SOME CHEMICAL AND PHYSICAL PROPERTIES OF THE MERCURIC CHLORIDE-SOLUBILIZED GEL PROTEIN FROM DIFFERENT WHEAT VARIETIES[1]

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ABSTRACT

Mercuric chloride-solubilized gel proteins (MCS-gel proteins) from nine different wheat varieties with good and poor bread-baking qualities were reduced with mercaptoethanol and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fifteen different subunits (approximate molecular-weight (mol wt) range of 14,000–105,000) were detected. MCS-gel proteins from strong flours (hard red spring and hard red winter) had higher concentrations of the subunits in the mol wt range of 77,000–95,000 than those from weak flours (soft white winter). Also, MCS-gel proteins from strong flours had greater sensitivity to precipitation by salt solutions than those from weak flours. Comparisons were made between the subunits of the MCS-gel proteins and glutenin.

In a continued effort to identify those constituents in wheat flours responsible for dough quality in breadmaking, various investigators (1,2) have shown that the glutenin proteins exert a pronounced effect on dough behavior, and differ in their physical and chemical nature from one flour variety to another. Huebner (3) isolated glutenins from several varieties of wheat flours and correlated the salt sensitivities of the fractions with bread-baking quality of the parent flour. Glutenins from those flours recognized for higher quality in making bread products yielded steeper salt precipitation curves than poorer quality wheats. Also Huebner (3), and later Bietz and Wall (4), showed that significant differences in the number and amounts of polypeptide subunits of the glutenin fraction were characteristic of a wheat flour variety, but no definite relationship could be established between the number and mol wt of subunits and breadmaking quality of the flour.

We had studied some glutenin-like constituents, the mercuric chloride-solubilized gel proteins (MCS-gel proteins). These proteins were highly insoluble and occurred in the hydrated residue obtained when flour is exhaustively extracted with dilute acetic acid. Also, these proteins were not subjected to the flour-water dough-mixing and gluten formation of the usual glutenin isolation procedure. The results of that work (5) showed that a relationship existed between the concentration of MCS-gel protein in a flour, and the mixing time required to reach the flour's optimum dough development.

We have now used chemical reduction and electrophoresis to study these MCS-gel proteins and examined the sensitivity of these proteins, and two of their fractions, to precipitation by salt solutions. These studies show that varietal differences in the MCS-gel protein properties other than dough-mixing characteristics (5) exist among these flours. The results are reported and discussed in this paper.

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MATERIALS AND METHODS

Wheat flours used in this study were obtained from the following varieties: hard red spring (HRS): Ceres, Marquis, Rescue, and Red River; hard red winter (HRW): Scout, Warrior, and Winalta; soft white winter (SWW): Genesee and Lemhi.

All of the wheat flours except Lemhi (SWW) were unbleached and milled experimentally. The latter was milled commercially. Mercuric chloride and urea were obtained from Baker Chemical Co. Sodium dodecyl sulfate (SDS) (Nutritional Biochem.) was recrystallized from absolute ethanol.

Preparation of Protein Samples

MCS-gel proteins were extracted from lyophilized gel solids (6) with dilute mercuric chloride by a method described previously (7), except that the step involving removal of residual carbohydrate was omitted. Gel permeation (GP) and salt-eluted (SE) fractions of gel protein derived from Red River flour (HRS) were prepared by agarose chromatography (7). Also, these fractions (in concentrations of 5% w/v) were applied to Sephadex G-200 columns and elution profiles were obtained by a previous procedure (8), except that the solvent used was 0.03*M* acetic acid-0.01*M* hydrochloric acid-4*M* urea, pH 3.0. Glutenin was isolated from Scout flour (HRW) using the procedure of Jones *et al.* (9), in which the gluten was dispersed in 70% ethanol (0.01*N* in acetic acid), followed by adjustment of the pH to 6.5 to precipitate the glutenin. Lyophilized glutenin samples were then extracted with dilute mercuric chloride, as described above for lyophilized gel solids.

