THE EFFECT OF VARIOUS EXTRACTANTS ON THE SUBUNIT COMPOSITION AND ASSOCIATIONS OF WHEAT GLUTENIN

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ABSTRACT

Wheat flours were extracted sequentially with 0.04M NaCl, 70% ethanol, 0.1N acetic acid (HOAc), 0.01N acetic acid-0.2mM. $HgCl_2$ ($HgCl_2$), and 0.1N acetic acid-0.1% 2-mercaptoethanol (ME). The distribution of nitrogen in each extract was determined, and subunit compositions were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Glutenins, found in the HOAc, $HgCl_2$, and ME extracts, differed significantly in subunit composition for each variety; thus glutenin is heterogeneous and can be partially fractionated by different solvents. The HOAc and $HgCl_2$ extracts, upon further fractionation in neutral 70% ethanol, were found to contain 30 to 51% gliadinlike proteins. Carefully defined conditions, including a precipitation step, must be used if purified glutenin is to be isolated from flour extracts.

Many solvents and extracting conditions have been combined in attempts to solubilize and separate all the proteins in wheat flour (1). Most extraction processes are based on the original solvents of Osborne (2): water is used to extract albumins, saline to solubilize globulins, 70% ethanol to isolate gliadin, and acidic or basic solutions to extract glutenins. However the sequence in which such solvents are used, the vigor of extraction (stirring, shaking, or blending), and the starting material (flour or gluten ball) all affect extraction yields of the various proteins due to their high insolubility, tendency to associate, and possible chemical interactions.

Glutenin is the most difficult protein class to solubilize and purify. After extraction with acetic acid, much glutenin may remain undissolved as either gel (3–6) or residue (7) protein, but it may be solubilized by $HgCl_2$ (5), surfactants (4), hydrogen bond-breaking agents (6), or reducing agents (8–9). Furthermore, extracts containing glutenin may also commonly contain other proteins, explaining reported inconsistencies in glutenin's composition and properties. Glutenin has been separated from such impurities by reprecipitation upon adjustment of pH (10,11), gel filtration chromatography (12), or by an SE-Sephadex C-50 procedure (13). No longer does it seem adequate to define glutenin by solubility in the classical manner. Rather, glutenin should be considered as a complex of flour proteins, with molecular weights (MW) ranging into the millions and with characteristic spectra of disulfide-bonded subunits, including some similar to gliadin and others with MW in excess of 100,000 (14–16).

Native glutenin molecules which have different sizes are known to have somewhat different subunit compositions (see ref. 14 for review). It is not known, however, if glutenins isolated by other procedures have identical subunit compositions. Separation and recognition of subunits of glutenin remain difficult by starch gel electrophoresis since many subunits have charge properties similar to one another and to gliadin. However, polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) has

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facilitated differentiation of glutenin subunits from one another and from other wheat proteins (15).

During recent studies of genetic control of wheat proteins, we extended the sequential extraction scheme of Osborne (2) by including additional extraction steps with solutions containing mercuric chloride and mercaptoethanol. Surprisingly, the glutenins extracted with different solvents from any variety differed in subunit composition, and were highly contaminated with lower MW proteins. This report describes these studies, and discusses their implications for further wheat protein research.

MATERIALS AND METHODS

Wheat Varieties

W. Bushuk[2] and E. R. Sears[3] generously provided samples of the hard red spring wheat varieties Prelude and Canthatch; Dr. Sears also provided the hard red spring variety Chinese Spring. Ponca, a hard red winter wheat variety of good quality used in previous research at the Northern Laboratory, was also studied.

We are grateful to E. W. Cole[4] for furnishing a sample of gel protein (5) from a commercial mixed hard red winter wheat flour suitable for bread baking.

Preparation and Defatting of Flours

Approximately 100 g. of each wheat was milled in a Brabender Quadruplex mill into flour, coarse flour, and bran. A small amount of bran was removed from the flour with a 50-mesh screen. Between 60 and 70% was recovered as flour and used for all further studies. Flours were defatted by triple extraction with 200 to 400 ml. 1-butanol (20 to 30 min. stirring per extraction), followed by one similar extraction with petroleum ether, and air dried.

