Hydrophobic Properties of Reduced and Alkylated Acetic Acid-Soluble Glutenins—Fractionation by Hydrophobic Interaction Chromatography[1]

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ABSTRACT

The proteins from flours of 13 wheat varieties representing different wheat classes were separated by a modified Osborne fractionation procedure. The reduced and alkylated acetic acid-soluble proteins were fractionated by hydrophobic interaction chromatography using phenyl-Sepharose CL-4B. The protein content of each fraction obtained from hydrophobic interaction chromatography was quantified by absorption at 280 nm. Often, the largest proportions of the protein were eluted with aluminum lactate buffer and with aluminum lactate buffer containing 30% 2-propanol. A larger proportion of protein was eluted with aluminum lactate buffer for soft red winter and Norwegian spring wheats than for hard red spring and hard red winter wheats, indicating lower hydrophobicity of reduced and alkylated glutenin from wheats of poor baking quality. The high-molecular-weight (HMW) glutenin subunits were also eluted with aluminum lactate buffer and with aluminum lactate buffer containing 30% 2-propanol. Sodium dodecyl sulfate electrophoregrams indicated that HMW glutenin subunits 8–10 and 12 had lower surface hydrophobicities than HMW subunits 1, 2, 2*, 5, and 7.

Wheat proteins are unique among the cereal proteins because of their ability to form a viscoelastic dough, which can be attributed to the ability to form gluten when flour and water are mixed. The viscoelastic properties of the gluten protein in a dough system are generally considered important in determining the baking properties of a wheat flour (Huebner and Wall 1976, Tatham et al 1984).

During the past two decades, the high molecular weight (HMW) glutenin subunits of wheat have been subjected to extensive research by many scientists in various countries. The presence or absence of certain HMW glutenin subunits in wheat varieties has been related to breadmaking properties (Orth and Bushuk 1973a,b; Payne et al 1980; Lawrence and Payne 1984; Branlard and Dardevet 1985; Khan et al 1989). However, the variability in HMW glutenin subunit composition between wheat varieties does not explain all of the variability in breadmaking properties between varieties (Orth and Bushuk 1973a,b; Khan et al 1989). Varieties of good and poor breadmaking properties may contain the same HMW glutenin subunits (Khan et al 1989). Other characteristics of the flour proteins apparently play a role in determining the breadmaking properties of a flour.

The number of hydrophobic sites and their location might be of importance for interactions among protein constituents, and between proteins and lipids, during the mixing and baking processes. Tatham et al (1984) considered the HMW glutenin subunits to be the major elastic components of gluten and proposed that several molecules form elastic fibrils. Hydrogen bonds and hydrophobic interactions could participate in the stabilization of the fibrils, which suggests that variability in the hydrophobic properties of the glutenin proteins might be a cause for variability in baking properties between flours.

Chung and Pomeranz (1979) examined the hydrophobic properties of the glutenin fractions of two flours, one with good and one with poor baking properties. They found less hydrophobic interaction between the glutenin from the poor-baking-quality flour and the hydrophobic gel than between the glutenin from the good-baking-quality flour and the gel. A more recent study by He and Hoseney (1991) found that the breadmaking quality of a poor-breadmaking flour was improved by the addition of chaotropic salts, probably due to increased hydrophobic bonding. These results suggest that a relationship might exist between the hydrophobic properties of flour proteins and the mixing and baking properties of a flour.