Electrophoretic Analyses

SDS-electrophoresis of the reduced flour proteins and reduced protein standards and calculation of mol wt were carried out according to a previous method (7). Time of runs varied from 3 to 7 hours, with a potential of 5 V/cm across the polyacrylamide gel slab. Protein standards (Pharmacia) used were as follows (mol wt in parentheses): ribonuclease (13,700), chymotrypsinogen A (25,000), and ovalbumin (43,000). Two procedures were used to produce high-mol wt polymer standards from ovalbumin. One method employed diethylpyrocarbonate (Pfizer) as catalyst (10), and the other used glutaraldehyde (Fisher) (11). Electrophoretic patterns of polymers produced by both methods were identical.

Measurement of Salt Sensitivities

MCS-gel protein sensitivities to salt solutions were measured according to the procedure of Huebner (3), with the following modifications: MCS-gel protein (0.6% w/v) from each variety was dissolved in 2*M* urea-0.03*M* acetic acid. Four dilutions for each variety were then made with the 1*M* sodium chloride-urea-acetic solution, so that the final concentration of protein was 0.12%, and the salt concentration varied from 0 to 0.04*M*. The solutions were agitated on a Vari-Whirl Mixer while the salt solution was added, and their absorbances were read immediately in a Beckman DU spectrophotometer at 520 nm. MCS-gel protein solutions without salt served as blanks.

RESULTS AND DISCUSSION

As shown in Fig. 1, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) subunit patterns, obtained for MCS-gel proteins from nine different wheat varieties, had at least 15 different subunits of the following mol wt: band 1, 105,000; band 2, 95,000; band 3, 86,000; band 4, 80,000; band 5, 77,000; band 6, 70,000; band 7, 64,500; band 8, 60,000; band 9, 55,500; band 10, 50,000; band 11, 47,000; band 12, 43,000; band 13, 38,500; band 14, 15,500; and band 15, 14,000. In MCS-gel proteins, subunit bands 6 through 9 occurred in low concentrations and were not clearly defined. These particular subunits will be discussed later in this paper.

Much similarity existed among the SDS-PAGE patterns of these proteins, but there were also differences, particularly among the slower moving subunit bands 1 through 5 (approximate mol wt range 105,000–77,000). Band 1 occurred only in the MCS-gel proteins of the SWW (poor breadmaking quality) flours, Lemhi