Sequential Extraction of Flour Proteins

The method chosen was basically that of Ewart (17), modified to include the procedure of Mecham et al. (5) for disruption of gel protein with $HgCl_2$. For each extraction, approximately 10 g. of defatted flour was placed in a 250-ml. screw-top plastic centrifuge bottle and 150 ml. of extractant was added. The mixture was shaken vigorously for 30 min. on a bottle shaker and then centrifuged for 30 min. at about 2,000 × g. The supernatant was decanted, and the precipitate mixed with the next extractant. In this manner, the flours were sequentially extracted twice with each of the following solutions: 0.04M NaCl (NaCl), 70% ethanol (EtOH), 0.1N acetic acid (HOAc), and 0.01N acetic acid-0.2 mM $HgCl_2$ ($HgCl_2$). When residues still contained appreciable nitrogen, they were additionally extracted with 0.1N acetic acid-0.1% 2-mercaptoethanol (ME). Dough formation during early extraction steps seemed minimal, since each centrifuged residue could be fully dispersed with the succeeding solvent. NaCl extracts were dialyzed for 24 hr. vs. two to three large excesses of distilled water; temperature was maintained at 4°C. to minimize possible proteolytic digestion. EtOH

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TABLE I
Distribution of Nitrogen in Wheat Flour Extracts

Flour	Percentage of Total N in Flour Extracts						Total N Recovered %
	NaCl	EtOH	HOAc	$HgCl_2$	ME[a]	Residue	
Ponca (2.29)[b]	17.6(10.90)	42.9(17.65)	6.2(11.30)	26.0(14.26)	...[c]	9.8(0.31)	102.5
Chinese Spring (3.23)	12.7(9.73)	20.5(13.23)	9.8(11.92)	19.0(12.37)	26.2(13.57)	11.2(0.50)	99.3
Canthatch (2.31)	13.9(7.67)	22.2(11.50)	8.4(8.92)	25.2(12.71)	10.5(10.52)	7.9(0.26)	88.0
Prelude (2.29)	16.5(8.97)	19.4(12.05)	12.0(9.65)	27.2(13.40)	12.2(11.04)	8.4(0.28)	95.7

[a]ME denotes 2-mercaptoethanol.
[b]Values in parentheses denote percentage nitrogen on a weight basis.
[c]Extraction step omitted.

TABLE II
Percentage by Weight of Glutenin in Extracts of Various Flours as Determined by Insolubility in Neutral 70% Ethanol

Variety	Extract	
	HOAc	$HgCl_2$
Ponca	63.6	70.0
Chinese Spring	57.6	51.3
Canthatch	49.0	58.4
Prelude	58.1	58.0

extracts were concentrated under reduced pressure in a rotary evaporator to remove ethanol. All extracts were lyophilized.

The HOAc and $HgCl_2$ extracts were further fractionated by dissolving or dispersing them in 0.7% acetic acid-70% ethanol and adjusting the pH to 6.6 with 2N NaOH (10). After cooling to 4° C., the precipitated glutenins were removed by centrifugation, and recoveries were determined gravimetrically after ethanol removal and lyophilization.

Other Analytical Methods

Nitrogen recoveries were determined by a modification of an automated Kjeldahl procedure (18). SDS-PAGE was performed on 5% polyacrylamide gels using a pH 8.9 0.125M *tris*-borate buffer containing 0.1% SDS, as described by Koenig et al. (19). Further experimental details and application of the method to wheat proteins were described previously (15,16). Reagent-grade chemicals and deionized water were used in all studies.

