STUDIES OF GLUTENIN. IX. SUBUNIT COMPOSITION BY SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS AT pH 7.3 AND 8.9[1]

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ABSTRACT

Cereal Chem. 54(3): 588-596

Glutenin subunit compositions of three Canadian hard red spring wheat cultivars, Prelude, Rescue, and Thatcher, their extracted AABB tetraploid lines, and several other wheats were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at pH 7.3 and pH 8.9. Results from these experiments differed from published results; five high-molecular weight subunits were found for the hexaploid wheats: 134,000 (1), 132,000 (2), 110,000 (3), 98,000 (4), 90,000 (5). Extracted tetraploids Tetraprelude and Tetrathatcher lacked subunits 1 and 5, whereas Tetrarescue lacked subunits 2 and 5. The natural AABB tetraploid, Stewart 63, a durum wheat cultivar, lacked subunits 1, 2, and 5. The variety Chinese Spring, a hexaploid cultivar used extensively in cytogenetic studies, lacked subunit 2 but contained the other four high-molecular weight subunits. The first two high-molecular weight subunits were better resolved at pH 7.3 for most of the hexaploid wheats examined, whereas subunits 4 and 5 were better resolved at pH 8.9. For complete resolution of the high-molecular weight subunits, it is necessary to use a combination of SDS-PAGE methods.

Hexaploid cultivars of common wheat (*Triticum aestivum* L. em Thell.) and their extracted AABB tetraploid lines have been used to study the contribution of the D genome to breadmaking quality (1,2), to the solubility, amino acid composition, and electrophoretic properties of endosperm proteins (3), and to the control of the synthesis of gluten proteins (4), gliadins (5), and glutenin subunits (6,7). A previous article from our laboratory (6) reported that in three cultivars, Prelude, Rescue, and Thatcher, removal of the D genome resulted in a deletion of three of the six largest subunits of glutenin. One cultivar, Canthatch, lost only two subunits in the same molecular weight (mol wt) region. These results were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) performed at pH 7.3. On the other hand, later work on the same wheat samples by Bietz *et al.* (7), with the same technique but at pH 8.9, showed that on removal of the D genome one high-mol wt subunit was deleted and one diluted in Tetracanthatch, two were deleted and one modified in Tetraprelude, three were deleted (or greatly diluted) in Tetrarescue, and two were deleted in Tetrathatcher (7).

The present study was carried out in an attempt to reconcile the discrepancies between the two previous studies (6,7). The results obtained are presented in this article.

MATERIALS AND METHODS

Wheat Samples

The grain samples of Prelude, Rescue, and Thatcher and their extracted AABB tetraploids, Tetraprelude, Tetrarescue, and Tetrathatcher, were portions

[1]Contribution No. 444 of the Department of Plant Science, University of Manitoba, with financial assistance from the National Research Council of Canada.

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of the same samples used by Orth and Bushuk (6) and Bietz *et al.* (7). Other cultivars used in this study for comparisons were the common wheats Chinese Spring, Ponca, Manitou, Red River 68, and Talbot, and Stewart 63, a Canadian durum wheat.

Extraction of Glutenin

Three grams of grain was ground with a mortar and pestle and glutenin was extracted from the resulting meal by the modified Osborne solubility fractionation procedure of Chen and Bushuk (8). Glutenin was also extracted from single kernels according to the method of Bietz *et al.* (7).

SDS-PAGE

SDS-PAGE was performed at pH 7.3 according to the method of Orth and Bushuk (9), using their phosphate buffer system. For the pH 8.9 gels, the tris-borate buffer system of Koenig *et al.* (10), modified for the E-C vertical slab-gel electrophoresis apparatus, was used. The gel solution consisted of 10.2 g acrylamide, 0.27 g methylenebisacrylamide, 50 mg sodium sulfite, 0.5 ml dimethylaminopropionitrile, and 4 ml of 2% ammonium persulfate solution, all dissolved in 200 ml of 0.125*M* tris-borate buffer containing 0.1% SDS to give a 5% gel. The size of the gel was 18 by 14 cm and the sample slots were 1 by 1 by 0.5 cm. Glutenin was solubilized in 0.125*M* tris-borate buffer solution containing 1% SDS and 1% β-mercaptoethanol. Both electrode buffers were 0.125*M* tris-borate containing 0.1% SDS. Electrophoresis time was from 3 to 5 hr on a thick slab gel (set with 6-mm spacers) at 100 volts or 50 mA.