The surface hydrophobicity of gliadin components has been studied extensively by Popineau and Godon (1982), Popineau (1985), and Popineau and Pineau (1987, 1988). Popineau and Pineau (1987) applied hydrophobic interaction chromatography (HIC) and reversed-phase high-performance liquid chromatography (RP-HPLC) and found that the surface hydrophobicity of gliadin components was greatest in γ-gliadins and less (in decreasing order) in α-, β-, and ω-gliadins. Popineau (1985) also fractionated glutenin obtained from the gluten of defatted flour. The fractionation of unreduced glutenin by HIC on phenyl-Sepharose CL-4B resulted in two major peaks. Comparison of the fractionation of gliadins and acetic acid-soluble glutenins suggested that some glutenin aggregates exhibited surface hydrophobicity properties similar to those of gliadins, whereas others had higher surface hydrophobicity properties. Their results differed slightly from the elution order of gliadins fractionated by HIC in the study by Caldwell (1979). Popineau (1985) also observed differences in subunit composition between glutenin aggregates having different surface hydrophobicity properties. The fractionation of glutenin is complicated by the presence of disulfide bonds between the glutenin subunits. The presence of large glutenin aggregates might affect the ability of the glutenins to bind to the hydrophobic gel matrix and thus affect the fractionation of glutenin components based on their surface hydrophobicities. Burnouf and Bietz (1984, 1985) separated reduced and alkylated (RA) glutenins by RP-HPLC and found that the subunits coded by the long arms of chromosome 1D had lowest surface hydrophobicities among the HMW glutenin subunits. Burnouf and Bietz (1985) also ranked the surface hydrophobicities of several HMW glutenin subunits (e.g., in increasing order, 12, 2, 8, and 7), based on their separation by RP-HPLC. Marchylo et al (1989) used sequential extraction and precipitation to isolate the glutenin fraction. An RP-HPLC procedure was devised to separate and quantitate the HMW glutenin subunits, which were reduced and alkylated in propanol-Tris-HCl. The elution order of the HMW subunits was very similar to that obtained in our study with phenyl Sepharose CL-4B.

The objectives of the present study were to isolate by solubility fractionation the acetic acid-soluble proteins from varieties with widely different baking properties and to determine the hydrophobic properties of RA (pyridylethylated) glutenins by HIC.

MATERIALS AND METHODS

Flour Samples

Flours from four hard red spring (HRS), three hard red winter (HRW), three soft red winter (SRW), and three Norwegian spring wheat varieties were used in this study. The HRS wheats were Butte 86, Celtic, Coteau, and Len; the HRW wheats were Centura, Cody, and Colt; the SRW wheats were Compton, Fairfield, and Titan; and the Norwegian spring wheats were T3042, T6001, and T7035.

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Protein Fractionation

The flours were subjected to protein fractionation according to the modified Osborne fractionation procedure of Chen and Bushuk (1970).

Reduction and Alkylation (Pyridylethylation) of Glutenin

The acetic acid-soluble glutenin fraction was initially reduced with 2-mercaptoethanol (Mallinckrodt, Paris, KY) and alkylated with 4-vinylpyridine (Sigma Chemical Company, St. Louis, MO) according to the method of Friedman et al (1970). Excessive amounts of reducing and alkylating agent were removed by dialysis against distilled water. Fractions were lyophilized after dialysis for subsequent analyses. Acetic acid-soluble glutenin was dissolved in 0.028*M* aluminum lactate buffer, pH 3.6, at a concentration of 10 mg/ml and was applied to the column.

HIC

The acetic acid-soluble fractions (soluble glutenin) were fractionated by HIC, using a Pharmacia column (180 mm long by 10 mm in diameter) packed with phenyl-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. In preliminary experiments, fractionation with octyl-Sepharose CL-4B as column packing material was compared with fractionation on phenyl-Sepharose CL-4B. The preliminary experiments indicated a better separation of the acid-soluble glutenins on phenyl-Sepharose than on octyl-Sepharose. Based on the results from the preliminary experiments, phenyl-Sepharose was routinely used as column packing material.

Initially, 0.028*M* aluminum lactate buffer with 2-propanol in 10% increments (0–50%), as described by Popineau and Godon (1982), were used as eluants. No protein was eluted with 10, 20, or 50% 2-propanol in aluminum lactate buffer. However, some proteins were still bound to the column after elution with 50% 2-propanol in aluminum lactate buffer. Therefore, the following sequence of eluants was used: first, two bed volumes of 0.028*M* aluminum lactate buffer, pH 3.6; second, 30% 2-propanol in 0.028*M* aluminum lactate buffer, pH 3.6; third, 40% 2-propanol in aluminum lactate buffer, pH 3.6; fourth, 60% 2-propanol in aluminum lactate buffer, pH 3.6; and then 0.015*M* Tris in 3*M* urea, pH 8.6, as the final wash. At least two bed volumes of each eluant was used. The elution of protein was monitored with an Isco UA-5 absorbance/fluorescence detector connected to a type 9 optical unit (Isco, Lincoln, NE). Fractions of 2 ml each were collected and pooled according to the elution profiles. The protein recovery from the column was determined by an assay using Coomassie Brilliant Blue G as described by Read and Northcote (1981). Fractions were dialyzed against distilled water and lyophilized.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

The freeze-dried glutenin fractions from HIC were subjected to analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli (1970), on 12% gels.

