Albumin Fraction from Spring, Winter, and Soft Wheats—Protein and Associated Carbohydrate Identified by Gel Filtration Chromatography and Gel Electrophoresis[1]

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ABSTRACT

Wheat flour proteins are a mixture of albumins, globulins, gliadins, and glutenins. The carbohydrates associated with some of the proteins of each class may be useful indicators of wheat quality. Therefore, the albumins of two hard red spring, one hard red winter, and two soft red winter wheats were fractionated by gel filtration chromatography in a solvent of 0.1*M* acetic acid and 8*M* urea on Sephacryl S-300 for characterization of protein and associated carbohydrate. Proteins eluted in peaks I, II, and III; carbohydrates eluted in peaks I, II, and IV. The quantity of the carbohydrates in peak I of albumin proteins was highest in the hard red spring wheats (Butte and Era) and lowest in the soft red winter wheats (Fairfield and Titan). The albumin proteins of peak II of soft red winter wheat seem to be associated with high levels of sugar. Peak III, a low molecular weight albumin subfraction, and peak IV, a low molecular weight carbohydrate fraction, do not seem to be associated with sugar or nitrogen material, respectively. Analysis of total albumin, and those fractions from gel filtration by urea-polyacrylamide gel electrophoresis using periodic acid-Schiff staining for carbohydrates and Coomassie Brilliant Blue staining for proteins, showed that the carbohydrate components were associated with the high molecular weight polypeptides. During gel filtration, protein and carbohydrate eluted in peaks I and II, and protein and carbohydrate comigrated on urea-polyacrylamide gel electrophoresis, which indicate that protein in those peaks may be covalently linked to carbohydrate.

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Some evidence indicates that functional gluten, produced during dough mixing and development, involves formation of aggregates through hydrogen bonds and hydrophobic, ionic, and polar interactions (Bushuk 1985a,b; Zawistowska et al 1985). However, specific nonprotein constituents of flour, such as carbohydrates and lipids, are also involved in the formation of aggregates (Bekes et al 1983; McMaster and Bushuk 1983; Zawistowska et al 1984, 1985). Lipids are bound to flour proteins during dough mixing (Chung et al 1979), and certain lipids (Bekes et al 1983; Zawistowska et al 1984, 1985) and carbohydrates (McMaster and Bushuk 1983) strongly bind to specific proteins in isolated fractions. A closer association has been observed between a carbohydrate and some high molecular weight glutenin subunits (Bushuk et al 1980). The carbohydrate appears to be involved in the aggregation of specific glutenin subunits into large complexes. Reduced glutenin from wheat, separated by gel filtration chromatography in a solvent of acetic acid and urea, showed three peaks. Peak I contained subunits with a strong tendency to aggregate (Huebner and Wall 1974, Arakawa and Yonezawa 1975, Hamauzu et al 1975, Arakawa et al 1977, Khan and Bushuk 1979). All of the carbohydrate in the glutenin fraction coeluted with fraction I (Khan and Bushuk 1979, McMaster and Bushuk 1983, Zawistowska et al 1984). Similarly, the lipid occurred only in fraction I (Zawistowska et al 1984).

Traditionally, only storage proteins (gliadins and glutenins) were thought to form aggregates. Recently, Gupta et al (1991) indicated that certain globulins (triticins) and high molecular weight albumins in wheat endosperm form disulfide-linked aggregates. Although aggregates are usually established by covalent disulfide bonds, in hydrophilic environments, monomeric gliadins might also form aggregates that are stabilized by hydrogen bonds and hydrophobic interactions (Shewry et al 1986). Gupta et al (1991) also indicated that the presence of specific albumins and globulins in the aggregated protein fraction could be explained if they were physically trapped within the aggregates or if they contained disulfide bonds that allowed them to be linked with aggregates or subunit components or with themselves to form polymers that coexist with other aggregates.

The present research with gel filtration chromatography and gel electrophoresis was undertaken to characterize the albumin protein fraction (water-soluble material) among wheat classes, and also to investigate possible association of carbohydrate constituents with the protein subfractions from gel filtration chromatography to provide information that may help in understanding wheat protein interactions.

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MATERIALS AND METHODS

Wheat Flour Samples

Two hard red spring (HRS) wheats (Butte, Era) and the hard red winter (HRW) wheat (Roughrider) used in this study were grown at different locations in North Dakota in 1988. The soft red winter (SRW) wheats (Fairfield and Titan) were grown in Ohio in 1987.

Isolation of Albumin Protein Fraction

Albumin protein fractions from three wheats were isolated according to the modified Osborne fractionation procedure of Chen and Bushuk (1970). The water-soluble albumins were freeze-dried and stored in sealed containers at 4°C.

Determination of Protein Content

Nitrogen content of flours and albumin fractions was determined using the Kjeldahl procedure according to AACC method 46-11A (AACC 1983). Protein content was also expressed as absorbance at 280 nm.

Estimation of Carbohydrate Content by Phenol-Sulfuric Acid Method

Carbohydrate content was estimated as total carbohydrate (pentoses and hexoses) using the phenol-sulfuric acid method (Dubois et al 1956). Glucose was used as the standard sugar.

Quantitative Analysis of Monosaccharides by Gas Liquid Chromatography

The neutral sugars in the carbohydrate hydrolysates were converted to their respective aldononitrile acetates using the method of McGinnis (1982). The aldononitrile acetate sugar derivatives were separated and identified by gas chromatography.

