Glutenin of Marquis Wheat as a Reference for Estimating Molecular Weights of Glutenin Subunits by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis[1]

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ABSTRACT

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Molecular weights of glutenin subunits of the wheat cultivar Marquis were determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) using characterized proteins from other sources as molecular weight markers. The subunits of Marquis were then used as secondary standard reference proteins for the determination of subunit molecular weights of other wheat cultivars. Statistical analysis of replicate data for one cultivar (Neepawa) showed that the precision of the proposed method is better than 1.5%. The procedure has several practical advantages over the standard procedure based on marker proteins from other sources. Furthermore, it is recommended that molecular mass in kilodaltons (kDa), instead of arbitrary numbers, be used to identify glutenin subunits to facilitate interlaboratory comparison of results.

It is generally accepted that the properties of proteins present in bread wheat flour dictate a wheat's suitability for processing into bread. These properties are jointly responsible for the so-called "protein quality" for breadmaking. It is now well established that glutenin contributes significantly to protein quality and is mainly responsible for the intervarietal differences in breadmaking potential (Orth and Bushuk 1973, Payne et al 1980).

Glutenin constitutes about 45% of the total endosperm protein and is made up of at least 15 polypeptide subunits obtained after reduction of its disulfide bonds (Khan and Bushuk 1978); some of the polypeptides are linked by interpolypeptide disulfide bonds. Orth and Bushuk (1972) reported that the loaf volume is inversely related to the proportion of soluble glutenin and directly related to the proportion of insoluble glutenin. However, they did not find any obvious relationship between subunit electrophoretic patterns and breadmaking quality. Subsequently, Payne et al (1979) reported a significant relationship between specific high molecular weight (HMW) subunits of glutenin and breadmaking quality of English bread wheat cultivars. To examine further the relationship between glutenin subunit structure and breadmaking quality, a practical procedure is needed for estimating the molecular weights (MWs) of the subunits. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has become the most commonly used method for estimating MWs of protein components in a complex mixture (Bunce et al 1985, Shapiro et al 1967, Weber and Osborn 1969). Several precautions must be taken to ensure a good degree of accuracy and precision when applying the method to cereal proteins (Bunce et al 1985).

In previous studies, MWs have been estimated from calibration curves obtained with primary standard reference proteins of known MW, usually from several foreign sources. In the present article, we describe use of the SDS-PAGE electrophoregram of reduced glutenin of the wheat cultivar Marquis, calibrated against primary standard reference proteins, as a secondary standard reference.

MATERIALS AND METHODS

Chemicals

Primary standard reference proteins (indicated below), glycine, Tris, glycerol, potassium hydroxide, and Coomassie Brilliant Blue G-250 were obtained from Sigma Chemical Company (St. Louis, MO). Acrylamide, bisacrylamide, and sodium dodecyl sulfate (SDS) were of electrophoretic grade and were obtained from Bio-Rad (Richmond, CA). All other chemicals used were of analytical reagent grade.

Wheat Samples

Grain of the Canadian hard red spring cultivar Marquis was from the International Association for Cereal Science and Technology Standard Sample (for variety identification by gliadin electrophoresis) maintained in the Department of Plant Science, University of Manitoba. Grain of the Canadian cultivar Neepawa was provided by A. B. Campbell (Agriculture Canada, Winnipeg), the British cultivar Holdfast by P. Payne (Plant Breeding Institute, Cambridge), the German cultivar Diplomat by B. Fretzdorff (Federal Research Institute for Cereal and Potato Processing, Detmold, FRG), and the Australian cultivar Halberd by C. W. Wrigley (CSIRO Wheat Research Unit, Sydney).

Preparation of Glutenin Extracts

Glutenin extracts were prepared by suspending 40 mg of flour in 1 ml of buffer solution, pH 6.8, containing 0.063*M* Tris/HCl, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) Pyronin Y. This glutenin is not identical with the classical glutenin obtained by Osborne's solubility fractionation procedure. Each flour-buffer mixture was allowed to stand at room temperature for 2 hr with occasional shaking, heated for 2.5 min in a boiling water bath, and allowed to cool to room temperature. The suspension was allowed to settle, and an aliquot of the clear top layer was used as the experimental protein extract. The extract can be frozen for future use.

The amount of solution that was loaded into each slot in the gel was 7.5 μl for Marquis and Neepawa, 10 μl for other cultivars (because of their lower protein content), and 5 μl of the solution of the primary standard reference proteins.

The primary standard reference protein mixture (3 mg), containing carbonic anhydrase (MW = 29,000), egg albumin (45,000), bovine serum albumin (66,000), phosphorylase B (97,400), β-galactosidase (116,000) and myosin (205,000), was dissolved in 1 ml of the same buffer mixture.