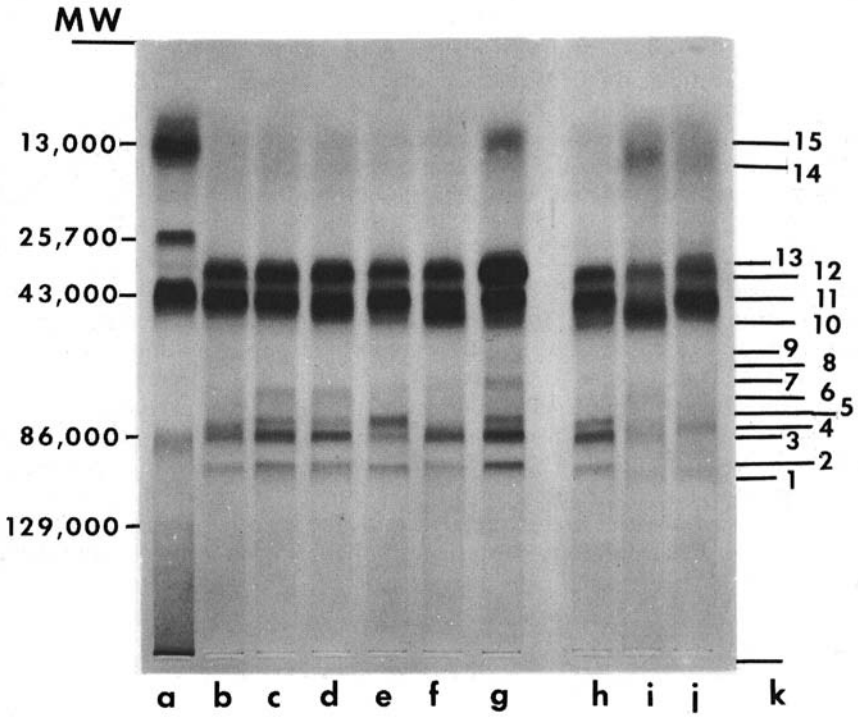

Fig. 1. SDS-PAGE pattern of MCS-gel protein subunits from several wheat flour varieties: a) standard protein calibration mixture (ribonuclease, chymotrypsinogen A, ovalbumin and its polymers); b) Ceres (HRS); c) Marquis (HRS); d) Rescue (HRS); e) Scout (HRW); f) Red River (HRS); g) Warrior (HRW); h) Winalta (HRW); i) Genesee (SWW); j) Lemhi (SWW); k) numbering system used to designate MCS-gel protein subunits (see text). MW = mol wt.

and Genesee, and not in the good breadmaking HRS and HRW wheat flours. Band 3 occurred in the hard wheat varieties and was less prominent in the soft wheats. Bands 4, 7, and 10 occurred at random in the MCS-gel proteins of both hard and soft wheat flours, and were not characteristic of any particular variety. Lemhi and Genesee MCS-gel proteins had significantly lower concentrations of the subunits in the higher mol wt range of 95,000–77,000 (subunits 2 through 5) than the HRS and HRW flours. Further reference will be made to these particular subunits later in this discussion.

In a previous paper (5), the MCS-gel proteins were shown to be similar to the glutenin fraction of wheat flour in their effect on the mixing tolerance of flour-water dough. The subunits (approximate mol wt range 70,000–55,500, bands 6 through 9) of the MCS-gel proteins were markedly lower in concentration than the corresponding ones in glutenin (Fig. 2, slots c and e, respectively). Also, the scarcity of these subunits is evident in the MCS-gel proteins of all nine flours in

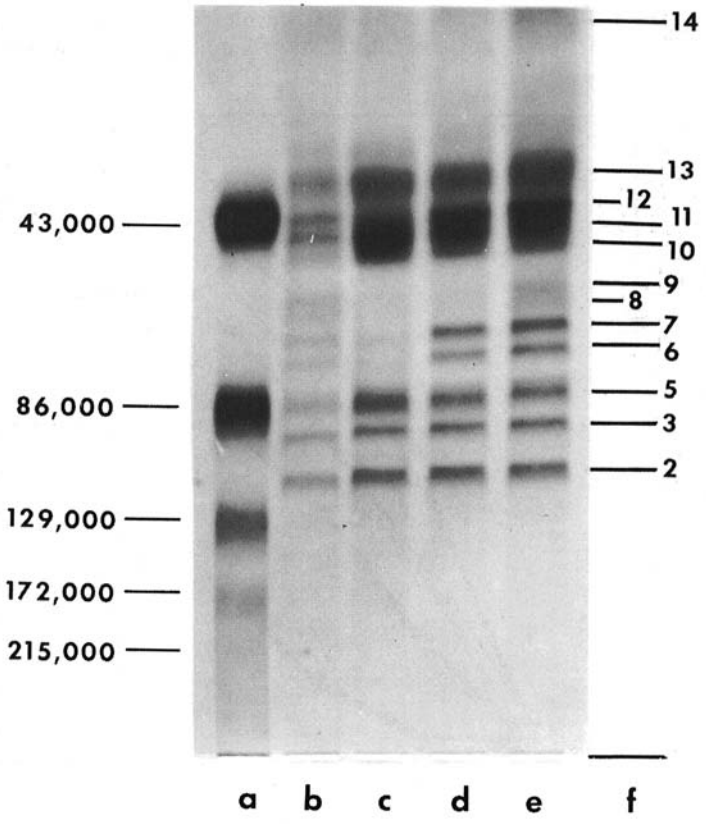

Fig. 2. SDS-PAGE pattern of subunits of MCS-gel protein fractions from Scout (HRW) flour: a) standard protein calibration mixture of ovalbumin and its polymers; b) crude gel; c) MCS-gel protein; d) MCS-glutenin; e) glutenin; f) numbering system used to designate protein subunits (see text).