RESULTS AND DISCUSSION

Establishing Conditions for HIC

First, octyl-Sepharose CL-4B and phenyl-Sepharose CL-4B were compared for their ability to separate RA-glutenins. The RA-glutenins appeared to be more tightly bound to octyl-Sepharose than to phenyl-Sepharose since solvents of lower polarity were required for elution of protein from the column packed with octyl-Sepharose than from the column packed with phenyl-Sepharose. RA-glutenins were, therefore, poorly separated on octyl-Sepharose. Similar results were obtained by Popineau and Godon (1982) for gliadin proteins. Caldwell (1979) found that gliadins were better separated on octyl-Sepharose CL-4B than on phenyl-Sepharose CL-4B. Chung and Pomeranz (1979) reported no major differences in protein binding capacities between phenyl- and octyl-Sepharose CL-4B for unreduced glutenins.

Second, when RA-glutenins containing reducing and alkylating agents in solution were applied to the hydrophobic column, poor separation resulted. Also, the presence of the reducing and alkylating agents appeared to alter the properties of the gel matrix, so that the column packing material had to be discarded and the column repacked between fractionation of each sample. Also, glutenin reduced with up to 5.6% dithiothreitol in aluminum lactate buffer was poorly separated by HIC using a column packed with phenyl-Sepharose CL-4B. Excessive (5.6%) amounts of dithiothreitol altered the properties of the gel matrix. Reduction with 2-mercaptoethanol (1.5%) and alkylation with 4-vinylpyridine was tried and was found to give good separation of glutenins on Sepharose CL-4B. Reducing the glutenins with 2-mercaptoethanol (Friedman et al 1970), followed by alkylation with 4-vinylpyridine and removal of the reducing and alkylating agents by dialysis permitted a successful fractionation of the proteins, with no apparent adverse effects on the column packing material. Therefore, this procedure was used routinely to obtain RA-glutenin.

HIC—Elution Profiles and Quantitation of Fractions

Representative HIC elution profiles of the glutenin components of Celtic (HRS), Colt (HRW), T6001 (Norwegian spring), and Titan (SRW) wheats are shown in Figures 1–4. The elution profiles show that the protein eluted with aluminum lactate buffer formed a relatively distinct peak. The peaks corresponding to the protein eluted with 30, 40, and 60% 2-propanol in aluminum lactate had several shoulders. The protein eluted with 60% 2-propanol in aluminum lactate exhibited high UV absorbance, indicating a large quantity of protein in this fraction. However, quantitation of the 60% 2-propanol fraction using the Coomassie Brilliant Blue assay of Read and Northcote (1981) indicated that only a small proportion of the total protein was eluted in this fraction, possibly indicating the presence of nonprotein material with high absorbance at 280 nm in this fraction. Also, the 60% 2-propanol fraction did not dry properly upon freeze-drying; oily droplets were formed.

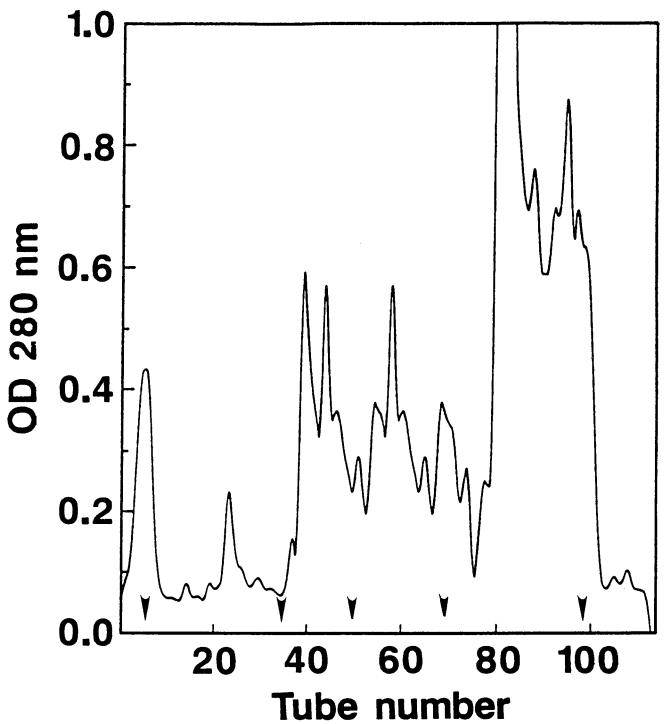

Fig. 1. Hydrophobic interaction chromatography elution profile of reduced and alkylated glutenin from the hard red spring wheat Celtic. (Arrow heads indicate where eluant was changed). 1–4, fraction eluted during sample application; 5–35, fraction eluted with aluminum lactate buffer; 36–50, 51–75, and 76–98, fractions eluted with 30, 40, and 60% 2-propanol, respectively, in aluminum lactate buffer; 99–113, fraction eluted with Tris-urea buffer.