Electrophoresis

SDS-PAGE was done on an LKB 2001 vertical electrophoresis unit. The procedure was that of Payne et al (1979 and 1980), with some minor modifications, based on the original procedure of Laemmli (1970). Our total gel size was 14 × 16 × 0.15 cm. The separating gel (lower 14 cm) comprised 172.55 g of acrylamide, 0.78 g of bisacrylamide, 1.00 g of SDS, and 0.375*M* Tris/HCl buffer solution (pH 8.8) per liter. The gel was polymerized by adding 0.25 g of ammonium persulfate and 0.5 ml of *N,N,N′,N′*-tetramethylethylenediamine per liter. The stacking gel (1 cm) contained 29.93 g of acrylamide, 0.43 g of bisacrylamide, 1.00 g of SDS, and 0.125*M* Tris/HCl (pH 6.8) per liter. The gel was polymerized by adding 0.375 g of ammonium persulfate and 0.75 ml of *N,N,N′,N′*-tetramethylethylenediamine per liter.

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Electrode buffer solution, pH 8.3, comprised 0.025*M* Tris, 0.192*M* glycine, and 1.00 g of SDS per liter. The gel, usually loaded with 13 samples, was subjected to electrophoresis for 22 hr at 20° C at a current of 5 mA (per gel) for the first 2 hr, followed by 18 hr at 10 mA, and a final 2 hr at 15 mA. The electrode buffer, maintained at 20° C, was circulated continuously during electrophoresis. After running time of 22 hr, the tracking dye, Pyronin Y, runs off the gel. It is included in the sample solution to facilitate addition of the otherwise colorless solution to the slot. The gels were stained overnight with Coomassie Brilliant Blue G-250 according to Blakesley and Boezi (1977), rinsed with distilled water, and photographed immediately through an orange filter (no. 02 from NPS Optical Filters, Japan).

Determination of MWs

In the first step, MWs of glutenin subunits of the wheat cultivar Marquis were determined in the usual manner using the primary standard reference proteins to develop a calibration curve. Marquis was selected as the reference cultivar because of its extensive use in wheat breeding programs and because it is used widely as the reference in cultivar identification by gliadin electrophoresis (Bushuk and Zillman 1978, Sapirstein and Bushuk 1985). It should be noted that with SDS-PAGE the MWs that are obtained are relative to the MWs of the markers; accordingly the MW so obtained is sometime referred to as relative molecular mass (Bunce et al 1985). In the present study, the determined MWs for Marquis subunits are actually relative values compared with published molecular weights of the marker proteins. We propose that the glutenin subunits of the cultivar Marquis be used as the secondary standard reference proteins for determining the MWs of glutenin subunits of other wheat cultivars. Samples of Marquis wheat can be obtained from the authors. Other local cultivars can be used as standards after calibration against Marquis and for this reason we include data for the international cultivars Diplomat, Halberd, and Holdfast.

To obtain the primary calibration curve, the relative mobility (R_m) of each of the primary standard reference proteins was calculated using the mobility of the smallest protein (carbonic anhydrase) as the front marker instead of the tracking dye band as in the standard procedures. Migration distances were determined from the photograph of the stained gel. The use of photographs obviates errors due to shrinkage or swelling of the gels.

To obtain the MW versus mobility calibration curve, two different relationships were examined (Fig. 1). In addition to the standard plot of log MW versus R_m, we plotted log MW versus log R_m. The regression line for the latter plot was slightly better than for the former ($r^2 = 0.99$ and $r^2 = 0.94$, respectively). Accordingly, the regression equation for log MW versus log R_m was used to estimate the MWs of the Marquis subunits (for subsequent use as the secondary standard reference) and of the subunits of other wheat cultivars. The purely mathematical treatment of log R_m did not introduce any additional error over that obtained for R_m because the MWs of subunits were calculated from the regression equation and not measured directly from the calibration curve. In the Marquis pattern, the 34,600 subunit was adopted as the front marker for calculating R_m values. If the reference subunit is also present in the experimental sample, its position in the gel serves as an additional check on the precision of the electrophoretic run. This subunit was present in all 35 varieties examined in our laboratory.

RESULTS AND DISCUSSION

Figure 2 shows SDS-PAGE patterns of Marquis glutenin subunits and the primary standard reference protein mixture. The major subunits, which will be used as the secondary standard reference proteins, are identified and their MWs indicated. The MWs were calculated from the regression equation for log MW versus log R_m for the primary standards. The R_m values for the Marquis subunits were calculated relative to the mobility of carbonic anhydrase.

