Studies of Glutenin. III. Identification of Subunits Coded by the D-Genome and Their Relation to Breadmaking Quality[1]

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ABSTRACT

Glutenins from four extracted AABB tetraploid wheats, their hexaploid (AABBDD) common wheat counterparts, a synthetic (AABBDD) hexaploid, its parents, and seven accessions of *aegilops squarrosa* were isolated, reduced, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The glutenin subunits of the synthetic hexaploid were simply inherited from its tetraploid (AABB) and diploid (DD) parents. Each extracted tetraploid lacked three glutenin subunits and showed a decrease in the amount of one electrophoretic band present in its hexaploid parent. Three of the affected subunits were of the same molecular weight (MW) for each hexaploid-tetraploid pair, the fourth being different for one of the four pairs. In each pair the four deleted (or diluted) subunits were of the same MW as subunits present in the glutenin of *ae. squarrosa* samples studied. The electrophoretic patterns of the reduced glutenin of the varieties *typica, anathera,* and *strangulata* were almost identical. The two highest-MW glutenin subunits of a fourth variety, *meyeri*, were smaller than the analogous subunits in the other three varieties. Common (bread) wheats contained glutenin subunits of high MW which were absent in durum wheats; their presence appears to be a necessary condition for breadmaking quality.

Previous work (1,2) demonstrated that the D-genome, and particularly chromosome 1D (3), was important in the determination of the breadmaking properties of wheat flour. The baking quality of synthetic and natural AABBDD hexaploid wheats was generally superior to that of extracted and natural AABB tetraploids (1,2). Subsequently (4,5) chromosomes 1B, 4B, and 5D were found to be critical to the baking potential of the bread wheat variety Cheyenne.

Since quality is related to the protein solubility distribution (2,6-10) and in particular to the relative proportions of acetic acid-soluble and -insoluble protein fractions (both are glutenins), analysis of glutenin is an essential step in the understanding of the molecular basis of breadmaking quality.

Until recently, electrophoresis of glutenins was very difficult because of their high molecular weights (MWs) and insolubility in aqueous systems. Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE), as first applied to flour protein by Bietz and Wall (11), allows electrophoresis of glutenin subunits obtained by reduction of disulfide bonds. Apart from providing excellent resolution of the glutenin subunits this technique also allows determination of the MW of each subunit with an accuracy of ±10% (12). Using this technique Bietz and Wall (11) found that the reduced glutenins of a number of classes of hexaploid bread wheat had similar patterns. The one durum wheat used in this study was deficient in two high-MW subunits and a subunit of MW 32,600. The absence of these high-MW subunits in the durum wheat suggests their possible relationship to breadmaking quality.

Using 26 hexaploid bread wheats of diverse baking quality, Orth and Bushuk (6) showed that there was no obvious relationship between their quality differences and the SDS-PAGE patterns of their reduced glutenins. The glutenins from all of

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these wheats contained high-MW subunits, regardless of quality. They suggested the presence of these subunits may be a necessary, but insufficient, condition for the breadmaking quality of common wheats.

The present study examines the inheritance of glutenin subunits and their relation to baking quality using varieties in which the D-genome has been added or removed cytogenetically. Glutenins from four hexaploid-extracted (AABB) tetraploid pairs, a synthetic hexaploid (AABBDD), its tetraploid (AABB) and diploid (DD) parents, and six other accessions of *aegilops squarrosa* were analyzed by SDS-PAGE.

MATERIALS AND METHODS

Wheat Samples

Canthatch, a Canadian hard red spring wheat of excellent milling and breadmaking properties, Tetracanthatch, the AABB component of Canthatch, and the synthetic hexaploid produced by crossing Tetracanthatch with *ae. squarrosa* variety *strangulata* (RL5271) were used for the first part of this study. The hexaploids and the extracted tetraploid were grown at one location in Winnipeg in 1967. The grain of the D-genome donor *ae. squarrosa* variety *strangulata* was obtained from plants grown under similar conditions. The same samples were used by Kerber and Tipples (1) in their study of the effect of the D-genome on baking quality. An additional six samples of four varieties of *ae. squarrosa* were obtained from the University of Manitoba seed stocks. These comprised two accessions of variety *strangulata*, one of *typica*, two of *anathera*, and one of *meyeri*.