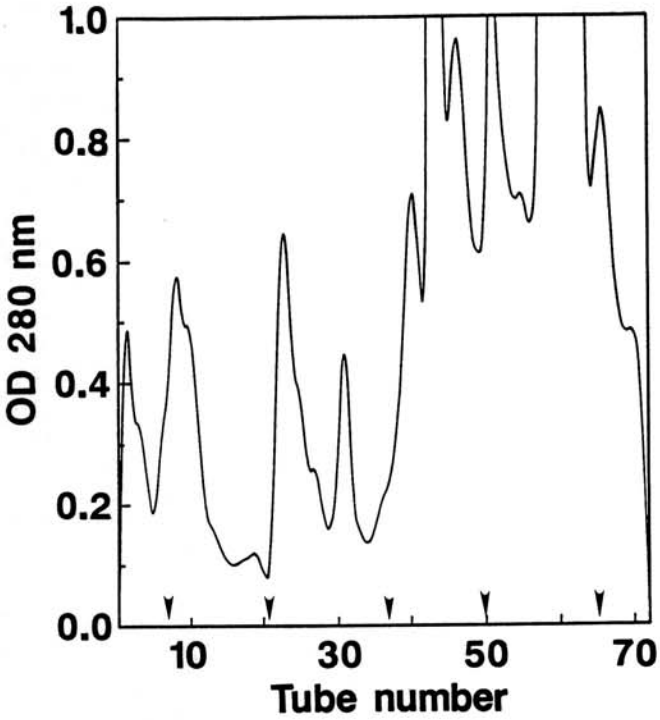

Fig. 2. Hydrophobic interaction chromatography elution profile of reduced and alkylated glutenin from the hard red winter wheat Colt. (Arrow heads indicate where eluant was changed). 1–6, fraction eluted during sample application; 7–20, fraction eluted with aluminum lactate buffer; 21–36, 37–49, and 50–64%, fractions eluted with 30, 40, and 60% 2-propanol, respectively, in aluminum lactate buffer; 65–73, fraction eluted with Tris-urea buffer.

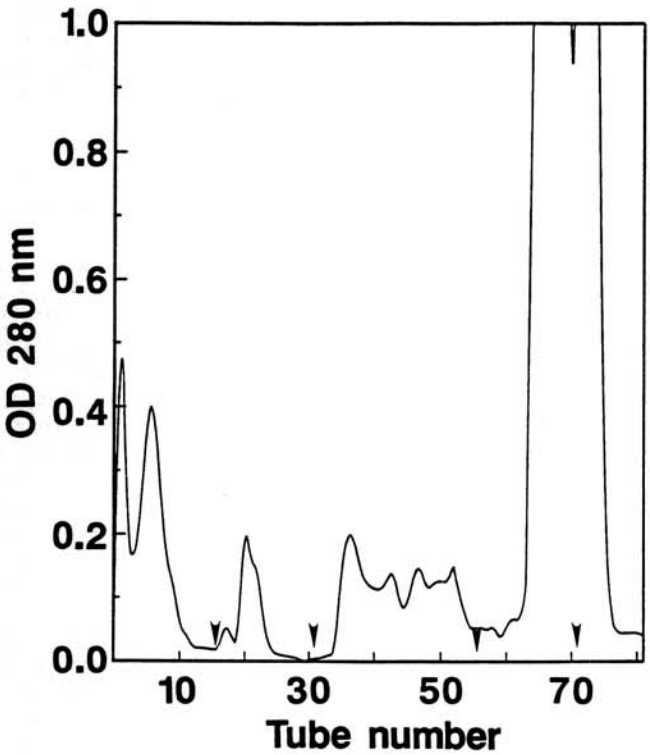

Fig. 3. Hydrophobic interaction chromatography elution profile of reduced and alkylated glutenin from the Norwegian spring wheat T6001. (Arrow heads indicate where eluant was changed). 1–15, fraction eluted with aluminum lactate buffer; 16–30, 31–55, and 56–70, fractions eluted with 30, 40, and 60% 2-propanol, respectively, in aluminum lactate buffer; 71–82, fraction eluted with Tris-urea buffer.