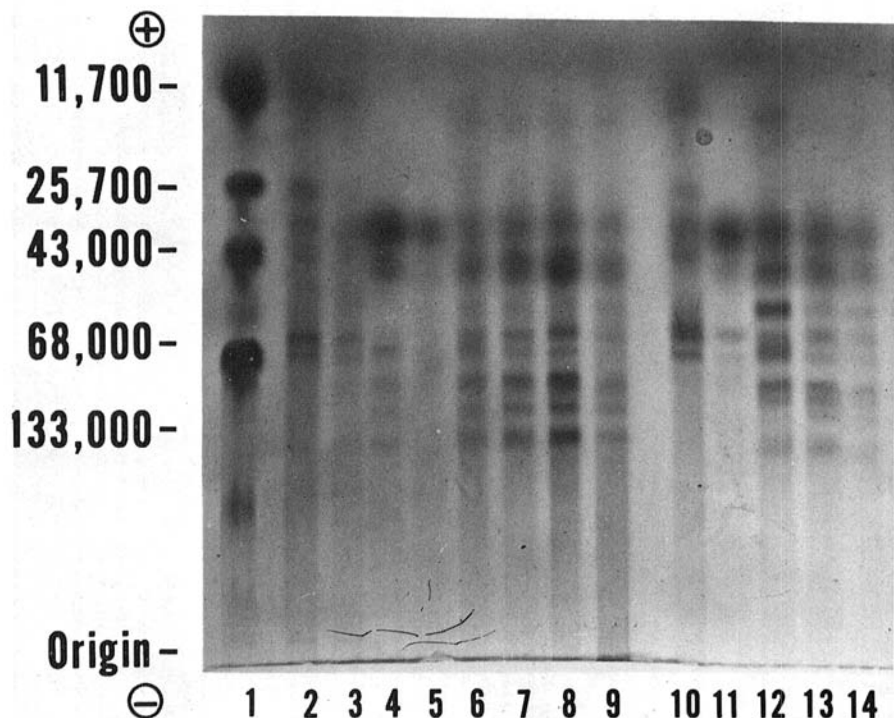

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of subunits of all proteins extracted from wheat varieties Ponca and Chinese Spring. SDS-PAGE was performed on 5% gels using 0.125M *tris*-borate, pH 8.9. (1) Standard proteins; (2–9) Ponca flour extracts from two extractions each with (2–3) NaCl, (4–5) EtOH, (6–7) HOAc, and (8–9) $HgCl_2$; (10–14) Chinese Spring flour extracts with (10) NaCl, (11) EtOH, (12) HOAc, (13) $HgCl_2$, and (14) 2-mercaptoethanol (ME). Each Chinese Spring pattern is of two combined extracts with each solvent. Scale on the left indicates molecular weight.

RESULTS

Extraction Studies

The amounts of nitrogen extracted from flours with each solvent are listed in Table I, expressed as percentage of total flour nitrogen in two combined extracts each of NaCl, EtOH, HOAc, and $HgCl_2$, one ME extraction, and in the final residue. Nitrogen contents of individual fractions are also indicated.

The amounts of protein nitrogen extracted with NaCl (albumins plus globulins) and EtOH (gliadins) are similar to those reported in other studies (7,17,20,21). However, considerably more protein was extracted from Ponca with EtOH than from the other varieties, a condition indicating either that more gliadin is present or that it is more easily extracted.

HOAc, a common solvent for purified glutenin, extracted a relatively small part of the glutenin known to be present in wheat (Table I). Presumably the lack of denaturants and dissociating agents plus the mild mechanical force used in the extraction resulted in much glutenin in flour being insoluble. Similarly, Orth and