The other extracted tetraploids that were used were derived by Kaltsikes from the hard red spring varieties Prelude, Rescue, and Thatcher. The three pairs were grown together on an experimental plot at the University of Manitoba. A detailed description of the genetic derivation of the tetraploids, together with pertinent quality data, was reported by Kaltsikes et al. (13).

All chemicals used were reagent grade.

Preparation of Glutenins

Glutenins were prepared by the pH precipitation method described previously (14). Glutenins of Canthatch, Tetracanthatch, the synthetic hexaploid, and the samples of *ae. squarrosa* were extracted directly from ground grain, since the quantities available were insufficient to mill into flour. The starting material for the preparation of glutenins of the hexaploids and extracted tetraploids of Prelude, Rescue, and Thatcher was flour milled on the Buhler experimental mill.

SDS-PAGE

SDS-PAGE was performed by the method of Koenig et al. (15) with slight modifications. The modified method was described previously (6). Electrophoresis was carried out at 130 v. in a 5% acrylamide gel using a phosphate buffer of pH 7.3. It was terminated when the bromophenol blue marker had migrated 7.0 cm.

The proteins used to calibrate the gels, with their MW and source in parentheses, were as follows: γ-globulin (160,000, Sigma), bovine serum albumin (132,000 and 66,000, Calbiochem.), ovalbumin (45,000, Sigma), chymotrypsinogen A (23,000, Schwarz/Mann), myoglobin (17,000, Calbiochem), and ribonuclease (13,000, Schwarz/Mann). A plot of log MW versus mobility produced a line with the

characteristic, but as yet unexplained, slight downward curvature found for SDS-PAGE of other proteins in 5% acrylamide gels (11).

The term baking quality as used in this article refers to breadmaking quality.

RESULTS AND DISCUSSION

Synthetic Hexaploid and Its Parents

Figure 1 gives the SDS-PAGE patterns of the reduced glutenin of the following genetically related grain species: a) Canthatch, a natural AABBDD hexaploid wheat; b) Tetracanthatch, tetraploid AABB component of Canthatch; c) synthetic 6N, synthetic AABBDD hexaploid derived from Tetracanthatch and *ae. squarrosa* variety *strangulata* (DD); and d) Squarrosa, *ae. squarrosa* variety *strangulata* (diploid DD).

Because photographic reproduction of these patterns results in a loss of detail, and in order to highlight the bands important to this discussion, both the photographs of the gels and schematic representations of the bands under discussion are shown in this figure and in Fig. 2.

Reduced glutenin from Canthatch gave an SDS-PAGE pattern typical of bread wheats (6,11). Eleven bands were evident with MWs ranging from 13,000 to 152,000. It was reported by Weber and Osborn (12) that SDS-PAGE yields MWs with an accuracy of better than ±10% for polypeptides with MWs between 15,000 and 150,000. Although the MWs quoted in this article may be subject to this error, they can still be used to identify the bands, since the major error involved in this technique is not its reproducibility for a particular polypeptide, but the inherent error in the assumption that the polypeptide-SDS complexes will migrate solely according to MW (12).

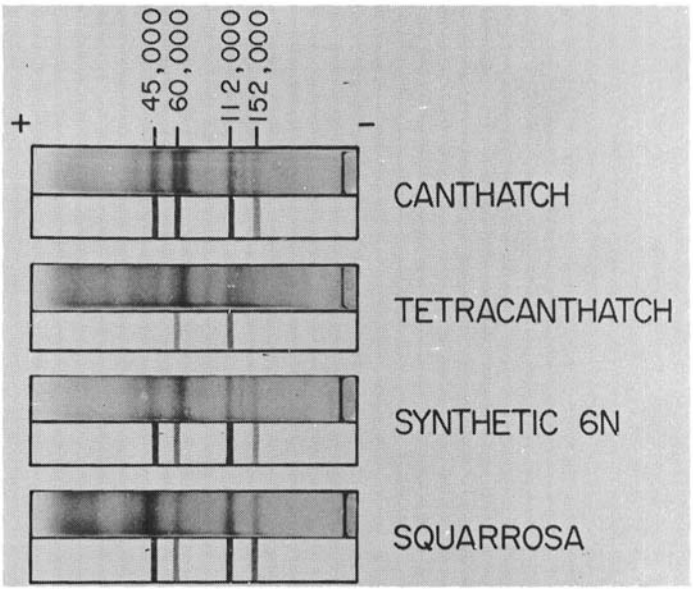

Fig. 1. SDS-PAGE patterns of reduced glutenin of Canthatch, Tetracanthatch, a synthetic hexaploid, and *aegilops squarrosa* variety *strangulata*.