Gel Filtration Chromatography

Sephacryl S-300 Superfine gel (Pharmacia Fine Chemicals) was equilibrated with a solvent of 8*M* urea and 0.1*M* acetic acid; it was packed in a 100- × 2.5-cm column following the manufacturer's procedure. Fractions (3 ml) were collected at 10.6-min intervals with a fraction collector. Protein and carbohydrate were determined on individual tubes. Appropriate fractions were pooled, dialyzed for 24 hrs, and freeze-dried for further analysis.

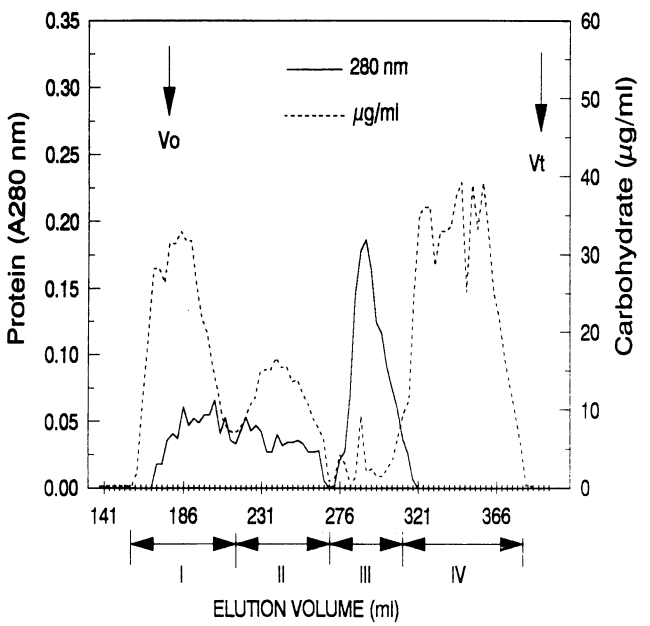

Fig. 1. Elution profile from gel filtration chromatography of unreduced albumin fraction of a hard red spring wheat (Era) with a solvent of 8*M* urea and 0.1*M* acetic acid on Sephacryl S-300 gel. Subfractions were pooled as indicated by the horizontal arrows; —— = protein; · · · = carbohydrate. Flow rate: 17.3 ml/hr; 3.0-ml fractions collected per tube.

The excluded volume (void volume, V_0) of the Sephacryl S-300 column was determined as the elution volume of blue dextran. The total volume (V_t) was determined by the peak of tryptophan. Sample application was 50 mg of unreduced albumin dissolved in 5 ml of a solvent of 8*M* urea and 0.1*M* acetic acid.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) procedure of Laemmli (1970) was modified for characterizing the wheat proteins. Gels containing 12% acrylamide (separating gels) were prepared from a stock solution of 30% (by weight) of acrylamide and either 0.8% or 0.28% (by weight) on N,N',-methylenebisacrylamide. For the final concentration, 1.5*M* tris-HCl (pH 8.8) and 0.1% SDS were added. Gels with 0.04% (v/v) N,N,N',N',-tetramethylethylenediamine (TEMED) were polymerized with 0.03% (w/v) ammonium persulfate.

The 4% acrylamide stacking gels were made from a stock solution of 30% (by weight) acrylamide and 0.6% (w/v) N,N'-methylenebisacrylamide. For final concentration, 0.5*M* tris-HCl (pH 6.7) and 0.1% SDS were added. The gels with 0.13% (v/v) TEMED were polymerized with 0.04% (w/v) ammonium persulfate.

Sample Preparation for Gel Electrophoresis

Freeze-dried protein sample (20–50 mg) on equal protein basis was dissolved with 1 ml of prepared sample buffer. Sample buffer stock solution contained 20 ml of glycerol, 12.5 ml of 1*M* tris-HCl (pH 6.8), 4 g of SDS, 24.1 ml of distilled water, and 20 mg of Pyronin Y tracking dye. Sample buffer was made fresh each time by combining 2.5 ml of sample buffer stock solution, 6 ml of distilled water, and 0.45 ml of 2-mercaptoethanol (2-ME) (4% v/v). Sample application was 10 μl per slot.

Staining and Destaining of Gels

The SDS gels were stained overnight by gentle shaking with Coomassie Brilliant Blue R-250 (CBB) solution. The staining solution consisted of 2 g per liter of CBB, 10% acetic acid, 50% methanol, and 2% trichloroacetic acid. Destaining was carried out with several changes of destaining solution (40% methanol and 5% glacial acetic acid) until the gel background was clear. Gels were stored in the destaining solution until photographed and dried.

Detection of Glycoproteins on Gels

Glycoproteins were detected specifically by the periodic acid-Schiff (PAS) procedure described by Leach et al (1980). However, SDS may interfere with the stain (Fairbanks et al 1971, Leach et al 1980), so a detergent-free urea-polyacrylamide gel electrophoresis (urea-PAGE) procedure was used.

Separating gels of 10% acrylamide were prepared from a 30% acrylamide stock solution (as indicated above for the SDS-PAGE separating gel), 5% (v/v) glacial acetic acid, and 2*M* urea. After degassing for a few minutes, 0.5% TEMED was added and, finally, polymerization was initiated with addition of ammonium persulfate (0.2%, w/v).

