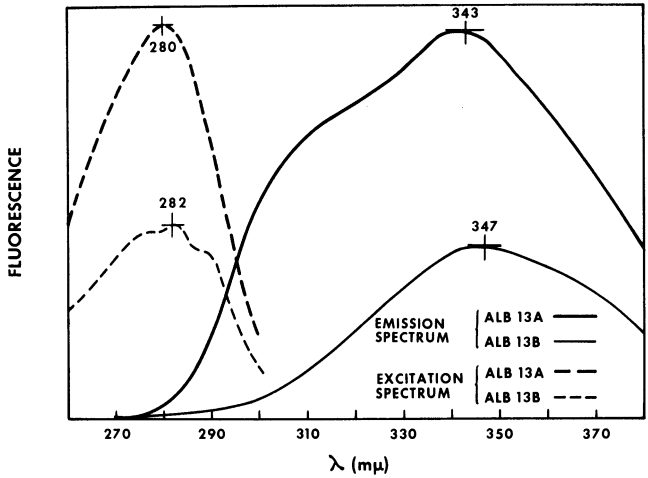

Fig. 15. Emission and excitation fluorescence spectra of ALB 13A and ALB 13B (in water solution).

Fluorescence Spectra. The emission and excitation fluorescence spectra of ALB 13A and ALB 13B are shown in Fig. 15. The position of emission maxima, 343 mμ for ALB 13A and 347 mμ for ALB 13B, suggests that most of the tryptophan residues are in a hydrophilic environment on the surface of the two proteins. The shoulder in the 300- to 310-mμ region for ALB 13A suggests fluorescence from tyrosine residues, in contrast to the usual complete dominance of tryptophan fluorescence in proteins containing both amino acids.

Circular Dichroism. The circular dichroism measurements of ALB 13A and ALB 13B in water solutions are shown in Fig. 16 (range 190 to 250 mμ) and in Fig. 17 (range 250 to 300 mμ).

The ellipticity bands in the range 190 to 250 mμ result primarily from the peptide bonds, although they could be modified or distorted by underlying aromatic or disulfide bands. The spectrum of ALB 13A is characterized by two negative bands at 208 and 220 to 222 mμ with a cross-over from negative to positive ellipticity at 220 mμ and a positive band lying just below this point. Such a spectrum is indicative of some alpha-helix structure. The ellipticity 10,000 (deg. $cm.^2$) d $mol.^{-1}$ at 220 to 222 mμ suggests about 25 to 30% helical structure in ALB 13A. The spectrum of ALB 13B in this range is very similar, but the ellipticity 15,500 at 220 to 222 mμ suggests about 40% helical structure.

The bands in the range 250 to 300 mμ most likely result from tyrosine or tryptophan, but these could conceivably be a contribution from disulfide bands. The spectra of ALB 13A and ALB 13B are completely different, in agreement with the commonly accepted idea that such a spectrum is characteristic for each protein.

Comparison between ALB 13A and Fish's Protein

As shown in Tables I and II, the properties of ALB 13A and 13B, the proteins described by Kelley (8) and Tkachuk and Tipples' beta-amylases (9) are distinctly different. On the other hand, however, the properties of ALB 13A and the protein

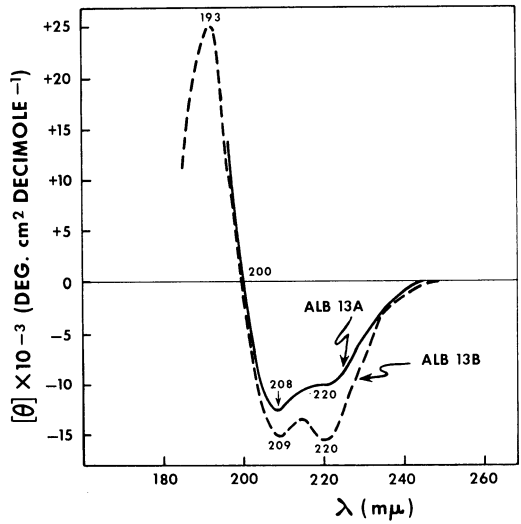

Fig. 16. Circular dichroism of ALB 13A and ALB 13B (in water solution). Range: 190 to 250 mμ ([θ] = mean residue ellipticity).