On termination of electrophoresis, both the pH 7.3 and pH 8.9 gels were immersed for 45 min in aqueous trichloroacetic acid (8–12%), rinsed with distilled water, and stained overnight in 150 ml of dye solution. The dye solution was prepared by mixing 1.6 g Coomassie Brilliant Blue R (Sigma), 800 ml ethanol (95% v/w), 800 ml distilled water, and 176 ml glacial acetic acid. The stained gels were destained by soaking in a solution of 400 ml 10% acetic acid and 100 ml methanol until the background was sufficiently clear for identification of the protein bands.

Chemicals and Reagents

Acrylamide, methylenebisacrylamide, N,N,N^1N^1-tetramethylethylenediamine, and dimethylaminopropionitrile were obtained from Eastman Chemical Company and used without additional purification; SDS was from Pierce Chemicals; ammonium persulfate, sodium sulfite, sodium mono- and diphosphates, boric acid, and tris hydroxymethylaminomethane were obtained from Fisher Chemical Company.

RESULTS AND DISCUSSION

The SDS-PAGE patterns of reduced glutenins from the common (hexaploid) wheats, Prelude, Rescue, and Thatcher, and from their AABB extracted tetraploids, Tetraprelude, Tetrarescue, and Tetrathatcher, are shown in Fig. 1 (pH 7.3) and Fig. 2 (pH 8.9). The figures also show, for comparison, the patterns for the hexaploid cultivar, Chinese Spring, and the natural tetraploid cultivar, Stewart 63.

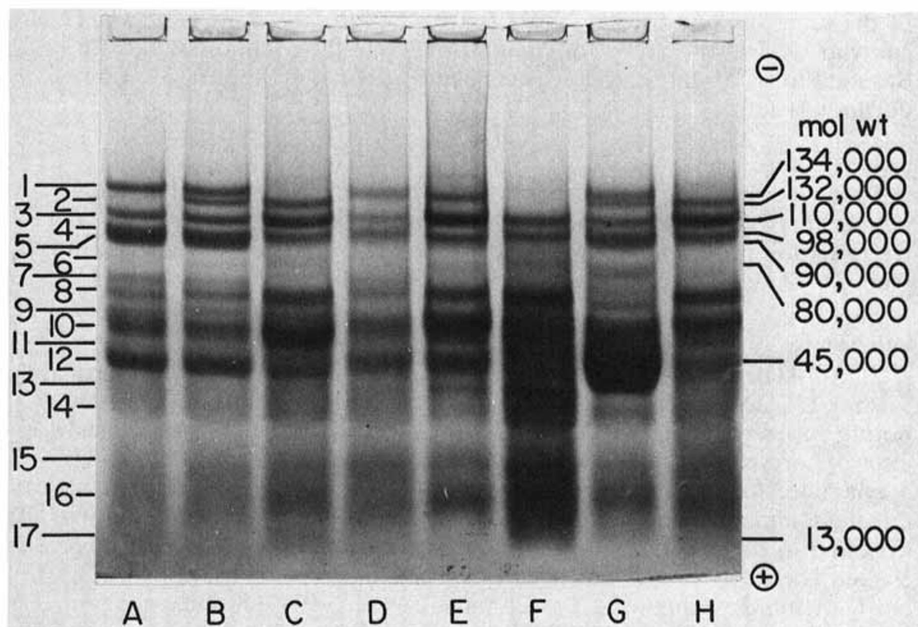

Fig. 1. SDS-PAGE electrophoretograms of reduced glutenin from the modified Osborne procedure at pH 7.3: A, Chinese Spring; B, Prelude; C, Tetraprelude; D, Rescue; E, Tetrarescue; F, Stewart 63; G, Thatcher; H, Tetrathatcher.