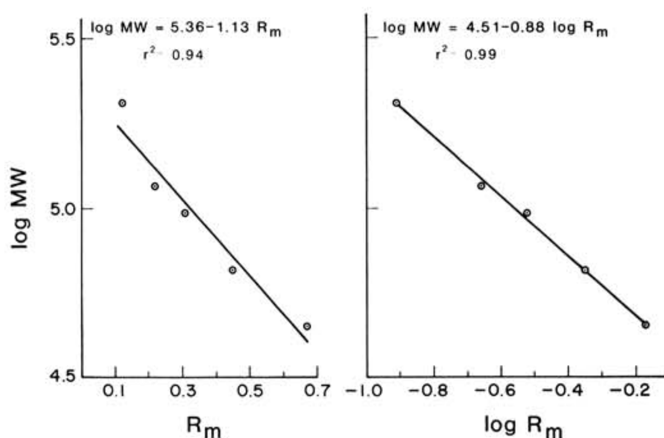

Fig. 1. Relationship between log MW vs. R_m (relative mobility) and log MW vs. log R_m for reference proteins.

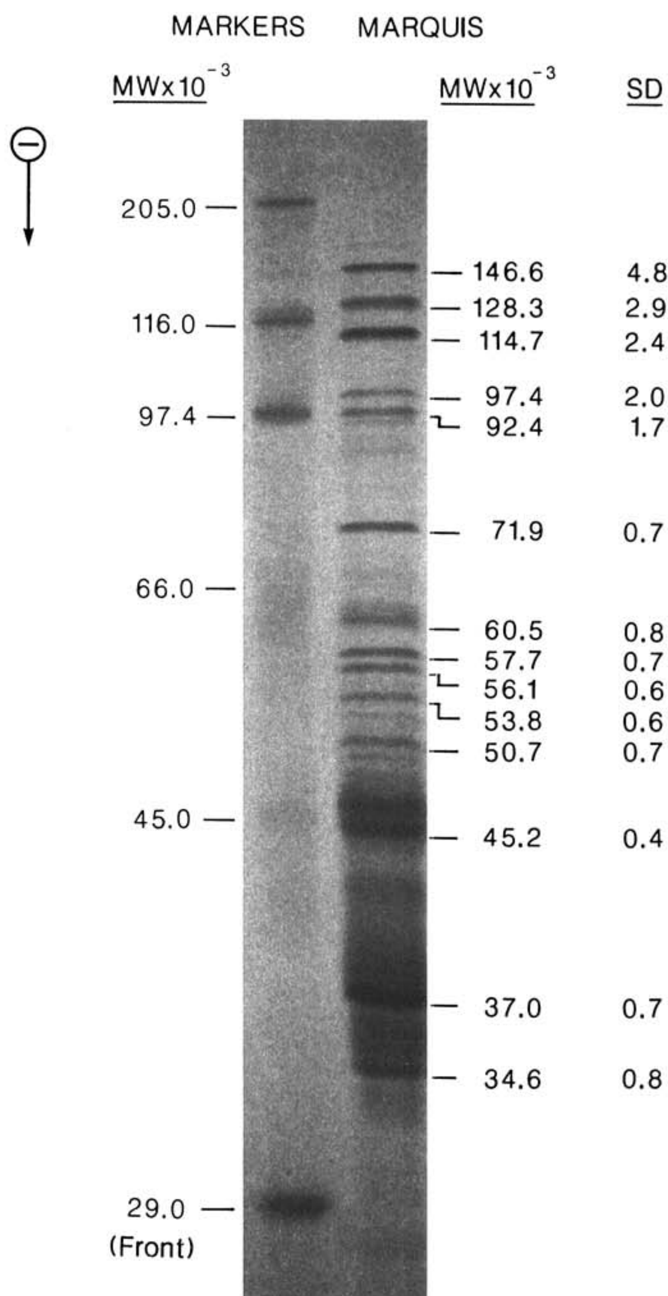

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns for molecular weight reference proteins and subunits of Marquis glutenin. **SD**, standard deviation ($n = 6$).

Four separate analyses of Neepawa glutenin were made to check the reproducibility of subunit patterns and to determine the reproducibility of MW values by the proposed method. In each analysis, glutenin extracts of Marquis (the secondary standard reference) and the experimental wheat variety (Neepawa) were run together. For each Marquis pattern, R_m values were determined relative to the position of the 34,600 subunit in the same pattern. Then, the regression equation for log MW versus log R_m was determined for each analysis. This regression equation was then used to estimate the MWs of the HMW glutenin subunits of the experimental variety. The estimated MWs of the experimental variety were averaged for the four separate runs. Molecular weights ($\times 10^{-3}$) and standard deviations ($n = 4$) for HMW glutenin subunits of Neepawa were 136.6 ± 1.4, 127.8 ± 0.9, 115.0 ± 0.4, 96.5 ± 0.3, and 91.7 ± 0.5. The four MWs for each subunit so obtained differed by less than 1.5%. On the basis of these results, it was concluded that the Marquis glutenin subunit pattern could be reproduced with sufficient precision to warrant its use as a reference for determination of MWs of "glutenin" subunits of other wheat cultivars.