The 4% acrylamide stacking gel was prepared from a 30% acrylamide stock solution (as indicated above for SDS-PAGE stacking gel), 0.13% (v/v) TEMED, and 0.004% (w/v) ammonium persulfate.

The electrode chambers were filled with 5 L of 5% glacial acetic acid solution.

Sample Preparation for Urea-PAGE

A 20-mg sample was prepared by adding 0.250–1 ml of urea stock solution: 8*M* urea in 5% acetic acid, 0.02% Pyonine Y tracking dye (4%), with and without 2-ME. The sample was boiled for 2.5 min in boiling water, and 5–20 μl of sample was applied to each slot. The electrophoresis was carried out with a current of 20 mA per gel until the Pyronin Y tracking dye migrated off the gels (~5 hr).

Staining Procedures for Urea-PAGE

Protein staining was performed as indicated above for SDS-PAGE, followed by the PAS staining procedure. Fuchsin-sulfite (Schiff reagent) contained basic fuchsin (2 g) (pararosaniline P-4896, Sigma Chemical Co.) dissolved in 40 ml of distilled water, which was then warmed, cooled, and filtered; 10 ml of 2*N* HCl and 4 g of sodium metabisulfite were added to the solution, which was then kept cool and dark overnight in stoppered bottles. Activated charcoal (1 g) was stirred in and filtered; 2*N* HCl (10 ml or more) was added to the point where one drop of the solution no longer turned red on the glass slide. The clear solution was stored in a cool dark place in a stoppered bottle.

After electrophoresis, the gels were immersed in 12.5% trichloroacetic acid for 34 min, rinsed with distilled water, and shaken for 1.5 hr with 5% phosphotungstic acid in a 2*N* HCl solution. The gel was then washed overnight by shaking in an orbital shaker with a solution of 40% methanol and 7% acetic acid (with at least two changes of solvent) to remove the phosphotungstic acid. Gels were immersed in 1% periodic acid and 3% acetic acid for 50 min and then washed in distilled water for 1 hr. A 0.5% sodium metabisulfite and HCl solution was added for 1 or 2 hr. Finally, the fuchsin-sulfite solution (Schiff reagent) was added and left overnight.

The glycoprotein bands became pink or red after 3–12 hr, depending on the carbohydrate concentration in the glycoprotein. When the background of the gel became red, 0.5% sodium metabisulfite-HCl solution was added once more to reduce the background until it became transparent.

RESULTS AND DISCUSSION

Gel Filtration Chromatography of Albumin Fraction (Water-Soluble Material)

The elution profiles of albumin obtained by gel filtration chromatography are shown in Figures 1–3. The protein of the eluted fractions was estimated by absorbance at 280 nm. Total carbohydrate content was determined by the phenol-sulfuric acid method of Dubois et al (1956). Horizontal arrows on the elution profiles indicate the fractions that were pooled to obtain material for SDS-PAGE, urea-PAGE, and sugar analysis.

The albumin proteins were separated into four peaks (I–IV) according to the order of elution. Protein, measured at 280 nm absorbance, was found in peaks I, II, and III. The majority of the carbohydrates, detected by the phenol-sulfuric acid method, eluted in peaks I, II, and IV for spring and winter wheats and in peaks II, III, and IV for soft wheats. Peak I eluted in the V_0 of the column, indicating that the material in this peak was of high molecular weight and excluded from the Sephacryl matrix.

The quantity of the carbohydrate in peak I (Figs. 1–3) decreased from an average of 34% in HRS wheats, to 30% in HRW wheats, to 9.7% in SRW wheats. The albumin proteins of peak II were associated with higher levels of sugar in SRW wheats (28.3%); the level was lower in HRW (21.1%) and HRS (18.2%) wheats, respectively. Peak III had much lower levels of carbohydrate than

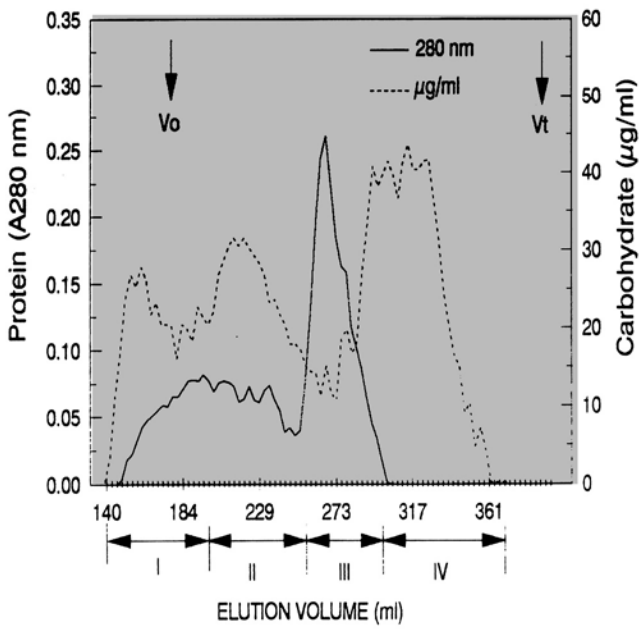

Fig. 2. Elution profile from gel filtration chromatography of unreduced albumin fraction of a hard red winter wheat (Roughrider) with a solvent of 8*M* urea and 0.1*M* acetic acid on Sephacryl S-300 gel. Subfractions were pooled as indicated by the horizontal arrows; —— = protein; · · · = carbohydrate. Flow rate: 17.3 ml/hr; 3.0-ml fractions collected per tube.