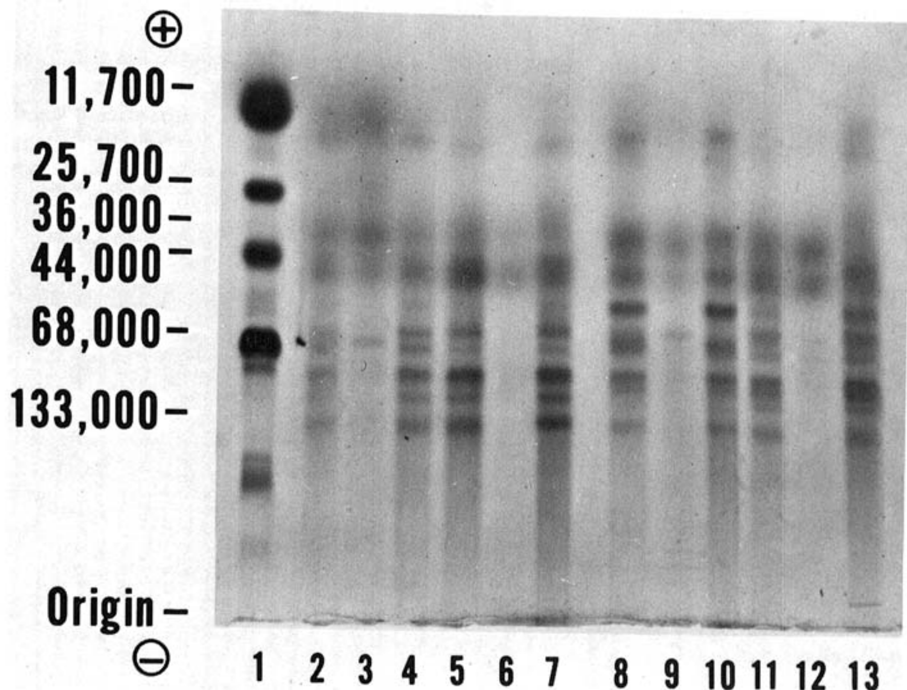

Fig. 2. SDS-PAGE patterns on 5% gels using 0.125M *tris*-borate, pH 8.9, of HOAc and $HgCl_2$ extracts of Ponca and Chinese Spring flours, and of the fractions obtained from each extract which were soluble and insoluble in neutral 70% ethanol. (1) Standard proteins; (2) Ponca HOAc extract, and (3) its ethanol-soluble and (4) ethanol-insoluble fractions; (5) Ponca $HgCl_2$ extract, and (6) its ethanol-soluble and (7) ethanol-insoluble fractions; (8) Chinese Spring HOAc extract, and (9) its ethanol-soluble and (10) ethanol-insoluble fractions; and (11) Chinese Spring $HgCl_2$ extract, and (12) its ethanol-soluble and (13) ethanol-insoluble fraction. Scale at left indicates molecular weight.

Bushuk (20) found little glutenin in HOAc extracts, particularly for wheats of good baking quality.

Mecham et al. (5) noted that protein not solubilized after repeated extraction of flour with dilute acetic acid formed a gel which, for unknown reasons, could be disrupted by $HgCl_2$. When we extracted the material insoluble in HOAc with $HgCl_2$, we found two to four times more protein nitrogen solubilized by $HgCl_2$ than by HOAc (Table I). These $HgCl_2$ extracts generally correspond to gel protein (5) although a voluminous gel did not always form after only two HOAc extractions. Similarly, Clements (22) noted that gels disintegrate to yield glutenin-containing precipitates if minute amounts of salt are added. The lack of gel formation in some of our studies is apparently due to the ionic strength still exceeding a critical level following our sequence of extractions.

After $HgCl_2$ extraction, 9.8 to 37.4% of the nitrogen was still insoluble (Table I). To obtain a sample of these insoluble proteins for comparison to HOAc and $HgCl_2$ extracts, the residues from Chinese Spring, Prelude, and Canthatch flours were extracted with ME, which cleaves disulfide bonds. Much of the remaining nitrogen was solubilized, but 7.9 to 11.2% still remained undissolved. The ME extraction was omitted for Ponca, in which significantly less nitrogen resisted extraction by $HgCl_2$. More complete ME extraction may require alkaline pH under denaturing conditions, but some remaining protein may also be glycoprotein, not subject to solubilization by ME. Total recoveries varied between 88.0 and 102.5%. Although the N recovery for Canthatch (88%) was lower than for the other three varieties (Table I), values of 84.4 to 90.5% were also obtained for four other varieties not included in this report.

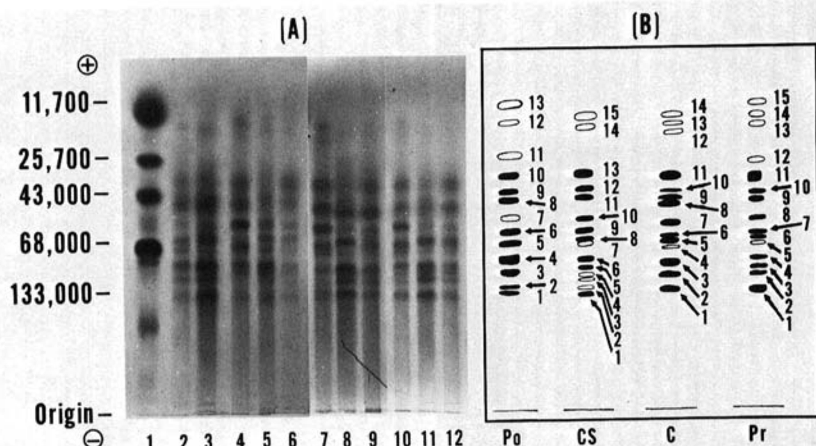

Fig. 3. **A)** SDS-PAGE comparison on 5% gels using 0.125M *tris*-borate, pH 8.9, of HOAc, $HgCl_2$, and ME extracts of each variety studied: (1) Standard proteins; (2–3) Ponca (Po) (2) HOAc and (3) $HgCl_2$ extracts; (4–6) Chinese Spring (CS) (4) HOAc, (5) $HgCl_2$, and (6) ME extracts; (7–9) Canthatch (C) (7) HOAc, (8) $HgCl_2$, and (9) ME extracts; and (10–12) Prelude (Pr) (10) HOAc, (11) $HgCl_2$, and (12) ME extracts. Scale at left indicates molecular weight. **B)** Tracings of electrophoretic patterns for each variety, incorporating all observed bands.