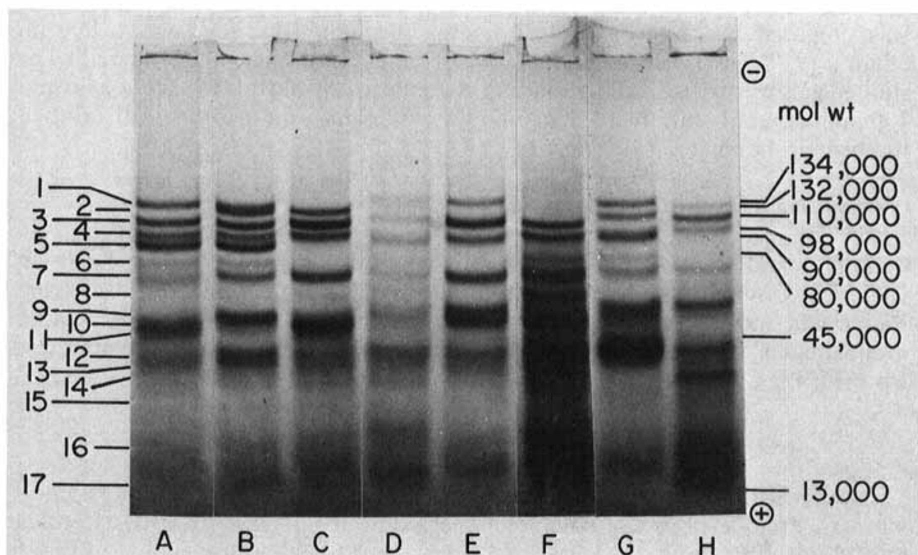

Fig. 2. SDS-PAGE electrophoretograms of reduced glutenin from the modified Osborne procedure at pH 8.9: A, Chinese Spring; B, Prelude; C, Tetraprelude; D, Rescue; E, Tetrarescue; F, Stewart 63; G, Thatcher; H, Tetrathatcher.

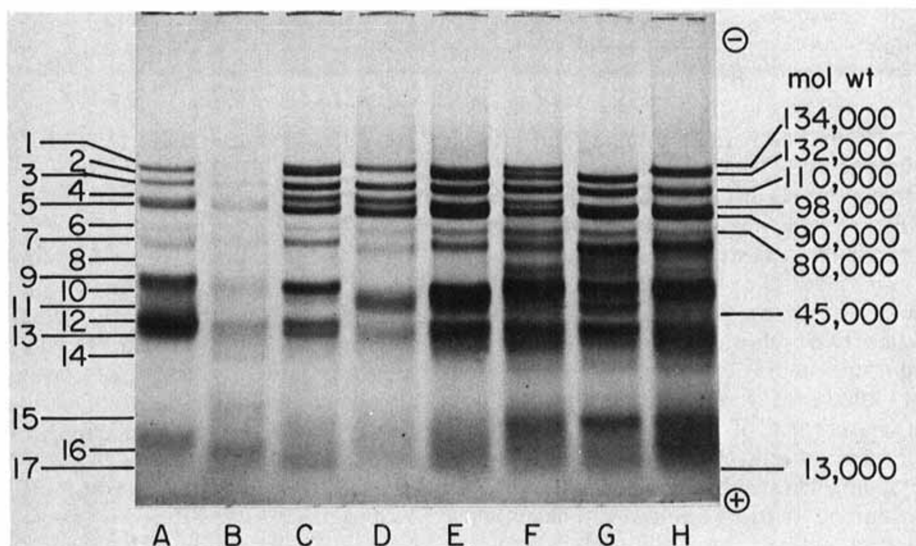

Fig. 3. SDS-PAGE electrophoretograms of reduced glutenin from the modified Osborne procedure at pH 8.9: A, Thatcher; B, Rescue; C, Prelude; D, Chinese Spring; E, Ponca; F, Red River 68; G, Manitou; H, Talbot.