The proportions of protein recovered in each fraction as a percent of total protein applied to the column are shown in Table I. The recovery of protein from the phenyl-Sepharose CL-4B column was between 78 and 100%. Some protein was probably lost when fractions with very low absorption at 280 nm were not pooled and collected but were discarded. The largest proportion of protein was often eluted with 0.028*M* aluminum lactate buffer; the next largest proportion was often eluted with 30% 2-propanol in aluminum lactate buffer. Chung and Pomeranz (1979) found low yields of protein eluted from phenyl-Sepharose columns, indicating strongly hydrophobic proteins. They also reported that complete elution could not be accomplished without the use of solvents destructive to the gel matrix. Results from the present study showed that practically all the protein could be eluted with 2-propanol in aluminum lactate buffer (at 2-propanol concentrations up to 60%) or with 0.015*M* Tris in 3*M* urea as a final column wash. In some of the samples investigated in this study, all of the proteins applied to the column interacted with the column packing material; in other samples, some proteins did not bind to the column but were eluted during sample application, indicating the presence of protein components of very low surface hydrophobicity. None of the Norwegian spring wheat samples contained any protein that eluted during sample application. The glutenins from samples representing the other three wheat classes showed some variability in the amount of protein eluted during sample application.

The SRW and Norwegian spring wheats generally contained a larger proportion of proteins in the fractions eluted with aluminum lactate buffer than did the HRS and HRW wheats (Table I). The HRS wheats differed considerably in the amount of protein eluted with 40% 2-propanol in aluminum lactate buffer. However, no statistically significant differences were detected among the wheat classes in amount of protein eluted with the various solvents. The HRS and HRW wheats represent the varieties with good breadmaking quality. The slightly larger amount of more hydrophobic proteins (those eluted in the 40 and 60% propanol and Tris fractions, Table I) found among the HRS and HRW wheat varieties might indicate a possible

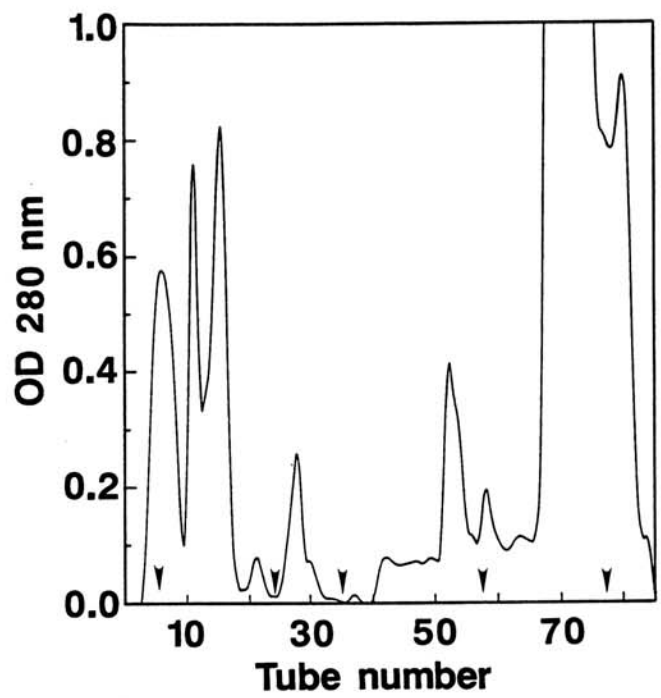

Fig. 4. Hydrophobic interaction chromatography elution profile of reduced and alkylated glutenin from the soft red winter wheat Titan. (Arrow heads indicate where eluant was changed). 1–6, fraction eluted during sample application; 6–23, fraction eluted with aluminum lactate buffer; 24–33, 34–57, and 58–76, fractions eluted with 30, 40, and 60% 2-propanol, respectively, in aluminum lactate buffer; 77–85, fraction eluted with Tris-urea buffer.