In the final part of this study, MWs of HMW glutenin subunits of the Australian cultivar Halberd, British cultivar Holdfast, German cultivar Diplomat, and Canadian cultivar Neepawa were determined using the proposed Marquis reference (Fig. 3). Five of the HMW glutenin subunits of cultivar Holdfast (Fig. 3, lane 3) are subunits 1, 5, 7, 8, and 11 according to Payne et al (1980). MWs of subunits 1 and 8 of 145,000 and 106,000, respectively, determined by SDS-PAGE by Payne et al (1980) compare well with 147,000 and 101,100 obtained in the present study in view of the overall precision of the SDS-PAGE method (Bunce et al 1985). It should be noted that MW values obtained in the present study apply only to the gel system used; other conditions would require a separate calibration.

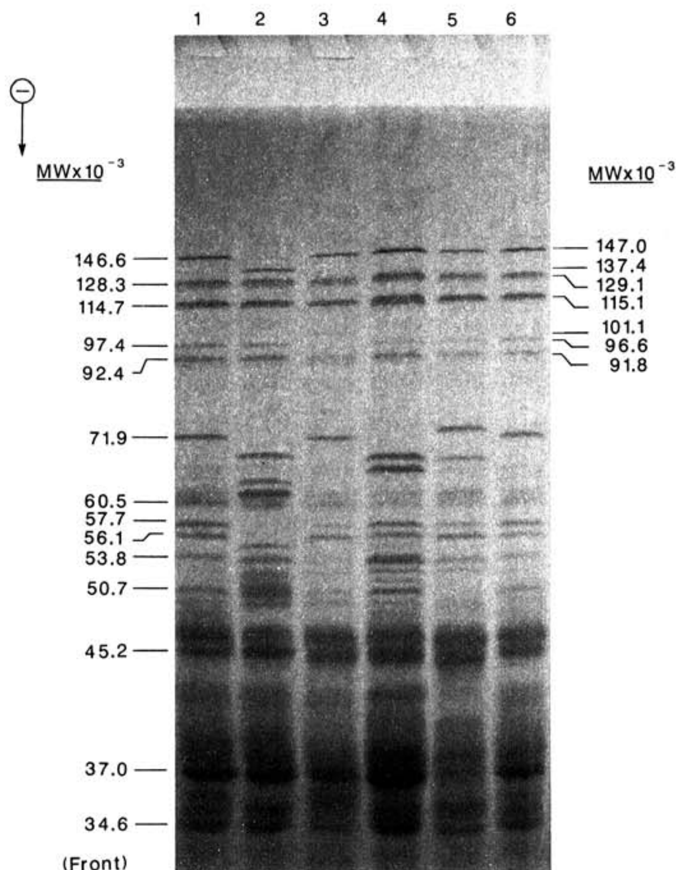

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns: lanes 1 and 6 = Marquis, 2 = Neepawa, 3 = Holdfast, 4 = Halberd, and 5 = Diplomat. Molecular weights of Marquis subunits listed on left; other cultivar high molecular weight glutenin subunits listed on right.

Currently, most published reports on glutenin subunits use the numerical nomenclature of Payne et al (1980 and 1981) for the HMW subunits. This system of nomenclature leads to some confusion when new subunits with mobilities between those for already named subunits are discovered. For example, subunit 2* has mobility in between subunits 1 and 2 (Payne et al 1981) and subunit 2.2 has slower mobility than subunit 1 (Payne et al 1983). Furthermore, a different numbering system was used by Moonen et al (1983). Accordingly we propose that MWs be used to identify the HMW glutenin in subunits to obviate the difficulties encountered with the "closed" systems referred to above.

CONCLUSIONS

The use of glutenin subunits of Marquis (or any other cultivar) as reference proteins for determination of MWs of glutenin subunits of other wheat cultivars by SDS-PAGE described in the present study has several practical advantages. The reference proteins are similar to the unknown proteins in chemical and physical structure, the entire range of MWs is covered by a large number of reference proteins, and the source of reference proteins is inexpensive. Although the electrophoresis time in our method is longer than the commonly used SDS-PAGE procedure, operators would only require two to three actual working hours to prepare materials for the electrophoresis. The long running time and low current of the electrophoresis gave higher resolution of bands. The proposed open system for identifying subunits by their MW, instead of an arbitrary number, will make possible addition of new subunits without any confusion as to their position (or MW) relative to previously identified subunits. Although the MWs determined by SDS-PAGE appear to be overestimated (Bunce et al 1985), the calibration procedure that we used gave MWs that are valid because they are determined relative to the values for standard proteins. All the MW values can subsequently be corrected when exact values for a few of the subunits are determined from complete amino acid sequences.

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