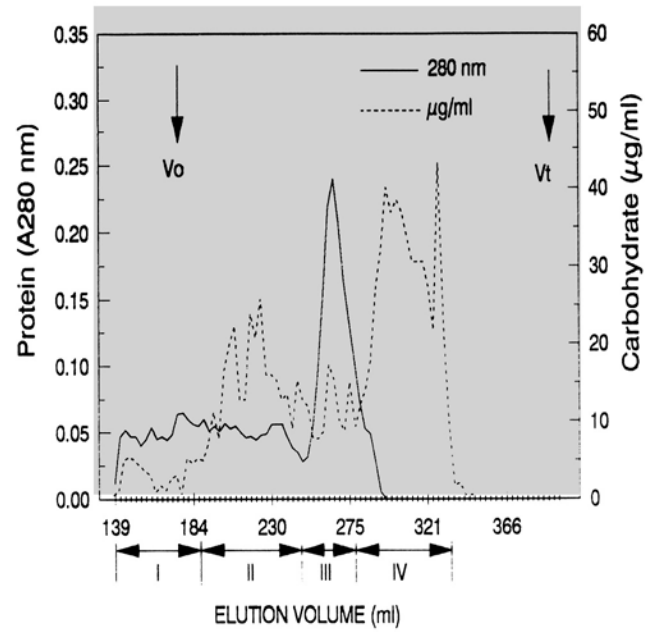

Fig. 3. Elution profile from gel filtration chromatography of unreduced albumin fraction of a soft red winter wheat (Titan) with a solvent of 8*M* urea and 0.1*M* acetic acid on Sephacryl S-300 gel. Subfractions were pooled as indicated by the horizontal arrows; —— = protein; · · · = carbohydrate. Flow rate: 17.3 ml/hr; 3.0-ml fractions collected per tube.

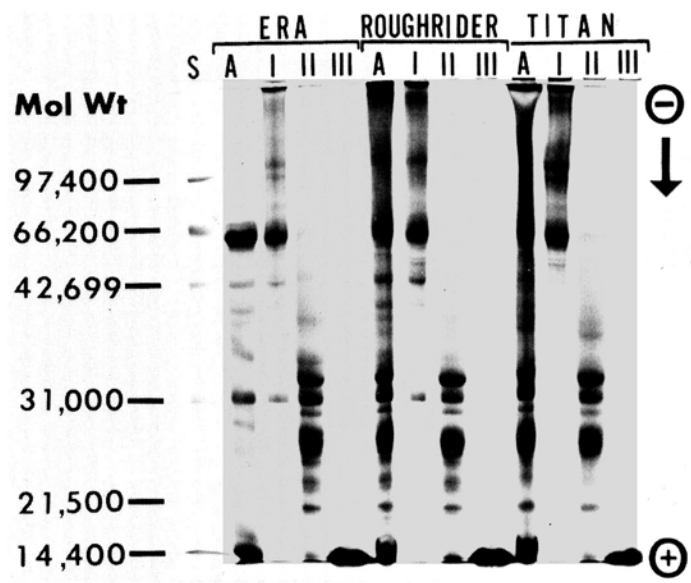

Fig. 4. Patterns of sodium dodecyl sulfate-polyacrylamide gel electrophoresis containing urea without the reducing agent 2-mercaptoethanol for Era (hard red spring), Roughrider (hard red winter), and Titan (soft red winter) wheat. A = albumin fraction; I, II, III = subfractions from gel filtration on Sephacryl S-300 gel; S = molecular weight standard.

the other peaks; however, the levels increased from 6.6% in HRS and 13.2% in HRW to 16.4% in SRW wheats. The majority of the carbohydrate material was found in IV, a nonprotein peak: 41.2%, 35.4%, and 45.7% in HRS, HRW, and SRW wheats, respectively.

Preliminary qualitative analysis of the albumin subfractions by gas chromatography indicated the presence of major sugars. Peak I contained xylose as a major sugar. Peak II contained xylose and galactose. Sugars could not be detected in III. Peak IV showed xylose as the major sugar.

SDS-PAGE of Fractions from Gel Filtration Chromatography

SDS-PAGE of unreduced (without 2-ME) and reduced (with 2-ME) fractions in the presence of acetic acid-urea solvent (Figs. 4 and 5, respectively) show that peak I contained major bands with molecular weights of approximately 66,000, 42,699, 40,000 (reduced), and 31,000. Part of the material remained at the origin of sample application, especially in the unreduced samples. There is streaking in the patterns of the unreduced samples above molecular weight 66,000. Also, two bands appear in the 97,000 molecular weight region (Fig. 4). These two bands are most likely the triticins identified by Singh and Shepherd (1985) that disappeared in the reduced sample (Fig. 5). Peak I of the reduced samples, however, showed two other bands of low mobility, present in all three wheats, near the origin of the resolving gel with molecular weights above 100,000.

Peak II contained lower molecular weight subunits ranging from 42,699 to 21,500 and an overlap of the 14,000 subunit, which is much more abundant in peak III (Fig. 5). Peak III contained one 14,400 band and had very low or no carbohydrate material. Peak IV, however, contained carbohydrate material that did not appear to be associated with protein. Therefore, it was not included in the electrophoretic studies. Also, it was impossible to recover peak IV after freeze-drying because the material adhered to the wall of the container.