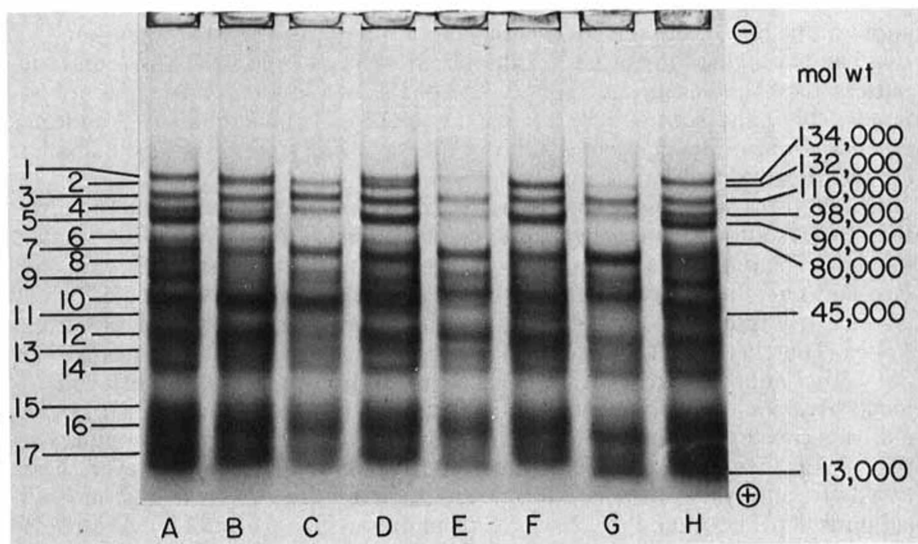

Fig. 4. SDS-PAGE electrophoretograms of reduced glutenin prepared from single kernels at pH 8.9: A, Chinese Spring; B, Prelude; C, Tetraprelude; D, Rescue; E, Tetrarescue; F, Thatcher; G, Tetrathatcher; H, Chinese Spring.

Five subunits can be identified in the high-mol wt region (90,000–134,000) of all the hexaploid wheats except Chinese Spring. Chinese Spring lacks one of the two largest subunits. These two subunits were better resolved at pH 7.3 than at pH 8.9 for most of the hexaploid cultivars, with some important exceptions (discussed below).

Compared with their hexaploid counterparts, Tetraprelude and Tetrathatcher lack the 134,000 subunit but have the 132,000 subunit; this can be readily seen from the results of Fig. 1 (pH 7.3) and Fig. 2 (pH 8.9). On the other hand, Tetrarescue has the 134,000 subunit and lacks the 132,000 subunit; this was particularly clear from the results at pH 8.9. The pattern for Rescue is faint in Figs. 2 and 3 at pH 8.9. However, the 134,000 and 132,000-mol wt bands were clearly seen on the gels, as in Fig. 4D. Rescue is the only cultivar that showed these two high-mol wt bands clearly resolved in the patterns of Bietz *et al.* (7). The pattern at pH 7.3 shows that Tetrarescue has a high-mol wt subunit of about 133,000 mol wt. From these results at pH 7.3, one could erroneously conclude that removal of the D genome from the cultivar Rescue has no effect on the largest subunit(s).

The 90,000 subunit is absent in the patterns of all three extracted tetraploids obtained at pH 7.3 and 8.9. The 80,000 subunit appears to be diluted (in some patterns it was completely deleted) by the removal of the D genome. There could be two subunits of this mol wt, one coded by the D and the other by either the A or B genome.

Of the five subunits, Stewart 63, a natural AABB tetraploid wheat, lacks the 134,000, 132,000, and 90,000 subunits; this is apparent from the results shown in Fig. 1 (pH 7.3) and Fig. 2 (pH 8.9).

A number of anomalies were observed for the hexaploid wheats (same genomic composition). The pH 7.3 pattern for the cultivar Red River 68 (Fig. 5F) showed one broad, intense band with a mol wt of about 133,000 rather than the two bands obtained for other hexaploids. Results of Fig. 5 also show that the pattern for Chinese Spring has only one of the two low mobility high-mol wt bands; this band seems to be equivalent to the 134,000 subunit of Manitou, Prelude, Ponca, Thatcher, and Talbot. Chinese Spring glutenin lacks the smaller (132,000) of the two largest subunits.