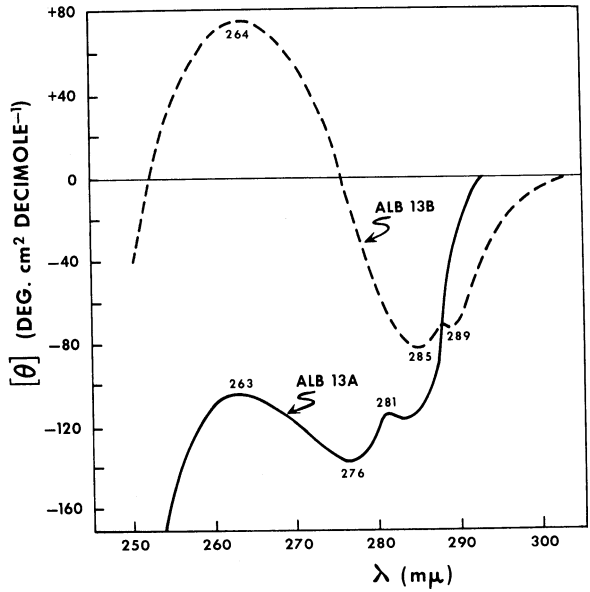

Fig. 17. Circular dichroism of ALB 13A and ALB 13B (in water solution). Range: 250 to 300 mμ ([θ] = mean residue ellipticity).

of Fish (11) are very similar. To compare these in more detail, the carboxymethyl cellulose chromatography step of Fish was used on a water extract of flour. ALB 13A was eluted at 0.15M NaCl-acetate buffer in the stepwise procedure, whereas the Fish protein appeared in the 0.28M NaCl step. However, we did not obtain the same elution curve profile in either the stepwise elution or in an attempt to reproduce the gradient elution described by Fish. Therefore, we cannot at present conclude anything definite about the identity of these two preparations.

Occurrence of ALB 13A and ALB 13B in Common and Durum Wheats

A limited amount of work with gel-electrophoresis, gel-permeation, and ion-exchange chromatography, comparing a few varieties of common and durum wheat, gave results supporting the previous conclusion (3,4) that ALB 13A and ALB 13B do not appear in durum wheats. Further work is necessary to evaluate this possibility.

Clayton (29) found that one peak, in CM-cellulose chromatography of buffer-soluble proteins, was present in common wheats, and absent in durum wheats. Resmini (30), using polyacrylamide-gel disc electrophoresis, found that one band in salt-soluble proteins of common wheat was absent from similar preparations from durum wheat. In both cases, no other evidence is given that would permit comparison to albumins 13A and 13B.

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NOTE

While this manuscript was in press, two articles appeared with descriptions of wheat flour albumins of similar electrophoretic "mobility" and amino acid composition (31,32). The results of the Fish-Abbott work (31) show molecular-weight and mole values of amino acids different from those in the cited thesis (11), but our comments on the relation of ALB 13A to this protein need no real change. Values in Tables I and II could not be changed to conform to the published molecular weight. The protein of Ewart (32), while similar to our ALB 13B in amino acid composition and "mobility," is reported as about twice the molecular weight of 13B. While such a difference could result from the difference in pH and composition of sedimentation solvents in the two laboratories, additional evidence, from fingerprinting, is offered for the higher value for Ewart's protein. With the present evidence, the difference in molecular-weight results cannot be explained, if indeed the two reports concern the same protein. Our results differ from these cited by showing the presence of two components of the same mobility but quite different size and composition in the major albumin band of a HRW wheat flour.

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