Tetracanthatch lacked the highest-MW subunit when compared to Canthatch. This subunit, MW 152,000, has been found in all hexaploid bread wheats studied (6,11) and is absent in all the durum wheat glutenins studied thus far. The second and third components affected by removal of the D-genome are those of MWs 112,000 and 60,000. Both appear to be diluted rather than absolutely deleted. The fourth change is a deletion of the 45,000 band.

The dilutions that were observed could result from a decrease in the amount of the same subunit, coded by the same gene in different genomes (redundancy), or from a deletion of a subunit of different amino acid composition and sequence but of the same MW. If the latter is true, then the bands representing the 112,000 and 60,000 subunits which showed a dilution upon removal of the D-genome would comprise two or more different polypeptides.

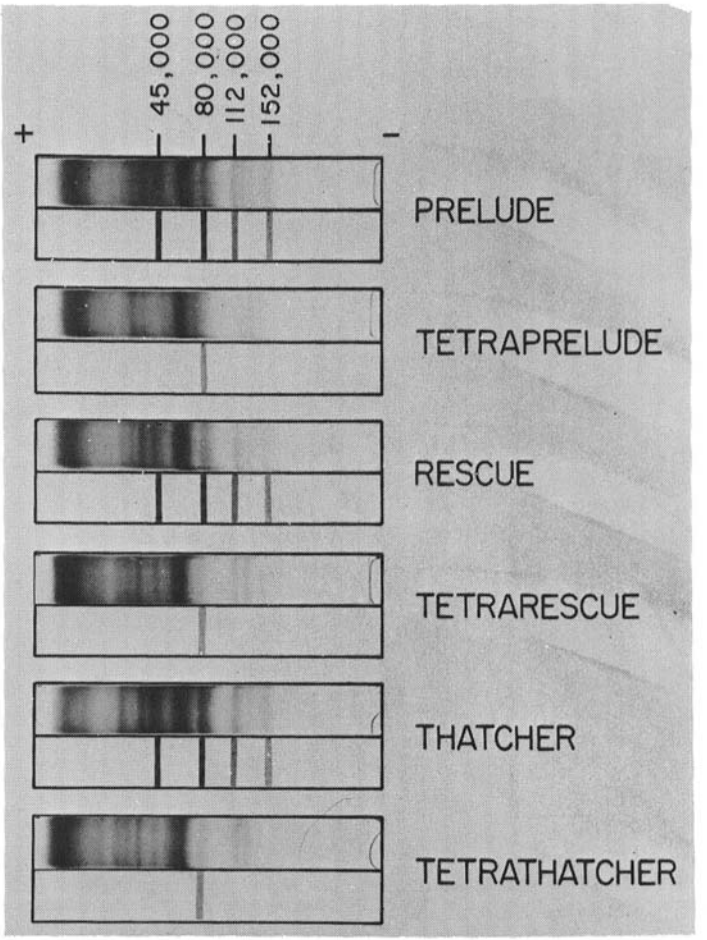

Fig. 2. SDS-PAGE patterns of reduced glutenin of Prelude, Tetraprelude, Rescue, Tetrarescue, Thatcher, and Tetrathatcher.

The synthetic hexaploid contained the same number of bands at mobilities corresponding to the same MWs as those of Canthatch. A band of moderate intensity is present at 60,000 for Canthatch, whereas the corresponding band in the synthetic hexaploid is weak. Otherwise, corresponding bands are of comparable intensity.

Reduced glutenin from the *squarrosa* contained seven subunits at MWs of 152,000, 112,000, 80,000, 60,000, 45,000, 39,000, and 14,000. Five of these subunits appear to be the same as those affected by removal and addition of the D-genome.