Fig. 1. In contrast, the protein present in the crude gel prior to mercuric chloride extraction (Fig. 2, slot b) contained all of the above subunits, as did the glutenin solubilized by mercuric chloride. Protein components having subunits in the mol wt range of 55,500–70,000 were more readily extracted from glutenin than from the crude gel. One reason for the selective extractability shown by dilute mercuric chloride may perhaps be found by examining the history of the crude gel and the glutenin preparations used in this work. The preparation of the latter involved flour-water dough-mixing to develop the gluten, followed by washing and extracting with 70% ethanol (9), whereas the former was prepared by mild extraction of flour with large volumes of dilute acetic acid (6). During its preparation, the glutenin fraction may have been altered in some way to change the solubility of some of its protein components in dilute mercuric chloride.

Bietz and Wall (4) studied the glutenin subunits prepared from nine different flour classes and varieties and found at least 15 components of different mol wt ranging as high as 133,000. In contrast, our mol wt values obtained for the largest subunit in glutenin (Fig. 2, slot e) were of a lower magnitude (mol wt 105,000). No reason for this difference in mol wt values is apparent, insofar as the glutenin subunit patterns of these workers looked very similar to ours. Evidently, the

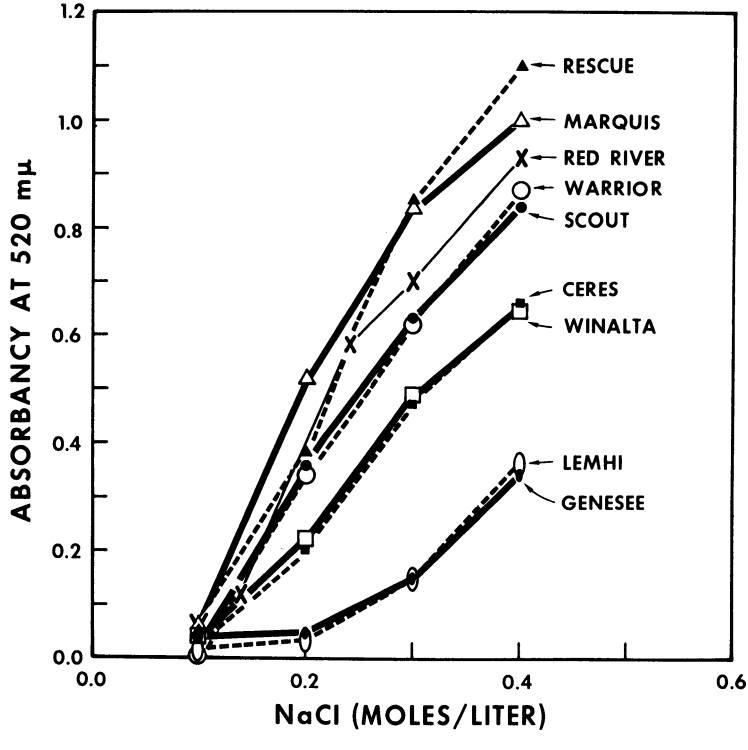

Fig. 3. Effect of sodium chloride on the absorbance of different wheat flour MCS-gel proteins dissolved in 2*M* urea-0.03*M* acetic acid. HRS: Ceres, Marquis, Rescue, and Red River; HRW: Scout, Warrior, and Winalta; SWW: Genesee and Lemhi.

