Use of Multistacking Gels in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis to Reveal Polydispersity, Aggregation, and Disaggregation of the Glutenin Protein Fraction[1]

To the Editor:

A multistacking gel procedure in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), based on the Laemmli procedure (1970), was devised to characterize the glutenin protein fraction.

We made five stacking gels at pH 6.9 of 4, 6, 8, 10, and 12% (w/v) acrylamide concentration while keeping the bisacrylamide concentration constant at 0.6% (w/v). Each stacking gel was approximately 0.5 cm high. The resolving gel (12 cm high) consisted of 14% acrylamide with 0.28% bisacrylamide. These gels were 1.5 mm thick and were made in a Hoefer vertical electrophoresis apparatus (Hoefer Scientific, San Francisco, CA). The gel plate dimensions were 18 cm high × 16 cm wide. The 14% resolving gel was polymerized first and then the 12% stacking gel. A snug-fitting, frosted polyethylene plastic plate (1.5 mm thick) was used to obtain a smooth surface on the stacking gels. It was lowered at an angle unto the surface of the gel to eliminate air bubbles and then straightened. After polymerization, the plastic plate was carefully removed and any polymerized gel stuck to the glass surfaces above the gel was scraped off with a Teflon spatula and washed clean with distilled water. The next stacking gel was poured and this procedure was continued for the 10, 8, and 6% stacking gels. For the 4% stacking gel, the sample slots were formed by insertion of the slot former. The time to polymerize the entire gel system was about 4 hr. Samples were loaded on the gel, electrophoresed overnight, stained, and destained as described by Khan et al (1989).

We devised this SDS-PAGE procedure to characterize native (nonreduced) glutenin with respect to the size differences of its molecules. The size of the gel pores becomes smaller from the 4% to the 12% acrylamide stacking gel. This retards molecules that are larger than the pore size of the particular stacking gel at each gel interphase. In this way, molecules of various sizes can be separated and further characterized.

Figure 1 shows the SDS-PAGE patterns of total protein extracts from a dough made in the absence of reducing agent (control) and equivalent doughs mixed with 20, 40, and 80 μmol of the reducing agent dithiothreitol (DTT) according to Ng et al (1991). The label "nonreduced" in Figure 1 refers to freeze-dried ground dough samples that were extracted with phosphate-SDS buffer (Singh et al 1990) in the absence of reducing agent by magnetic stirring and that were run on SDS-PAGE in the absence of reducing agent. At each gel interphase, labeled 4% (each gel is labeled at its origin) to 14% in Figure 1 (nonreduced), there is protein material that did not enter the corresponding gel. This protein material is the glutenin aggregate. Therefore, the protein material at each interphase is a different size. The larger glutenin aggregates remained at the origin of the largest gel pore (4%) while the smallest aggregates migrated to the interphase of the smallest gel pore, the 14% resolving gel. We can see from these separations that the glutenin fraction is polydisperse with molecular aggregates of different sizes.

Figure 1 also shows the effect of the reducing agent DTT on the partially reduced glutenin aggregates (Ng et al 1991). The amount of material remaining at the origin of the 4% stacking gel decreased from the control (pattern 1, no reducing agent) to the 80 μmol DTT-treated sample (pattern 4). At the same time, the material remaining at the other gel interphases increased from the control to the 80 μmol DTT-treated sample. It is clear from these results that the larger glutenin aggregates have been depolymerized to smaller aggregates by the use of low concentrations of reducing agent. None of the brightly stained high molecular weight (HMW) subunits of glutenin that are produced in excess reducing agent (5%, w/v, labeled reduced in Fig. 1) are evident in the resolving gel. However, there are some faint bands in the nonreduced samples, in the 97,000 molecular weight region, that may be depolymerized HMW glutenin subunits. The arrows in Figure 1 indicate the low molecular weight (LMW)

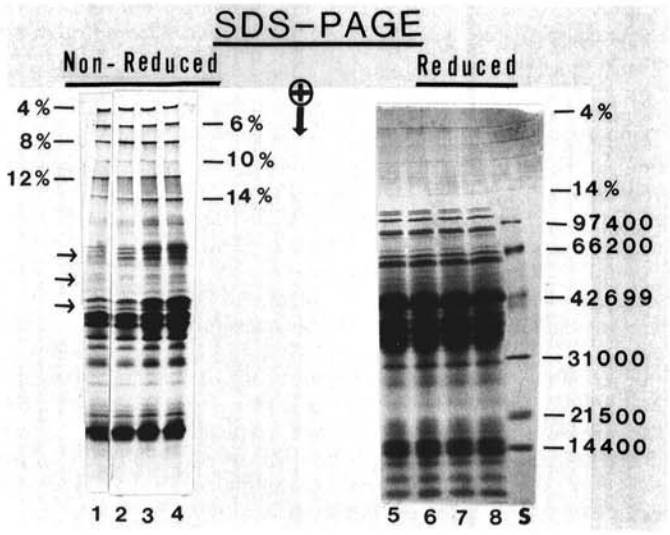

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using multistacking gels of nonreduced (patterns 1–4) and reduced (patterns 5–8) protein extracts of dough treated with low concentrations of dithiothreitol (DTT) (Ng et al 1991). Patterns 1 and 5, control dough with *N*-ethylmaleimide. Patterns 2 and 6, dough made with 30 μmol of DTT. Patterns 3 and 7, dough made with 40 μmol of DTT. Patterns 4 and 8, dough made with 80 μmol of DTT. The origins of the various stacking gels are labeled 4, 6, 8, 10, and 12%, in addition to the 14% resolving gel. S = molecular weight standards. Arrows indicate the low molecular weight region.