The glutenin from the synthetic hexaploid contained the major subunits present in its parents. No new bands were evident, and all the major bands of both parents were present in the progeny. Accordingly, it is concluded that the glutenin subunits of the synthetic hexaploid are simply inherited from its parents.

Boyd et al. (16) used starch-gel electrophoresis to compare the grain proteins soluble in 2M urea for the same four species used in this part of the present study. There was no difference in the patterns of Canthatch and the synthetic hexaploid; the patterns of the tetraploid and diploid parents were themselves different and also differed from the two hexaploids.

Extracted AABB Tetraploids and Their Hexaploid Parents

The effect of removal of the D-genome was studied further by analyzing the reduced glutenins of three hexaploid varieties and their extracted AABB tetraploids. Previous workers (2) have found differences in the protein solubility distribution between these two groups, but could find no qualitative differences by conventional PAGE of their water-, salt-, ethanol-, and dilute acetic acid-soluble proteins.

Figure 2 shows the SDS-PAGE patterns of the reduced glutenins of the three hexaploid-tetraploid pairs. For each variety, extraction of the D-genome removed three of the high-MW bands present in each hexaploid and decreased the intensity of a fourth. The three deleted subunits are characterized by the following MWs: 152,000, 112,000, and 45,000. The dilution occurred in the 80,000 subunit. These MWs are the same as those for four of the seven bands present in the reduced glutenin of the *ae. squarrosa* variety *strangulata* (see Fig. 1).

The results presented here differ slightly from those obtained for the Canthatch-Tetracanthatch pair (see above). The 112,000 subunit was deleted in the former, but diluted in the latter. Also, the conversion of Canthatch to Tetracanthatch produced a dilution in the 60,000 subunit, whereas in the other three pairs the dilution was in the 80,000 subunit.

The SDS-PAGE patterns of the reduced glutenins of seven accessions of *ae. squarrosa* are shown in Fig. 3. Four varieties are represented: *strangulata, meyeri, anathera,* and *typica*. There is a marked similarity of all the SDS-PAGE patterns, but minor differences in the mobility and number of visible bands are evident.

The three accessions of *strangulata* each have four major glutenin subunits with MWs of 152,000, 112,000, 60,000, and 45,000. Minor bands at 39,000 and 30,000 and a broad band of mobility corresponding to a MW of approximately 14,000 are all common to the three *strangulata* samples. A faint band at 80,000 is also evident.

Anathera and *typica* patterns were similar to those of *strangulata*. Subunits that are common to all three varieties have MWs of 152,000, 112,000, 60,000, 45,000, and 14,000 (broad band). Some minor differences among these varieties were

observed. Both samples of *anathera* had bands of MWs of 80,000 and 39,000, but these subunits appear absent in the *typica* studied.

The pattern of the variety *meyeri* was significantly different from the patterns of the other three varieties studied, particularly in the region of the slowest- moving subunits. The two largest subunits of *meyeri* were distinctly of lower MW than the analogous subunits of the other three varieties. In the lower MW region (higher mobility) the pattern of *meyeri* was essentially identical to that of the other varieties, with major bands at mobilities corresponding to MWs of 60,000, 45,000, 39,000, and 14,000 (broad band).

The similarity of subunit compositions of glutenin of the D-genome donors examined makes it impractical to use this criterion in the identification of the variety of *ae. squarrosa* involved in the natural evolution of hexaploid bread wheats. The high-MW subunits absent in AABB tetraploids and present in hexaploids (AABBDD) were present in the glutenin of the varieties *anathera, typica*, and *strangulata*, making either (or all) of these possible D-genome progenitors of the bread wheats.