Comparison of the results of Fig. 5 (pH 7.3) with those of Fig. 3 (pH 8.9) shows that the resolution of the two largest subunits is different in the two buffer systems. The difference varies among cultivars. In the patterns for Rescue and Red River 68, the first two high-mol wt subunits were not resolved at pH 7.3 but were clearly resolved at pH 8.9. On the other hand, the patterns for Manitou, Ponca, Thatcher, and Talbot (Fig. 5) show that the two subunits were resolved at pH 7.3 but not resolved at pH 8.9 (Figs. 2 and 3). Longer electrophoretic runs (5 hr) at pH 8.9 did not resolve the 134,000 and 132,000-mol wt subunits for these cultivars (results not shown) but did improve the resolution of these subunits at pH 7.3 (Fig. 5) except for the cultivars Red River 68 and Rescue. However, these two latter cultivars show complete resolution of the first two high-mol wt subunits at pH 8.9 after only 3 hr of electrophoresis (Figs. 3F and 4D). As already mentioned, the pattern for Chinese Spring has only one (134,000) band at both pH 7.3 and 8.9. It is apparent from these results that the same cultivar can give different results at different pH insofar as the resolution of the two largest glutenin subunits is concerned.

The 90,000 and 98,000-mol wt subunits of the hexaploid wheats were generally better resolved at pH 8.9 than at pH 7.3 (compare Figs. 3 and 5). At the lower pH, these two subunits for most cultivars run together and appear as an intensely stained broad band. The patterns for Prelude and Chinese Spring, however, showed a clear resolution of these two bands both at pH 7.3 and 8.9.

In addition to the cultivar-pH anomalies discussed above, it was necessary to reconcile possible discrepancies in the two previous studies (6,7) due to differences in the methods of glutenin preparation. Accordingly, the glutenins prepared by the modified Osborne solubility fractionation procedure (8) and by the single-kernel procedure of Bietz *et al.* (7) from the three hexaploid wheats and their AABB tetraploids were examined by SDS-PAGE. The patterns obtained at pH 8.9 for the two preparations were essentially the same (Figs. 2 and 4). However, there were obvious differences in the intensity of the stained bands in the region below 40,000 mol wt. These differences appear to be dependent on the isolation and purification procedure employed to obtain the glutenin. The staining was more intense in the patterns of glutenin prepared by the single kernel (7) and the pH precipitation (11) procedures and less intense in the patterns of the other glutenin preparations examined (Fig. 6). Unreduced glutenin prepared by the single kernel procedure (7) showed a number of fairly prominent bands that entered the electrophoresis gel (Fig. 6A). On the other hand, unreduced glutenin that was purified by the sulfoethyl-Sephadex procedure (13) showed only a few very faint bands (Fig. 6H). These are considered to be impurities that aggregate strongly with glutenin, which can only be removed by relatively strong