The separation of the subfractions was good, except for a subunit of 31,000 in peak I that overlapped with II (Fig. 5). However, in the SDS-PAGE procedure, sample preparation included 2-ME (Fig. 5), which cleaves disulfide bonds, thereby releasing individual subunits of similar molecular weight among the fractions, most likely contributing to overlapping of protein components. The data also show that the use of urea for sample buffer (acetic acid-urea solvent) in SDS-PAGE improved the separation and also decreased the streaking of protein subfractions of the unreduced albumin sample, especially below 42,000 (compare Figs. 4 and 5). In addition, the reduced samples showed more changes in mobility and molecular weight of components than did the unreduced samples. For example, peak II (reduced) shows a prominent band around 35,000 for all wheat samples; this was absent or very faint in the unreduced samples.

Peak I was separated into 10–12 major bands; peak II was separated into 12–14 major bands; and III had one major broad band. Peak IV, not included in SDS-PAGE studies, eluted (V_e about 310 ml) just before the total volume of the column (V_t 391 ml). Most of the carbohydrate material of this peak remained in the dialysis tubing, which retained materials from 8,000 to 6,000. It was, therefore, assumed that this carbohydrate complex had a molecular weight of 6,000–13,000.

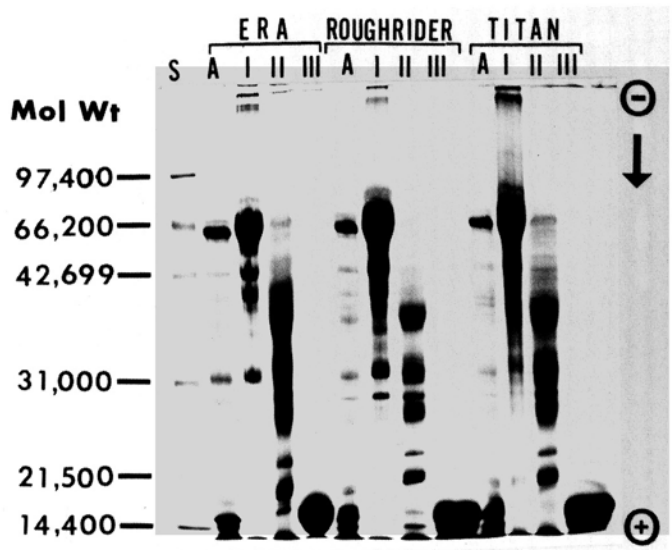

Fig. 5. Patterns of sodium dodecyl sulfate-polyacrylamide gel electrophoresis containing urea and reducing agent 2-mercaptoethanol for Era (hard red spring), Roughrider (hard red winter), and Titan (soft red winter) wheats. A = albumin fraction; I, II, III = subfractions from gel filtration on Sephacryl S-300 gel; S = molecular weight standard.

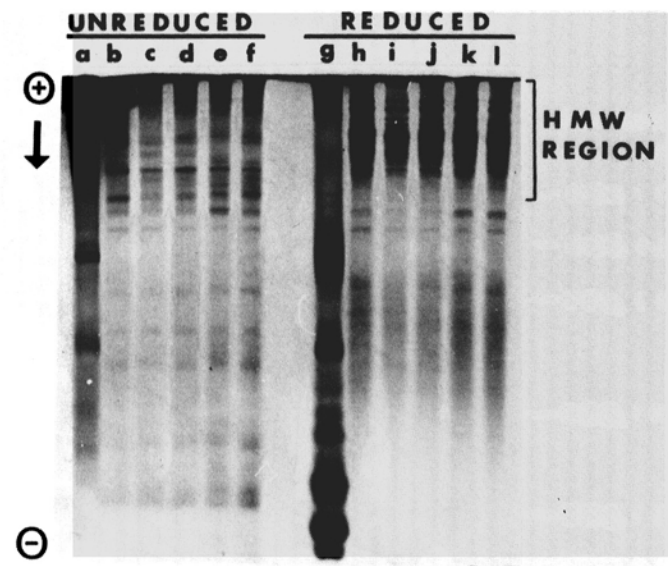

Fig. 6. Protein from total flour extracts characterized by urea-polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for proteins with Coomassie Brilliant Blue R-250. Unreduced (**a–f**): **a,** fetuin glycoprotein standard; **b,** Butte; **c,** Era; **d,** Roughrider; **e,** Fairfield; **f,** Titan. Reduced (**g–l**): **g,** fetuin glycoprotein standard; **h,** Butte; **i,** Era; **j,** Roughrider; **k,** Fairfield; and **l,** Titan. HMW = high molecular weight.