The SDS-PAGE patterns of subunits of the proteins extracted from Ponca and Chinese Spring flours (Fig. 1) are representative of all varieties studied. There is little cross-contamination between different types of extracts, as demonstrated by the lack of several albumin and globulin polypeptides and the presence of only small amounts of glutenin (as evidenced by subunit bands with MW over 100,000) in the EtOH extracts. Since there was little difference between the first and second extracts of Ponca flour with each solvent (Fig. 1, patterns 4–9), extracts were combined for the other varieties.

The NaCl extracts contain primarily albumins and globulins, whereas EtOH extracted mainly gliadin, as seen by comparing these gel patterns to those obtained previously (15). Gel patterns of proteins extracted with HOAc, $HgCl_2$, and ME are characteristic of glutenin prepared by direct extraction of gluten (15), in that high-MW subunits are present; these extracted glutenins will be compared in detail in a later section.

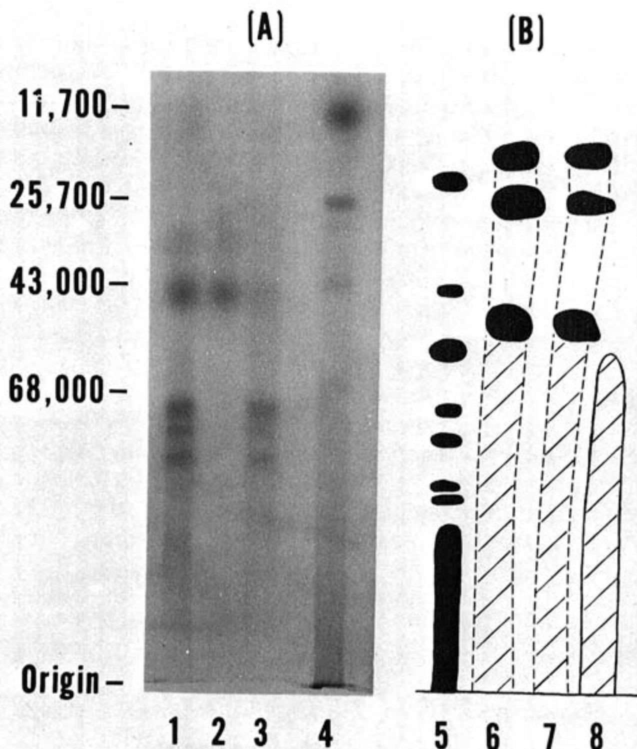

Fig. 4. SDS-PAGE examination on 5% gels using 0.125M *tris*-borate, pH 8.9, of gel protein and its fractions which were soluble and insoluble in neutral 70% ethanol. The gel protein was isolated from a commercial mixed hard red winter wheat flour (ref. 5). **A**) Subunit patterns of reduced proteins: (1) gel protein; (2) ethanol-soluble gel protein; (3) ethanol-insoluble gel protein; and (4) standard proteins. Scale on left indicates molecular weight. **B**) Sketch of precipitation bands observed upon SDS-PAGE of unreduced proteins: (5) ovalbumin, MW 43,000; (6) gel protein; (7) ethanol-soluble gel protein; and (8) ethanol-insoluble gel protein.

The HOAc and $HgCl_2$ extracts (Fig. 1) contain subunits of the same MW (64,300 and 71,000) as found in the NaCl and EtOH extracts. Because these glutenin subunits are not soluble in 0.5M NaCl (16) however, it is unlikely that they are identical to albumins or globulins of corresponding MW, but it is possible that they are denatured albumins or globulins of different solubility.

Association of Extracted Glutenins with Other Proteins

Some low-MW subunits in the HOAc and $HgCl_2$ extracts (Fig. 1) also correspond in MW to subunits in the NaCl and EtOH extracts. To determine whether these extracts contain any associated nonglutenin protein, they were dissolved or dispersed in acidic 70% ethanol and then adjusted to pH 6.6. From 49.0 to 63.6% of the HOAc extracts and 51.3 to 70.0% of the $HgCl_2$ extracts are, like glutenin, insoluble in neutral 70% ethanol (Table II). The ethanol-soluble proteins (36.4 to 51.0% of the HOAc extracts and 30.0 to 48.7% of the $HgCl_2$ extracts) are apparently only associated with glutenin, since they were not previously extracted with EtOH.