TABLE I
Peak-Area Values of Gel Origins of Nonreduced Control and DTT[a]-Treated Samples Scanned at 580 nm

Gel Origin[b] (%)	Peak Area (%)	
	Control Sample	Sample Treated with 80 μmol of DTT
4	42.3	15.6
6	14.5	17.1
8	13.3	15.2
10	2.2	7.9
12	20.7	26.9
14	6.9	17.3

[a]Dithiothreitol.
[b]See Figure 1 to locate origins of nonreduced samples.

[1]Published with the approval of the Director, Agricultural Experiment Station, North Dakota State University, as Journal Series No. 2048.

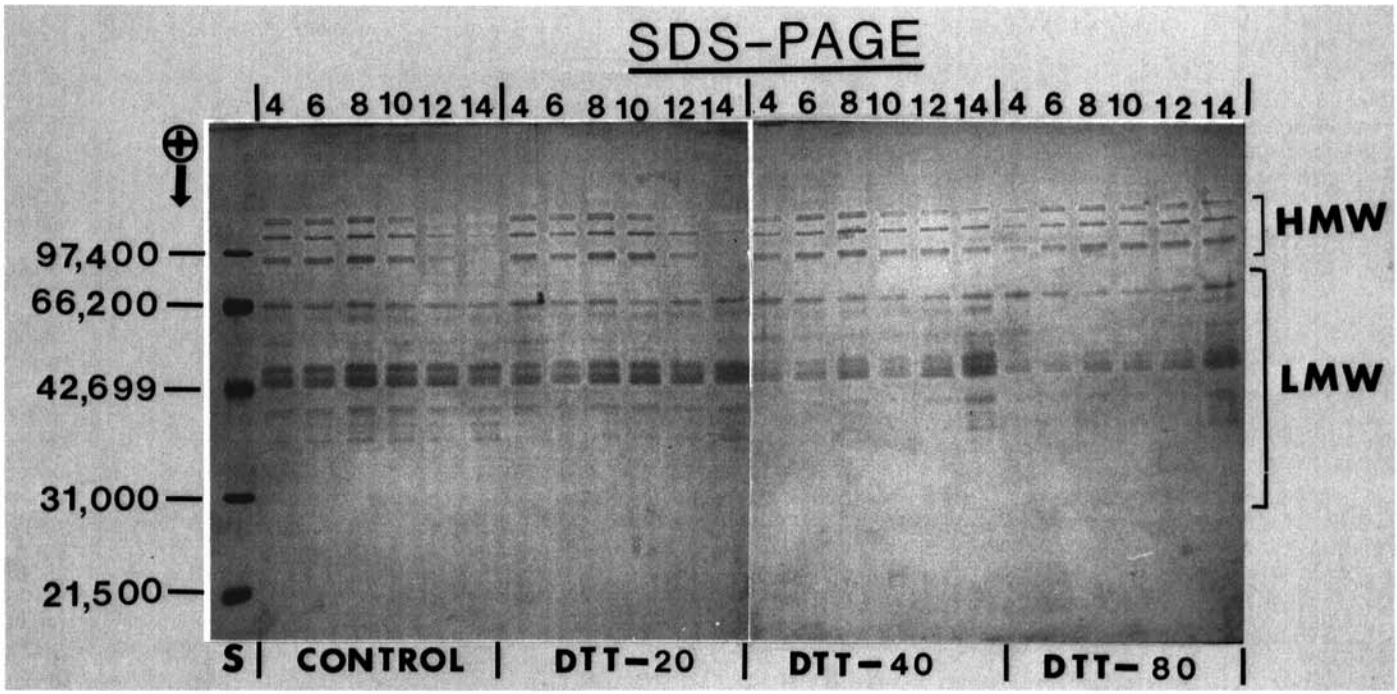

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of reduced (5% excess reducing agent) proteins eluted from the origins of the stacking gels and the resolving gel of Figure 1, patterns 1–4. The numbers 4–14 (top) indicate the origin of the gel concentration (Fig. 1) from which the pattern was obtained. The dough treatments used (bottom) are: control (with *N*-ethylmaleimide) and 20, 40, and 80 μmol of dithiothreitol (DTT). S = molecular weight standards. LMW = low molecular weight subunits. HMW = high molecular weight subunits.

region where staining intensity of bands increased with an increase in DTT concentration. (Differences in staining intensity are assumed to be due to different amounts of proteins present.) Patterns 5–8 show the subunit composition of glutenin of the totally reduced (with 5% reducing agent) dough extracts electrophoresed on the multistacking gel. They show that all reduced proteins enter into the 14% resolving gel, thereby negating the possibility of artifacts.