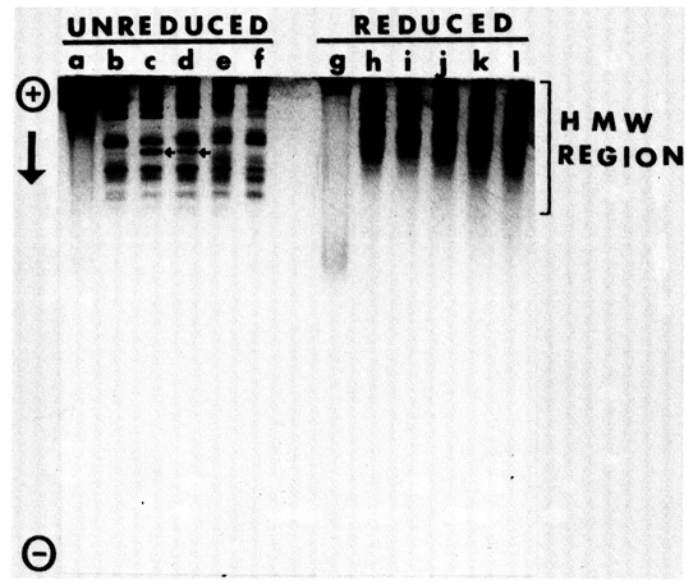

Fig. 7. Protein from total flour extracts characterized by urea-polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for carbohydrates with periodic acid-Schiff reagent. Unreduced (**a–f**): **a,** fetuin glycoprotein standard; **b,** Butte; **c,** Era; **d,** Roughrider; **e,** Fairfield; **f,** Titan. Reduced (**g–l**): **g,** fetuin glycoprotein standard; **h,** Butte; **i,** Era; **j,** Roughrider; **k,** Fairfield; and **l,** Titan. HMW = high molecular weight.

Urea-PAGE Detection of Sugar Components Comigrating with Proteins

Urea-PAGE (detergent-free) was employed in this study because the majority of the known glycoproteins were originally identified using the PAS staining procedure on polyacrylamide gels. Some researchers (Fairbanks et al 1971, Leach et al 1980) reported that SDS may interfere with PAS staining, therefore, it must be removed from the SDS gels before staining. Complete removal of SDS from its complexes with proteins is not easy to achieve due to the strong affinity of SDS to proteins (Kapp and Vinogradov 1978). The sugar content in protein fractions in cereals is relatively low, about 3–7%, compared with 10–41% reported for some animal glycoproteins (Green and Pastewka 1975, Leach et al 1980). Therefore, overloading of protein sample on acrylamide gels is usually required for detection of carbohydrates.

Urea-PAGE was used first for characterizing total protein extracts of the five samples (Butte, Era, Roughrider, Fairfield, Titan) stained for proteins (at equal protein content) with CBB (Fig. 6). Several bands appear on the gels stained with CBB of reduced and unreduced flour samples. However, in gels stained with PAS reagent (Fig. 7), it appears that the polypeptides associated with sugars are located at the high molecular weight (low mobility) region in both reduced and unreduced flour samples. CBB seems to have a weak affinity for the protein bands in the unreduced samples where sugar-protein components are found. The blue stain of these bands gradually disappear after a few hours of destaining, forming a whitish halo around the protein-containing sugar bands (Fig. 6). This observation agrees with Tsai and Frasch (1982) and Green and Pastewka (1975), who indicated that glycoproteins are poorly stained with CBB. In some instances, CBB-stained bands change to a permanent reddish-blue color. This metachromatic effect has been observed in wheat proteins (Minetti et al 1973, Cadwell and Kasarda 1978, Zawistowska et al 1984, Lupano and Anon 1985) and in collagen and histone 1 proteins (Duhamel et al 1980). In contrast, however, the protein bands are more brightly stained in the reduced samples. Some of the more brightly stained bands in the unreduced samples of the low mobility region seem to have disappeared in the reduced samples. These proteins are from total extracts of flour samples, thus the low mobility bands in the reduced samples are most likely to be the high molecular weight subunits of glutenin.

In Figure 7, gel stained for sugars showed more brightly stained bands at the high molecular weight region (sugar-protein bands) than the gel stained for protein using CBB (Fig. 6). The unreduced proteins of the flour samples of Era and Roughrider (Fig. 7c and d, respectively) stained with PAS, showed compositional differences in the bands (arrows) among the wheats. The SRW wheats (Fig. 7) did not show a stained band in this region. The reduced samples in Figure 7 (carbohydrate-stained) showed that some bands, which stained brightly in the unreduced samples in the region below the arrows, did not stain in the reduced samples. These bands were probably converted to lower molecular weight components upon reduction, with loss of associated carbohydrate because the lower molecular weight components did not stain with PAS. As in the reduced CBB-stained samples, the low mobility bands stained with PAS in Figure 7 are most likely the high molecular weight subunits of glutenin. Note that in Figure 7, the low mobility bands of the unreduced samples were stained to almost equal intensity as those in the reduced samples, unlike the CBB-stained samples (Fig. 6), which showed more faintly stained bands in the unreduced samples than in the reduced. In the reduced samples, however, the low mobility bands stained more intensely with PAS than with CBB.

The glycoprotein fetuin, containing about 23% carbohydrate (Green and Pastewka 1975, Leach et al 1980), was used as a glycoprotein standard applied in unreduced (Fig. 6a) and reduced state of the gel (Fig. 7g). With CBB, fetuin appears as a heavily stained protein in both its unreduced and reduced forms (Fig. 6). However, when fetuin is stained with the PAS reagent, only those protein components in the low mobility region stain positive (Fig. 7a and g). The low molecular weight bands of fetuin do not seem to be associated with sugars, as shown by the lack of detection by PAS of those bands (Fig. 7).