TABLE I
Protein Recoveries of Reduced and Alkylated Acetic Acid-Soluble Glutenins by Fractionation on Phenyl-Sepharose CL-4B[a]

Wheat Class	NB[b]	Elution Buffer					Protein Recovery
		AL[c]	Percent 2-Propanol in AL			Tris[d]	
			30	40	60		
Hard red spring	5.35	33.73	15.50	17.87	9.21	8.62	90.28
Hard red winter	13.47	36.46	22.27	6.25	4.88	8.56	91.88
Norwegian	0	64.03	20.19	8.23	1.00	0.42	93.87
Soft red winter	8.46	48.47	18.23	10.30	4.13	2.18	90.78
SD	10.24	18.02	8.23	10.96	7.23	9.04	7.53

[a]Mean values. Recoveries are reported in percent of total applied protein.
[b]Proteins not bound to the column during sample application (unbound proteins).
[c]Aluminum lactate buffer.
[d]Fraction eluted with Tris-urea buffer.

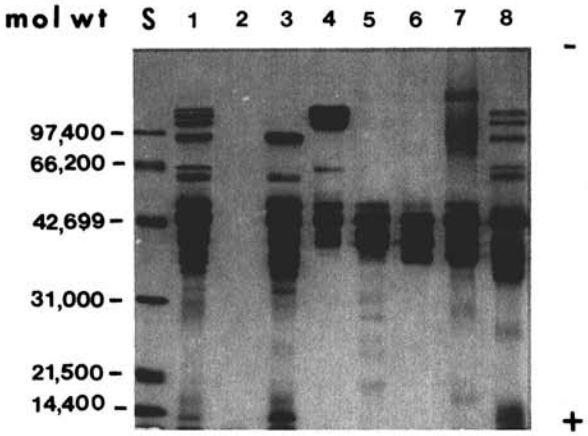

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of acetic acid-soluble glutenin from the hard red spring wheat Butte 86 fractionated by hydrophobic interaction chromatography. S, molecular weight standard; 1, glutenin before fractionation; 2, unbound proteins; 3, fraction eluted with aluminum lactate buffer; 4, fraction eluted with 30% 2-propanol in aluminum lactate buffer; 5, fraction eluted with 40% 2-propanol in aluminum lactate buffer; 6, fraction eluted with 60% 2-propanol in aluminum lactate buffer; 7, fraction eluted with Tris-urea buffer; 8, Len glutenin (standard).

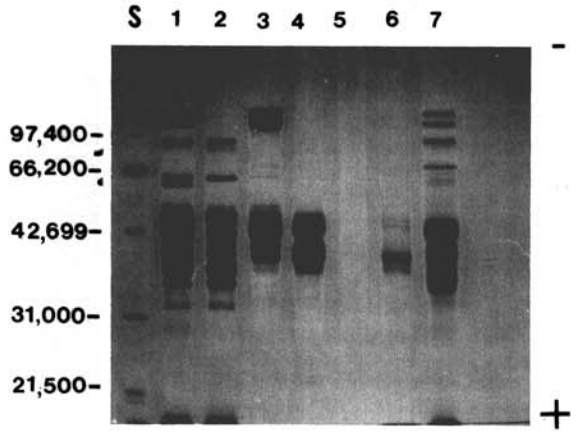

Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of acetic acid-soluble glutenin from the hard red winter wheat Colt fractionated by hydrophobic interaction chromatography. S, molecular weight standard; 1, glutenin before fractionation; 2, fraction eluted with aluminum lactate buffer; 3, fraction eluted with 30% 2-propanol in aluminum lactate buffer; 4, fraction eluted with 40% 2-propanol in aluminum lactate buffer; 5, fraction eluted with 60% 2-propanol in aluminum lactate buffer; 6, fraction eluted with Tris-urea buffer; 7, Len glutenin (standard).

relationship between the hydrophobicity of RA-glutenins and breadmaking quality. The SRW wheat class, not usually used for breadmaking, also showed relatively high hydrophobic properties. Perhaps each class of wheat, whether used for bread, pasta, or pastry, may have its own unique interaction properties. Therefore, to establish more firm relationships between hydrophobicity and breadmaking quality characteristics, further analysis must include more varieties from each class of wheat and much wider ranges of quality differences than used in the present study. Marchylo et al (1989) used RP-HPLC to quantitate the HMW subunits of glutenin in relation to breadmaking quality parameters. However, no definitive relationships were observed with the limited number of varieties examined.