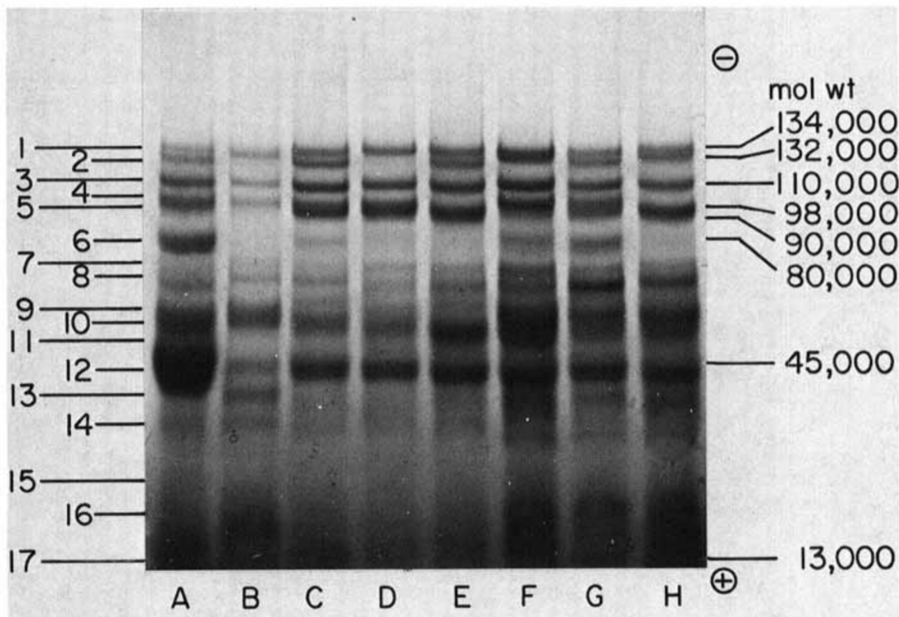

Fig. 5. SDS-PAGE electrophoretograms of reduced glutenin from the modified Osborne procedure at pH 7.3: A, Thatcher; B, Rescue; C, Prelude; D, Chinese Spring; E, Ponca; F, Red River 68; G, Manitou; H, Talbot.

purification procedures that can dissociate the noncovalent aggregates. If these "contaminants" are not removed, they are erroneously interpreted as glutenin subunits by the SDS-PAGE analysis.

The SDS-PAGE patterns of reduced glutenin for the mol wt region below 68,000 are qualitatively similar for the three hexaploid wheats and their AABB-extracted tetraploids that were examined (Figs. 1, 2, and 4). The patterns for Manitou (hexaploid) wheat glutenin prepared by six different fractionation procedures (Fig. 6) are qualitatively similar, with one minor exception. The 52,000-mol wt subunit in the pattern of the glutenin prepared by the modified Osborne procedure (8) (Fig. 6D) was extremely faint. Bietz and Wall (12), on the other hand, found that the 52,000-mol wt subunit (their subunit number 8) was deficient in reduced glutenin prepared from a washed gluten ball. It should be noted, however, that Bietz and Wall (12) obtained their gluten ball by washing a dough ball in 0.1% NaCl, whereas our gluten ball was obtained by washing a dough ball in distilled water, and this may account for the different results obtained.

GENERAL DISCUSSION

Results obtained in this study indicate that there is no single satisfactory SDS-PAGE procedure for the determination of the subunit composition of glutenin,

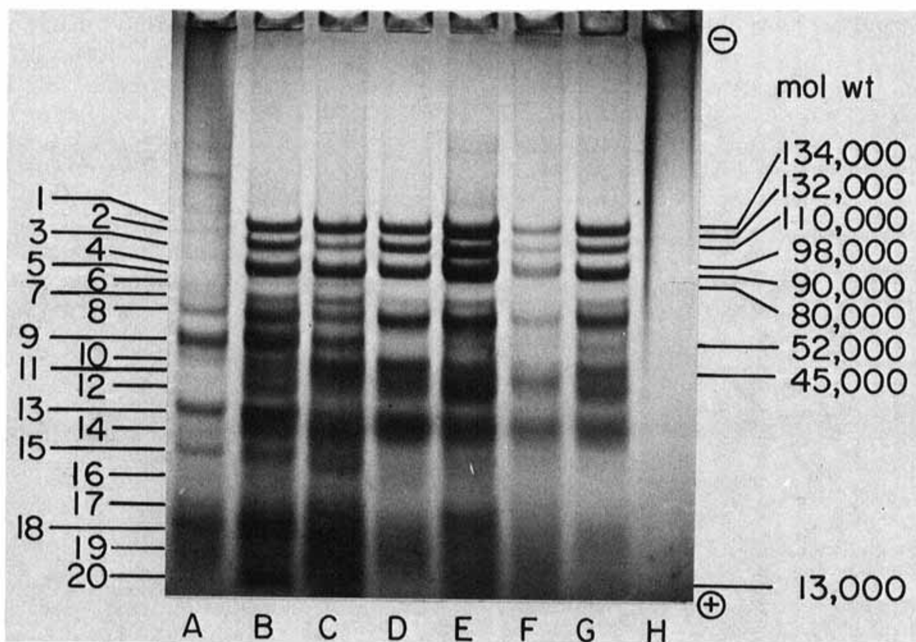

Fig. 6. SDS-PAGE electrophoretograms at pH 8.9 of reduced Manitou glutenin prepared by six different extraction procedures: A, B, from single kernels; C, by pH precipitation from gluten ball (11); D, soluble glutenin from the modified Osborne procedure (8); E, $HgCl_2$ extract of Bietz and Wall (12); F, β-mercaptoethanol extract of Bietz and Wall (12); G, H, from sulfoethyl-Sephadex purification from gluten ball (13).