SDS-PAGE patterns are shown in Fig. 2 of ethanol-soluble and insoluble fractions of HOAc and $HgCl_2$ extracts from Ponca and Chinese Spring, which are representative of all varieties studied. The ethanol-insoluble fractions retain all characteristic subunit bands of glutenin, but the ethanol-soluble fractions consist mainly of gliadinlike 36,000 and 44,000 MW subunits (15), along with small amounts of albumin- or globulinlike polypeptides. Some differences are apparent between the ethanol-soluble fractions of the Ponca and Chinese Spring $HgCl_2$ extracts: The ethanol-soluble fraction from Ponca contains primarily 44,000 MW subunits, like those present in high-MW gliadin, whereas that from Chinese Spring contains equivalent amounts of 36,000 and 44,000 MW polypeptides. The Chinese Spring $HgCl_2$ extract contains larger amounts of ethanol-soluble protein (Table II), although little protein was extracted from Chinese Spring flour with EtOH (Table I).

Examination of the ethanol-insoluble fractions of the HOAc and $HgCl_2$ extracts by SDS-PAGE in the absence of reducing agent (23) revealed that a single precipitation had separated all lower MW proteins from glutenin. Thus to free extracted glutenin from contaminating proteins, another purification step, such as this precipitation procedure, must be used. Conditions of isolation must be closely defined for any studies of glutenin.

Glutenins in HOAc, $HgCl_2$, and ME Extracts

SDS-PAGE patterns of glutenins in the HOAc, $HgCl_2$, and ME extracts from each variety are shown in Fig. 3, along with composite tracings of glutenin subunit bands from each variety, to which arbitrary numbers have been assigned for discussion purposes. Glutenins in the HOAc and $HgCl_2$ extracts had been purified by precipitation, but since ME reduces glutenin disulfide bonds, the ME extract could not be purified in this manner and may include some low-MW proteins in addition to glutenin and its subunits.

Within each variety, differences in subunit composition of glutenins extracted with different solvents are apparent. For Ponca, the HOAc and $HgCl_2$ extracts have slight differences in intensity of some bands, such as subunit Po-11 (Fig. 3). In the HOAc extract of Chinese Spring, subunits CS-3 and CS-8 (see Fig. 3B for subunit coding) are absent (or deficient) and CS-7 is uniquely present. The

prolaminlike subunits CS-10 and CS-12, of MW 44,000 and 36,000 (15), are deficient in Chinese Spring's $HgCl_2$ extract. The variety Canthatch has subunits C-6 and C-10 only in the $HgCl_2$ extract, but C-5 is absent in this fraction. The HOAc extract is the only one to contain subunit C-9, but it is deficient in C-8. Slight differences in subunits C-12, C-13, and C-14 may also occur between the extracts. In Prelude, subunit Pr-5 is absent from the ME extract, and Pr-7 from the HOAc extract. The $HgCl_2$ extract lacks Pr-6, and is deficient in Pr-8, but is the only extract to contain Pr-10.

Thus within several varieties, glutenins extracted with HOAc, $HgCl_2$, and ME have different subunit compositions. Nearly all subunits may differ in one variety or another; however, no correlation can be detected at present between glutenin's subunits and properties. Huebner and Rothfus (9) previously noted different subunit compositions for native glutenin molecules of different MW. Our studies confirm compositional differences between fractions of native glutenin from a single variety and suggest that if the two observations represent the same phenomenon, extractability of any glutenin molecule is determined primarily by its molecular weight and physical structure. Also, it is now apparent that complete extraction of glutenin yields a mixture of different types of molecules. To examine the relationship between glutenin structure and wheat functionality, sequential extraction and analytical methods, such as those presented here, could be used to fractionate and characterize native glutenin from wheats of different baking quality.

Gel Protein

Since our $HgCl_2$ extracts are similar to the gel protein of wheat flour, which is not solubilized by repeated extraction with acetic acid, we compared these fractions. Gel protein contains subunits with electrophoretic mobilities similar to glutenin subunits (3,4) but also has much 40,000 to 44,000 MW native protein (6,24). To examine these apparently different results, 64.4 mg. of gel protein (5) from a commercial mixed hard red winter wheat flour was fractionated in neutral 70% ethanol; 44.4%, by weight, remained soluble, and does not, therefore, appear to be glutenin; 29.7% precipitated (total recovery 74.1%).