SDS-PAGE of HIC Fractions

Figures 5–8 show representative SDS-PAGE patterns of the various fractions obtained by HIC. The SDS-PAGE patterns shown in these figures indicate that the proteins eluted during sample application included polypeptides of molecular weights between 14,000 and 95,000. This was generally true for those samples that showed unbound proteins. It should be noted, however, that these polypeptides stained rather faintly even though the protein content was appreciably high (Fig. 8 and Table I). The staining of these subunits seems to be similar to that found in the peak 1 proteins of Khan and Bushuk (1979b) from gel filtration of RA-glutenin on Sephadex G-200.

The majority of polypeptides of 14,000–35,000 mol wt present in glutenin fractions eluted in the aluminum lactate buffer (e.g., pattern 3, Fig. 5). The fractions eluted with aluminum lactate buffer also contained the HMW glutenin subunits in the 90,000–95,000 mol wt range, in addition to polypeptides in the 38,000–68,000 mol wt range. The fraction eluted with 30% 2-propanol contained the HMW glutenin subunits of 96,000–100,000 mol wt, a brightly stained band of 64,000 mol wt, and some bands of lower molecular weights in the 35,000–43,000 range.

The fraction eluted with 40% 2-propanol in aluminum lactate contained polypeptides of a much narrower range of molecular weights, 43,700–51,000, compared to the previous fractions. The fractions eluted with 60% 2-propanol in aluminum lactate and with the Tris-urea buffer wash consisted mainly of polypeptides of 37,000–48,000 mol wt. In some of the samples, however, faint bands of higher molecular weights in the 90,000–100,000 region were seen.

Based on the SDS-PAGE separations of the various protein peaks from HIC, it would seem that there is overlapping of some peaks, especially in the 30,000–45,000 mol wt region. This apparent overlap, however, is probably due to the complexity of the glutenin molecules, which contain subunits of similar molecular weights when separated by SDS-PAGE but different physicochemical properties when separated by other techniques such as isoelectric focusing (Khan and Bushuk 1979b, Ng et al 1989). It should also be noted that the more hydrophobic proteins of the glutenin fraction, that is, those subunits eluted with 40 and 60% propanol, have molecular weights in the 35,000–55,000 range, which is the

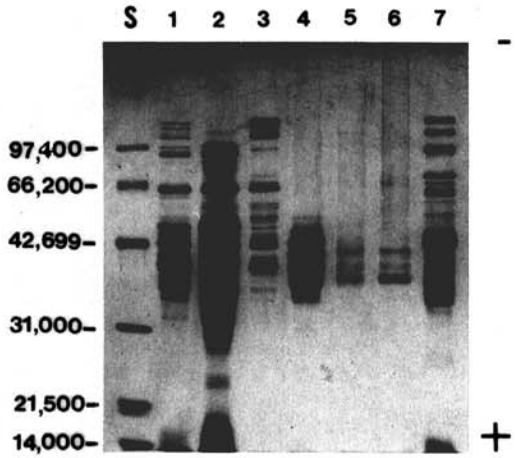

Fig. 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of acetic acid-soluble glutenin from the Norwegian spring wheat T6001 fractionated by hydrophobic interaction chromatography. S, molecular weight standard; 1, glutenin before fractionation; 2, fraction eluted with aluminum lactate buffer; 3, fraction eluted with 30% 2-propanol in aluminum lactate buffer; 4, fraction eluted with 40% 2-propanol in aluminum lactate buffer; 5, fraction eluted with 60 % 2-propanol in aluminum lactate buffer; 6, fraction eluted with Tris-urea buffer; 7, Len glutenin (standard).