Albumin Fractions on Urea-PAGE Stained for Protein and Carbohydrate

Unreduced and reduced albumin fractions of Butte, Era, Roughrider and Titan were characterized by urea-PAGE with CBB and PAS staining procedures (Figs. 8–9). Albumins were overloaded, as shown with the CBB stain (Fig. 8), to improve detection with the PAS staining procedure (Fig. 9). However, for better resolution of proteins with CBB, lower sample concentration should be applied. Figures 7 and 9 show clearly that some bands in the high molecular weight region are detected by PAS, but the proteins in this region do not seem to be stained strongly with CBB (Fig. 8). Conversely, the low molecular weight

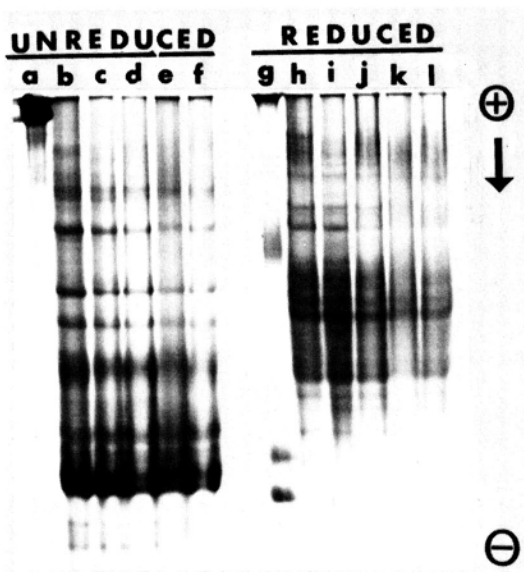

Fig. 8. Albumin protein fraction characterized by urea-polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for proteins with Coomassie Brilliant Blue R-250. Unreduced (**a–f**): **a,** fetuin glycoprotein standard; **b,** Butte; **c,** Era; **d,** Roughrider; **e,** Fairfield; **f,** Titan. Reduced (**g–l**): **g,** fetuin glycoprotein standard; **h,** Butte; **i,** Era; **j,** Roughrider; **k,** Fairfield; and **l,** Titan.

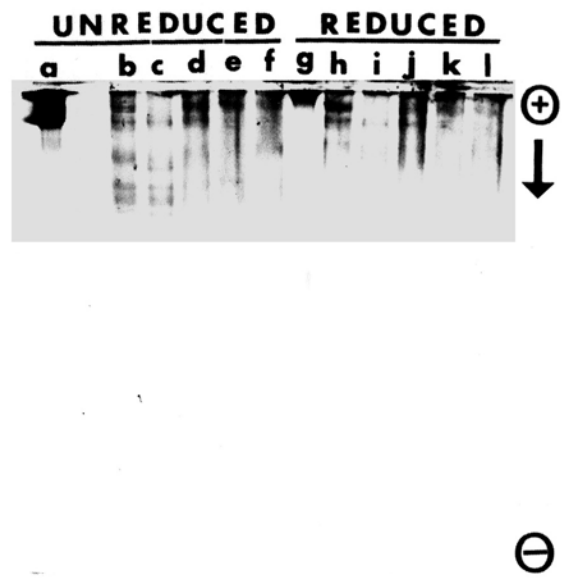

Fig. 9. Albumin protein fraction characterized by urea-polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for carbohydrates with periodic acid-Schiff reagent. Unreduced (**a–f**): **a,** fetuin glycoprotein standard; **b,** Butte; **c,** Era; **d,** Roughrider; **e,** Fairfield; **f,** Titan. Reduced (**g–l**): **g,** fetuin glycoprotein standard; **h,** Butte; **i,** Era; **j,** Roughrider; **k,** Fairfield; and **l,** Titan.

polypeptides stained heavily with CBB, but they did not give a positive stain with the PAS reagent. As noted for proteins from total flour extracts (Figs. 6 and 7), the albumin protein bands that contain carbohydrates stain very faintly with CBB (Fig. 8), but they stain more brightly with the PAS (Fig. 9).

Albumin Subfractions on Urea-PAGE Stained for Protein and Carbohydrate

The subfractions of albumin from gel filtration (peaks I, II, and III) of Era, Roughrider, and Titan were separated by urea-PAGE (Figs. 10–12). The separation of subfractions II and III, unreduced on urea-PAGE, did not show overlapping bands (Fig. 10). However, there seemed to be some overlap between subfractions I and II (Fig. 10). Because all tubes of each peak were pooled to obtain each specific subfraction, certain overlapping was expected. Subfraction III, however, was separated completely from subfraction II (Figs. 1–3). The subfractions (peaks) reduced with 2-ME (Fig. 11, overloaded), seem to increase in the number of minor bands (faint bands). Samples reduced with 2-ME resulted in more overlapping of protein components on urea-PAGE than did unreduced samples (Fig. 10) among the subfractions (e.g., II and III). In addition, streaking increased in reduced samples (Fig. 11). A similar effect was observed for SDS-PAGE of reduced and unreduced albumin subfractions (Figs. 4 and 5).