Gel protein and its fractions were compared by SDS-PAGE after reduction to determine the number, MW, and distribution of subunits, and also by SDS-PAGE without reduction (23) to examine MW of the native proteins (Fig. 4). The subunits of gel protein (Fig. 4A) are similar to those of glutenin in MW (15). The SDS-PAGE pattern is simpler than usual for glutenin (see Fig. 3); this may be a varietal difference, or could reflect effects of processing of the commercial flour sample. Cole et al. (6) failed to detect high-MW subunits in fractions of gel protein eluted from agarose presumably because 25% of the protein, which possibly is glutenin, was irreversibly bound. Our observation that 29.7% of gel protein is insoluble in neutral 70% ethanol is consistent with the idea that only 25 to 30% of gel protein is glutenin. The ethanol-soluble and -insoluble fractions of gel protein (Fig. 4, patterns 2 and 3) are equivalent to those fractions from the HOAc and $HgCl_2$ extracts (Fig. 2): the ethanol-soluble fraction contains entirely gliadinlike subunits, whereas the insoluble fraction contains the high-MW subunits characteristic of glutenin.

SDS-PAGE of unreduced gel protein fractions (Fig. 4B) reveals polypeptides of high mobility in gel protein and its ethanol-soluble fraction; these mobilities

are close to that of monomeric ovalbumin (MW 43,000), a similarity first observed by gradient ultracentrifugation by Cole et al. (24). In a separate experiment using SDS-PAGE, we also found the ethanol-soluble polypeptides of gel protein to have MWs similar to a 44,000 MW glutenin subunit (16). The ethanol-insoluble fraction of gel protein contains only streaking material, identical to that of glutenin known to be free of low-MW proteins. Thus, gel protein consists both of glutenin and low-MW proteins.

For the unreduced gel protein fractions (Fig. 4B), it was necessary to sketch the precipitation bands observed when the gel was viewed with oblique lighting against a dark background, since the 40,000 to 44,000 MW components of native gel protein seem to bind the stain, Coomassie brilliant blue (25), to a limited extent. Reduced subunits, however, stain normally.

DISCUSSION

Our extraction studies demonstrated that more than one solvent was required for almost complete solubilization of glutenin from flour under mild conditions. For example, HOAc extracted a relatively small part of the total glutenin from several wheat flours in this study, while with more vigorous mixing or blending nearly all glutenin in a gluten ball is soluble in dilute acetic acid. The previous extractions of albumins, globulins, and gliadins seem to make glutenin less extractable, possibly through partial dough formation during early extraction steps. $HgCl_2$ and ME extracted most of the remaining glutenin, but some protein resisted extraction even under reducing conditions. SDS-PAGE showed that the glutenins extracted with HOAc, $HgCl_2$, and ME differ significantly in subunit composition. Thus, in any studies seeking to relate glutenin's structure and functionality, several solvents must be used to extract all glutenin.

Gel protein, which remains insoluble after repeated extractions with acetic acid, is similar to our $HgCl_2$ extract in that it consists of much low-MW gliadinlike protein associated with glutenin. The gliadinlike fraction contains mainly 44,000 MW polypeptides which appear to be single-chained; previously (15), native gliadin proteins of this MW were not observed. The reason for the inextractability of gel protein by HOAc and the mechanism of its solubilization by $HgCl_2$ (5,6) still remain unknown. We did observe that native gel protein binds the electronegative protein dye Coomassie blue to a limited extent; therefore, the basic residues of gel protein may be largely inaccessible. Gel protein could therefore exist in an unusual conformation or state of aggregation which could partially be responsible for its observed physical properties.

Our studies also have shown that from 30 to 51% of the protein extracted with HOAc or $HgCl_2$ in a typical fractionation procedure, after prior extraction with NaCl and EtOH, is not glutenin. Rather, it appears to consist of unextracted gliadin or subunits of high-MW gliadin (26), plus some albumins and globulins which are probably associated with glutenin through noncovalent bonds. In our study, glutenin in the HOAc and $HgCl_2$ extracts was purified by precipitation from neutral 70% ethanol. Yonezawa et al. (11) and Orth and Bushuk (13) also have noted that extracted glutenin is contaminated with lower MW proteins, and have suggested methods for its purification. Certainly, the Osborne-type of fractionation procedure for separation of wheat protein classes is less successful than commonly supposed. Clearly a combination of methods must be used, and conditions must be fully defined to produce a suitable purified preparation of glutenin for any study.