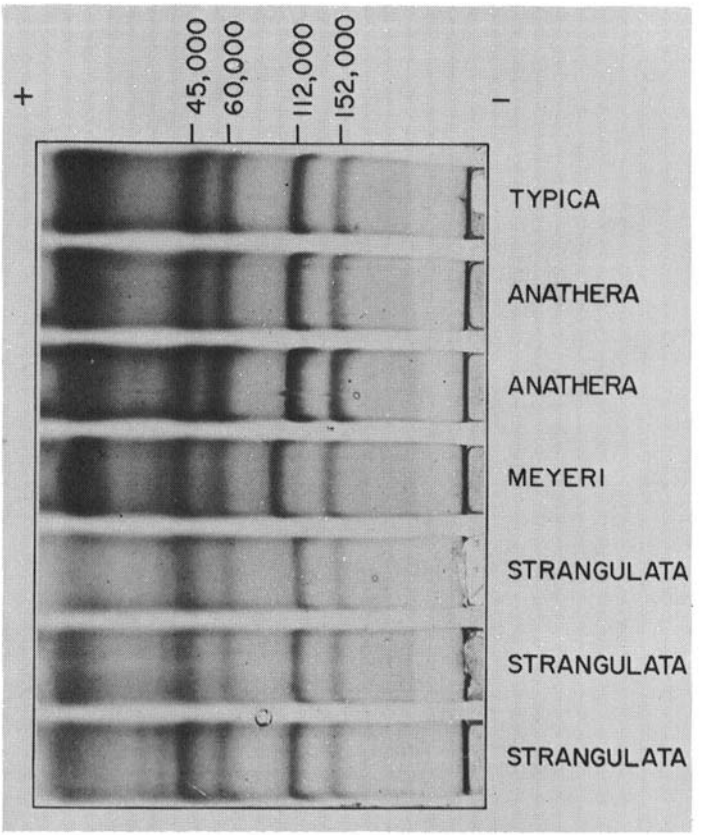

Fig. 3. SDS-PAGE patterns of reduced glutenin of seven accessions of *aegilops squarrosa*.

Relation of Patterns to Baking Quality

Relevant baking quality data for the wheats used in this study have been reported (1,13). Removal of the D-genome from Canthatch caused a marked decrease in quality (1) and, on the basis of the present study, is associated with the absence of glutenin subunits at MWs 152,000 and 45,000, and a decrease or deletion of the 112,000 and 60,000 subunits. Production of the synthetic hexaploid restored most of the lost baking quality and the lost glutenin subunits in the SDS-PAGE pattern.

Removal of the D-genome from Rescue and Thatcher caused a large decrease in baking quality (13). Although Tetraprelude gave a satisfactory loaf volume, this appears to be due to its relatively high protein content. When the baking results are expressed as loaf volume per unit protein, Prelude protein (59 cc. per percent protein on a 14% moisture basis) is of slightly better quality than that of Tetraprelude (55 cc. per percent protein).

The decrease in breadmaking quality of the four extracted tetraploid wheats examined in this study was accompanied by the loss of glutenin subunits of MWs 152,000, 112,000 (except for Canthatch where a dilution occurred), and 45,000, and a dilution in either the band at 80,000 or 60,000. Previous studies (11) showed that natural AABB tetraploids, i.e. durum wheats, lacked some of the high-MW subunits present in hexaploid wheats. This report was confirmed in the present study (results not shown). All AABB tetraploid wheats that have been studied, whether natural or synthetically produced, lack some of the high-MW glutenin subunits.

CONCLUSIONS

The most significant subunit deletions, resulting from the removal of the D-genome of common wheat, are those at MWs 152,000 and 112,000. Since these subunits have not been found in durum glutenins studied to date (11 and present study), their absence can be correlated to the lack of breadmaking quality in durum wheats. It might be speculated that these subunits are an essential part of the high-MW glutenin which is the elastic component of gluten. Breadmaking quality requires an optimal amount of this type of glutenin for a proper balance in rheological properties.

The high-MW subunits could play a dominant role in baking quality in either of two ways. First, they may merely increase the size of the glutenin by their direct contribution to the MW of the intact glutenin molecule. This possibility seems unlikely since these large subunits represent only a small percentage of the total protein in glutenin, as may be inferred from the low intensity of their bands in the SDS-PAGE patterns (Figs. 1 and 2).

The second possibility is that these subunits occupy a critical position in the structure of glutenin. For example, they may be located in regions of the molecule where branch points or other characteristic structural features occur, or they may be a requirement for a particular type of tertiary or quaternary structure. Determination of the actual role of these subunits in breadmaking quality will require delineation of the structure of glutenin.

The presence of these large subunits is not the only factor that controls baking quality, since all bread wheats contained them regardless of quality (6,11). Obviously, breadmaking quality requires more than merely their presence, but they appear to be necessary.

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