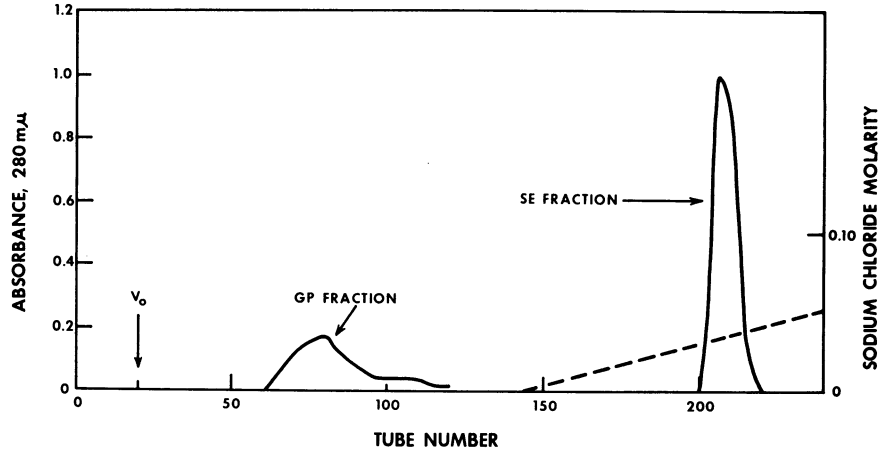

Fig. 4. Elution curve obtained in agarose chromatography of the MCS-gel protein (Red River flour) in 4*M* urea-0.03*M* acetic acid-0.01*M* hydrochloric acid, pH 3.0. GP fraction was obtained by gel permeation and SE fraction by sodium chloride gradient elution.

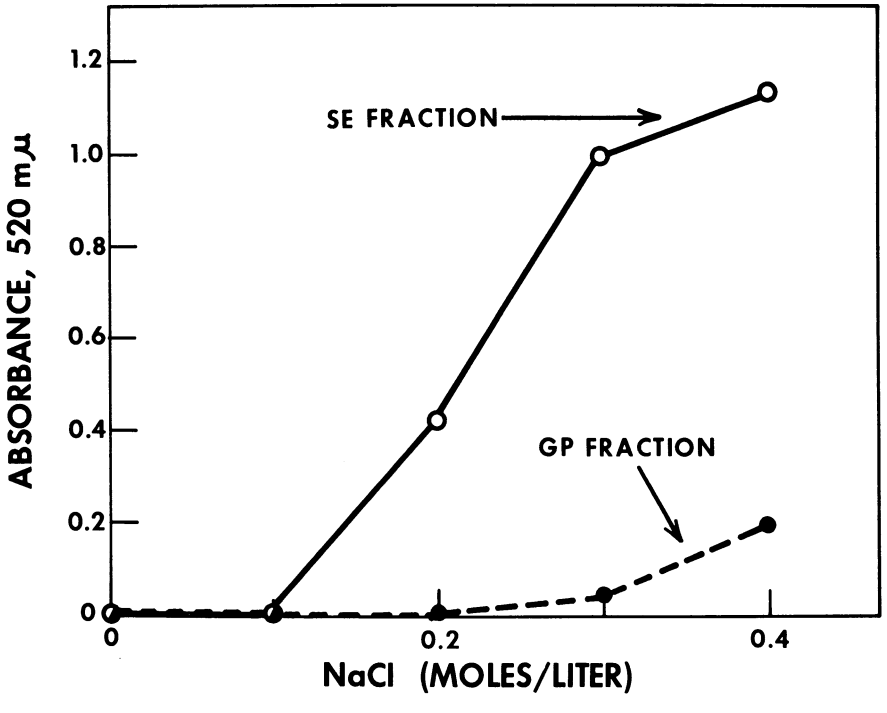

Fig. 5. Effect of sodium chloride on the absorbance of the gel permeation (GP) and salt-eluted (SE) fractions isolated from Red River flour MCS-gel protein. Samples were dissolved in 2*M* urea-0.03*M* acetic acid.

method for determining subunit mol wt by SDS-PAGE (7) is dependent on gel formulation and electrophoretic conditions.

Salt Sensitivities of Gel Proteins

The sensitivities of MCS-gel proteins to changes in salt concentrations varied considerably from one variety of flour to another (see Fig. 3). In this respect, these proteins were similar to the glutenin fraction described by Huebner (3). Within the same class, *e.g.*, for the HRS flours, Marquis, Rescue, and Ceres, there were distinct differences in the curves of the gel proteins. In this phase of work, we did not attempt to establish whether or not a correlation existed between the tendency to precipitate with increased salt and mixing time, and bread quality of a flour within a particular class. The MCS-gel proteins from hard wheat flours recognized for higher quality in making bread products invariably were more sensitive to salt than MCS-gel proteins from the poorer bread quality soft wheat flour. All of these curves were obtained with concentrations of MCS-gel protein at the same nitrogen level, so that the differences in the heights of slopes of these curves are due to qualitative differences among the MCS-gel proteins from the different varieties. This observation prompted us to fractionate a sample of MCS-gel protein in order to locate and further study the components that were the most sensitive to salt.