Literature Cited

1. KASARDA, D. D., NIMMO, C. C., and KOHLER, G. O. Proteins and the amino acid composition of wheat fractions. In: Wheat: chemistry and technology (2nd ed.), ed. by Y. Pomeranz. American Association of Cereal Chemists: St. Paul, Minn. (1971).
2. OSBORNE, T. B. The proteins of the wheat kernel. Carnegie Institution: Washington, D.C. (1907).
3. CLUSKEY, J. E., and DIMLER, R. J. Characterization of the acetic acid-insoluble fraction of wheat gluten protein. Cereal Chem. 44: 611 (1967).
4. INAMINE, E. S., NOBLE, E. G., and MECHAM, D. K. Solubilization and fractionation of wheat flour proteins insoluble in dilute acetic acid. Cereal Chem. 44: 143 (1967).
5. MECHAM, D. K., COLE, E. W., and NG, H. Solubilizing effect of mercuric chloride on the "gel" protein of wheat flour. Cereal Chem. 49: 62 (1972).
6. COLE, E. W., NG, H., and MECHAM, D. K. Further studies on the fractionation and characterization of the gel proteins of wheat flour by column chromatography. Cereal Chem. 50: 336 (1973).
7. CHEN, C. H., and BUSHUK, W. Nature of proteins in *Triticale* and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50: 9 (1970).
8. WREN, J. J., and NUTT, J. The effects of three thiols on the extractability of wheat-flour proteins. J. Sci. Food Agr. 18: 119 (1967).
9. HUEBNER, F. R., and ROTHFUS, J. A. Evidence for glutenin in wheat: stability toward dissociating forces. Cereal Chem. 48: 469 (1971).
10. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84: 363 (1959).
11. YONEZAWA, D., MIWA, T., AWO, Y., and SHIMOMURA, H. Studies on wheat gluten proteins. I. Purification of glutenin by precipitation. Bull. Univ. Osaka Prefect. Ser. B 18: 61 (1966).
12. HUEBNER, F. R. Comparative studies on glutenins from different classes of wheat. J. Agr. Food Chem. 18: 256 (1970).
13. ORTH, R. A., and BUSHUK, W. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50: 106 (1973).
14. BIETZ, J. A., HUEBNER, F. R., and WALL, J. S. Glutenin: The strength protein of wheat flour. Baker's Dig. 47(1): 26 (1973).
15. BIETZ, J. A., and WALL, J. S. Wheat gluten subunits: molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49: 416 (1972).
16. BIETZ, J. A., and WALL, J. S. Isolation and characterization of gliadin-like subunits from glutenin. Cereal Chem. 50: 537 (1973).
17. EWART, J. A. D. Fractional extraction of cereal flour proteins. J. Sci. Food Agr. 19: 241 (1968).
18. BIETZ, J. A. Micro-Kjeldahl analysis by an improved automated ammonia determination following manual digestion. Anal. Chem. 46: 1617 (1974).
19. KOENIG, R., STEGEMANN, H., FRANCKSEN, H., and PAUL, H. L. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207: 184 (1970).
20. ORTH, R. A., and BUSHUK, W. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49: 268 (1972).
21. DRONZEK, B. L., KALTSIKES, P. J., and BUSHUK, W. Effect of the D genome on the protein of three cultivars of hard red spring wheat. Can. J. Plant Sci. 50: 389 (1970).
22. CLEMENTS, R. L. Effects of prior salt treatment on gluten dispersibility. Cereal Chem. 50: 87 (1973).
23. DUNKER, A. K., and RUECKERT, R. R. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. 244: 5074 (1969).
24. COLE, E. W., NG, H., and MECHAM, D. K. Gradient ultracentrifugation in the characterization of the acetic acid-insoluble proteins of wheat flour. Cereal Chem. 49: 68 (1972).
25. FAZEKAS de ST. GROTH, S., WEBSTER, R. G., and DATYNER, A. Two new staining procedures for quantitative estimation of proteins on electrophoretic strips. Biochim. Biophys. Acta 71: 377 (1963).
26. NIELSEN, H. C., BECKWITH, A. C., and WALL, J. S. Effect of disulfide-bond cleavage on wheat gliadin fractions obtained by gel filtration. Cereal Chem. 45: 37 (1968).

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