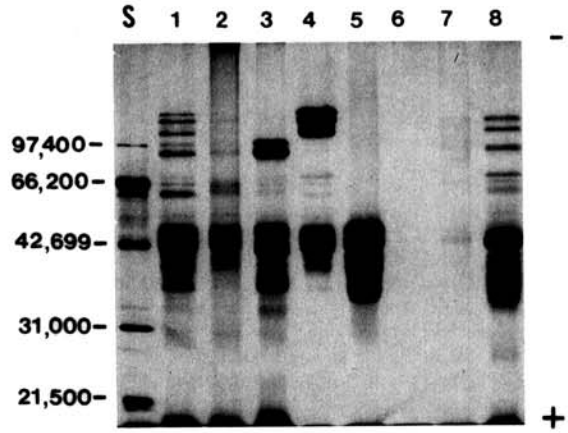

Fig. 8. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of acetic acid-soluble glutenin from the soft red winter wheat Fairfield fractionated by hydrophobic interaction chromatography. S, molecular weight standard; 1, glutenin before fractionation; 2, unbound proteins; 3, fraction eluted with aluminum lactate buffer; 4, fraction eluted with 30% 2-propanol in aluminum lactate buffer; 5, fraction eluted with 40% 2-propanol in aluminum lactate buffer; 6, fraction eluted with 60% 2-propanol in aluminum lactate buffer; 7, fraction eluted with Tris-urea buffer; 8, Len glutenin (standard).

low molecular weight (LMW) glutenin subunit region; these subunits were designated B and C by Graveland et al (1982). Our results are similar to those of Popineau (1985), who separated unreduced acetic acid-soluble proteins of wheat by HIC. When the fractions from HIC were separated by SDS-PAGE with reducing agent (Popineau's Fig. 5), the elution order of glutenin subunits was very similar to that obtained in our study; that is, the HMW glutenin subunits (his fractions 1, 3, and 4) eluted before most of the LMW subunits (his fractions 14, 16, and 17). Therefore, it seems that reduction and alkylation of glutenin do not appreciably change the hydrophobic behavior of its subunits during HIC separation. Since the LMW glutenin subunits seem to exhibit greater hydrophobicity than the HMW subunits, they may play an important role in the aggregative behavior of the glutenin complex, as previously speculated by Khan and Bushuk (1979a) in their model of the glutenin fraction. Recent reports by Payne et al (1987), Gupta et al (1989, 1991), and Pogna et al (1990) have indicated that the presence of certain LMW glutenin subunits is important in influencing gluten strength.

Hydrophobic Properties of the HMW Subunits of Glutenin

In the present study, wheat varieties containing a range of HMW glutenin subunits also were subjected to fractionation by HIC. However, the glutenin samples fractionated by HIC were not selected on the basis of their HMW glutenin subunit composition. Most of the samples contained the subunits 2*, 5+10, and 7+9 (subunit designation according to Payne et al [1980]), but samples containing subunits 1, 2, 8, and 12 also were fractionated. Subunits 1, 2, and 12 occurred in only one sample of the 13 samples used in this study.

Results obtained from HIC and SDS-PAGE indicated that the aluminum lactate fractions from all the HRS and HRW wheat samples had very similar SDS-PAGE patterns. The HMW glutenin subunits 8–10, and 12 were eluted with the polar solvent, aluminum lactate buffer. Subunits 1, 2, 2*, 5, and 7 were eluted with the more hydrophobic solvent 30% 2-propanol in aluminum lactate. The results from the present study differ somewhat from the RP-HPLC results of Burnouf and Bietz (1985). Fractionation by HIC on phenyl-Sepharose CL-4B in our study did not separate individual HMW glutenin subunits as obtained by RP-HPLC.

CONCLUSIONS

Results from the present study show that reduction and alkylation (pyridylethylation) of glutenin proteins permitted their separation based on surface hydrophobicity properties on a column packed with phenyl-Sepharose CL-4B. Our preliminary investigations with limited numbers of wheat varieties showed that, generally, the SRW and Norwegian spring wheats contained a larger proportion of total protein in the fractions eluted with the more polar solvents such as aluminum lactate buffer and aluminum lactate buffer with 30% 2-propanol, compared to the HRS and HRW wheats, suggesting that better bread-baking wheats contain more hydrophobic RA-glutenins than poorer bread-baking wheats. However, with the limited number of varieties investigated, no statistically significant differences were detected. SDS-PAGE of fractions from HIC showed that surface hydrophobicity properties differed among the HMW glutenin subunits. Results from the present study indicated that HMW glutenin subunits 8–10 and 12 were less hydrophobic than subunits 1, 2, 2*, 5, and 7. The LMW glutenin subunits seemed to be more hydrophobic than the HMW subunits, since the former eluted in the less polar solvents, i.e., aluminum lactate buffer containing 40 and 60% 2-propanol. These differences in hydrophobic properties of the HMW and LMW glutenin subunits may influence the structure of the native molecule which, in turn, would influence the functional properties of glutenin. More research is needed, however, on the structure-function relationship of glutenin in breadmaking to establish more definitive relationships.

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