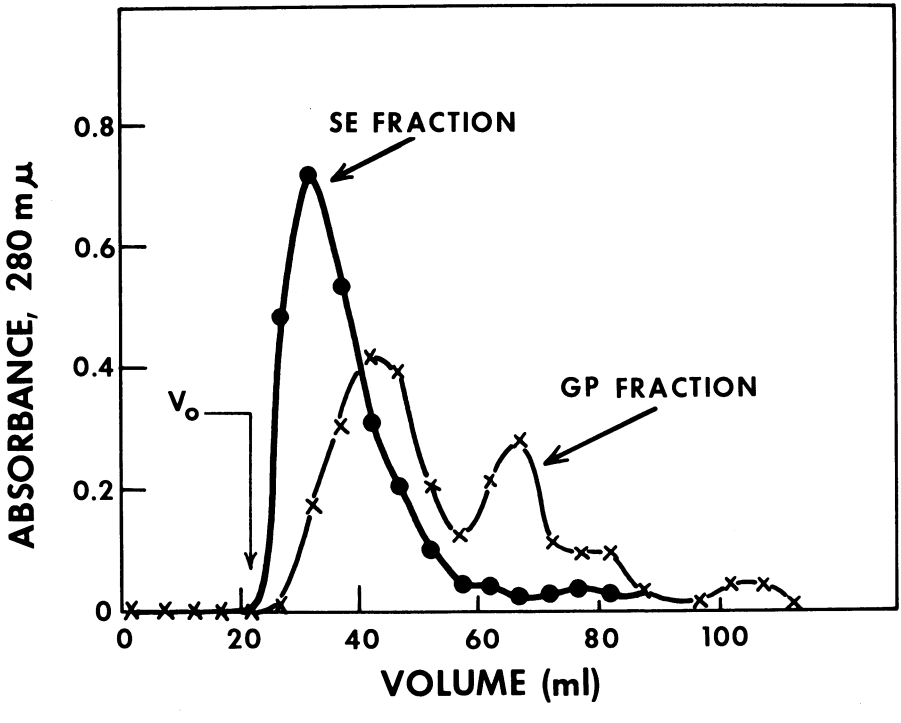

Fig. 6. Gel filtration on Sephadex G-200 of the salt-eluted (SE) and gel permeation (GP) fractions from Red River flour MCS-gel protein.

Since the MCS-gel protein from the HRS flour, Red River, had a relatively high salt sensitivity, it was used in these fractionation studies. When this MCS-gel protein was dispersed in 4*M* urea, pH 3.0, it could be separated in GP and SE fractions by agarose column chromatography (Fig. 4). Figure 5 shows the SE fraction was more sensitive to salt than the GP fraction. This difference, apparently, was due to differences in the sizes of protein molecules or aggregates in the two fractions. Chromatography on Sephadex G-200 gave the elution profiles for these fractions shown in Fig. 6. The GP fraction was retarded whereas the SE fraction was largely eluted at the void volume, indicating that the former fraction contained smaller or less-aggregated protein molecules than the latter.

In a previous paper (7), we showed SDS-PAGE patterns of the reduced SE and GP fractions. The SE fraction chiefly contained the higher mol wt subunits >70,000, whereas the GP fraction largely contained lower mol wt subunits <50,000. Earlier in this paper, it was noted that the subunits with mol wt greater than 70,000 occur in lower proportions in the MCS-gel proteins of the soft wheat flours (Genesee and Lemhi) possessing poor baking quality. Since higher mol wt subunits occur mainly in the SE fraction of the MCS-gel proteins, the presence or absence of this salt-sensitive fraction may be a varietal characteristic that differentiates strong from weak flours, and good from poor bread-baking flours. Therefore, further studies that attempt to relate the concentration of the salt-sensitive fraction of a wheat flour to its dough-mixing and bread-baking properties are warranted.

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