Figure 12 shows the urea-PAGE of unreduced albumin subfractions stained with PAS for glycoproteins. The first band in albumin subfraction I of Era and Roughrider stained more strongly than the first band of Titan, indicating less carbohydrate associated with protein in the SRW wheat. Subfraction II had at least seven major bands that stained positive for sugars. Subfraction II stained slightly brighter in the SRW wheat Titan than the HRS wheat Era. Subfraction III did not contain any bands that stained positive with PAS, which either confirms once more that III consisted largely of proteins of lower molecular weight that do not appear to be associated with sugars in the wheats studied, or that the sugar concentration was very low and not detectable by this method. Also in Figure 12, the intensity of the bands detected by PAS in I seemed to decrease from the HRS wheat Era to the SRW wheat Titan. The poor detection by PAS of the total albumin fraction (Fig. 12, lane A) compared with that of subfractions I and II in the same figure, indicates that, when using PAS staining, a step such as gel filtration chromatography is necessary for increasing sample concentration. Carbohydrate was associated with the lower mobility protein components of the albumin fraction and subfractions isolated by gel filtration chromatography.

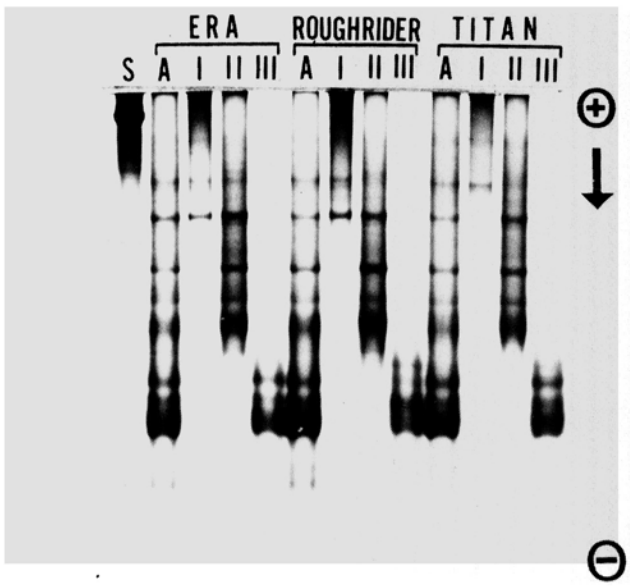

Fig. 10. Subfractions I, II, and III (unreduced) of Era, Roughrider, and Titan wheats characterized by urea-polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for protein with Coomassie Brilliant Blue R-250. A = albumin protein fraction; S = fetuin glycoprotein standard.

CONCLUSION

The results presented here are based on a limited number of samples from each wheat class. Carbohydrate was associated with the lower mobility (higher molecular weight) protein components of the albumin fraction and subfractions from gel-filtration chromatography. Protein and carbohydrate comigrated on urea-PAGE, as shown by PAS and CBB stains. On the basis of this information, our tentative conclusion is that the carbohydrate may be associated with albumin protein by a covalent-like linkage.

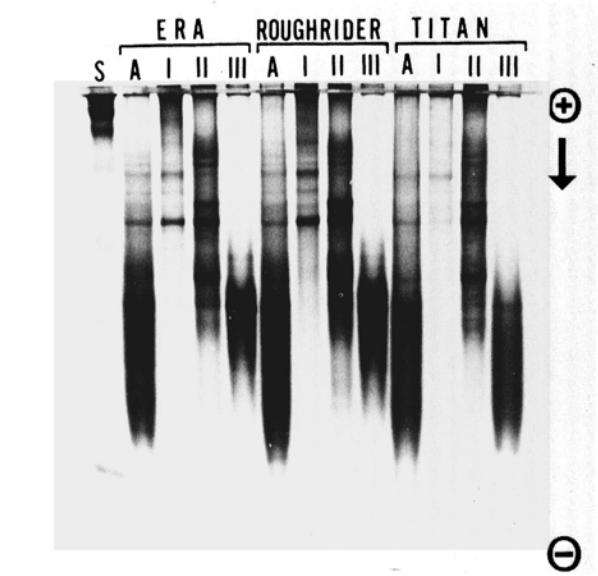

Fig. 11. Subfractions I, II, and III (reduced with 2-mercaptoethanol) of Era, Roughrider, and Titan wheats characterized by urea-polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for proteins with Coomassie Brilliant Blue R-250. A = albumin protein fraction; S = fetuin glycoprotein standard.

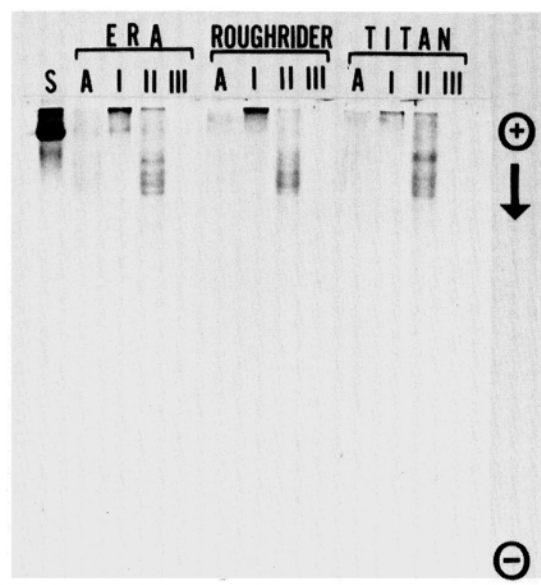

Fig. 12. Subfractions I, II, and III (unreduced) of Era, Roughrider, and Titan wheats characterized by urea–polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) stained for carbohydrate with periodic acid-Schiff reagent. A = albumin protein fraction; S = fetuin glycoprotein standard.

Further studies are needed to identify particular amino acids involved in the linkage and to confirm the nature of the bond.

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