To demonstrate quantitative differences, patterns 1 and 4 of Figure 1 (nonreduced), representing the control and the 80 μmol DTT-treated dough samples, respectively, were scanned at 580 nm using a gel scanner model 312 (ISCO Inc., Lincoln, NE). Peak-area values (Table I) show that the amount of protein at the 4% gel origin was greater in the nonreduced control than in the 80 μmol DTT-treated sample. At the 14% gel origin, the opposite effect was noted; that is, the 80 μmol DTT-treated sample contained more protein than the control sample. The amount of protein at the other origins in the DTT-treated sample was greater than the equivalent origin in the control sample (Table I). These results, therefore, confirm that the larger molecular weight glutenin aggregates are depolymerized into smaller ones at low concentrations of the reducing agent DTT.

To characterize the subunit composition of glutenin from each gel origin, the gel origins were cut and the protein material eluted with SDS-PAGE sample buffer containing reducing agent 2-mercaptoethanol, as in the original Laemmli (1970) procedure. For maximum extraction, gel pieces were allowed to stand at room temperature and shaken occasionally for 24–36 hr or put in a 40°C water bath and shaken overnight. Figure 2 shows the SDS-PAGE patterns of the material from the stacking gel interphases plus the resolving gel interphase. Many quantitative differences in the subunit composition of glutenin can be seen. (Samples were applied on an equal concentration basis.) For example, the HMW subunits became fainter in the 4% gel from the control to the 80 μmol DTT-treated sample; however, these subunits became more intensely stained in the other stacking gel interphases from the control to the 80 μmol DTT-treated sample. Especially interesting is the composition of the glutenin at the 14% resolving gel interphase of the control—it seems that LMW glutenin subunits predominate, with very faint HMW subunits, indicating strong aggregative properties of LMW subunits. Recent results of Gao (1992), from gel-filtration chromatography of a sample null for the HMW glutenin subunits, indicated an aggregated fraction (void volume fraction) that contained predominantly LMW glutenin subunits as shown by SDS-PAGE.

Therefore, this multistacking gel procedure can be used to separate the glutenin fraction into different size aggregates that can be recovered for further study. We think that this multistacking gel SDS-PAGE procedure can be useful for studying properties such as differences in size distribution of molecules of nonreduced glutenin, gluten, and other wheat protein fractions. This procedure can be coupled with other techniques such as solubility fractionation and column chromatography to characterize isolated protein fractions (e.g., for further electrophoresis, including 2-dimensional; amino acid composition; end-group analysis; and sequence analysis) to provide information on the structure of wheat proteins. An added advantage of the multistacking gels is the almost total elimination of severe streaking into the resolving gel of nonreduced glutenin that occurs with the single-stacking gel system of Laemmli (1970). We are presently using this procedure to investigate wheat protein samples solubilized by sonication according to Singh et al (1990).

The technique can be modified further by using different acrylamide concentrations, e.g., 1% (w/v) acrylamide concentration differences or by using a continuous gradient from 4 to 12% stacking gel composition at pH 6.9 to provide some more flexibility. It should be noted that other researchers such as Gupta and Shepherd (1990) have made improvements to the resolving gel portion of the Laemmli (1970) procedure. It is our hope that this communication would stimulate more research on the native (nonreduced) glutenin fraction, especially the influence of interactive properties, to provide information on the structure-function relationship of glutenin in breadmaking.

ACKNOWLEDGMENT

The Minnesota Wheat Research and Promotion Council, and the North Dakota Wheat Commission are thanked for financial assistance.

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LITERATURE CITED

GAO, L. 1992. Physicochemical and structural studies of glutenin in relation to breadmaking quality. Ph.D. thesis. University of Manitoba, Winnipeg, Canada.

GUPTA, R. B., and SHEPHERD, K. W. 1990. Two-step one dimensional SDS-PAGE analysis of LMW subunits of glutenin. Theor. Appl. Genet. 80:65.

KHAN, K., FROHBERG, R., OLSON, T., and HUCKLE, L. 1989. Inheritance of gluten protein components of high-protein hard red spring wheat lines derived from *Triticum turgidum* var. *dicoccoides*. Cereal Chem. 66:397.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680.

NG, P. K. W., XU, C., and BUSHUK, W. 1991. Model of glutenin structure based on farinograph and electrophoretic results. Cereal Chem. 68:321.

SINGH, N. K., DONOVAN, G. R., BATEY, I. L., and MacRITCHIE, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chem. 67:150.

[Received June 17, 1992. Accepted July 23, 1992.]