whether it is obtained from bulk grain or from single kernels. Cultivar and pH variability in resolution of subunits occur mainly in the high-mol wt region (90,000 to 134,000). Certain subunits are better resolved at pH 7.3 while others are better resolved at pH 8.9. Perhaps the pH of the buffer affects the amount of SDS bound to certain subunits or influences the conformation of certain SDS-subunits in such a way as to hinder or improve the resolution. Contrary to the first suggestions, Bietz and Wall (14) found that reduced glutenin and aminoethylglutenin bound approximately the same amount of SDS as serum albumin and ovalbumin at pH 7.2.

Orth and Bushuk (6) reported that removal of the D genome from three cultivars, Prelude, Rescue, and Thatcher, resulted in the deletion of the 134,000 (their 152,000) and the 90,000 (their 112,000) subunits. Bietz *et al.* (7), using the same wheat samples, found that the 90,000 (their 86,000)-mol wt subunit was deleted from the extracted tetraploids, but not a high-mol wt subunit, which had either slightly lower or higher mobility than their 133,000 subunit, depending on the cultivar. Bietz *et al.* (7) also found that the patterns obtained for glutenin prepared by their single kernel procedure and those for glutenin prepared by the pH precipitation procedure of Orth and Bushuk (6) were essentially the same, except that the high-mol wt bands separated at pH 7.3 stained less intensely than the equivalent bands separated at pH 8.9. The results from the two earlier studies (6,7) seem to be conflicting. This apparent conflict, however, could arise from misinterpretation of the patterns. The present study showed that the largest subunit (134,000 mol wt) was deleted in two of the three extracted tetraploids, while the second largest mol wt subunit (132,000) was deleted in the third extracted tetraploid (Rescue). This discrepancy could result from a minor deviation in resolution. Both studies showed four subunits of the high-mol wt group of five resolved in the present study. The two earlier studies each missed one (different) subunit. Orth and Bushuk (6,9) at pH 7.3 resolved the 134,000, 132,000, and 110,000 subunits, but not the 98,000 and 90,000 subunits, which migrated as a single broad band. The experiments of Bietz *et al.* (7), at pH 8.9, did not resolve the 134,000 and 132,000 subunits, which ran as a single band of 133,000, but resolved the 98,000 and 90,000-mol wt subunits. Orth and Bushuk (6) might have missed the 132,000 subunit of the extracted tetraploids because the stain of this subunit rapidly fades in the SDS-gels at pH 7.3. (The staining procedure used was published by Wasik and Bushuk, ref. 15.)The subunit can be seen at this pH if the destaining period is not excessively long.

Orth and Bushuk (6) and Bietz *et al.* (7) both found that the nulli 1D tetra 1A (N1DT1A) and nulli 1D tetra 1B (N1DT1B) aneuploid lines of Chinese Spring did not show any subunits in the 132,000–134,000-mol wt region. This seemed anomalous since, on the basis of results obtained for other hexaploids, these aneuploid lines would be expected to have the 132,000-mol wt subunit. The present study showed, however, that the cultivar Chinese Spring differs from other hexaploids in that it does not have the 132,000-mol wt subunit. Since the removal of the D genome (from the hexaploids Prelude, Rescue, and Thatcher) resulted in the deletion of one of the two high-mol wt subunits, the N1DT1A and N1DT1B substitution lines of Chinese Spring should lack both of the two largest subunits.

Stewart 63, a natural tetraploid (durum) wheat used as the source of marker subunits in Figs. 1 and 5, lacks the 134,000, 132,000, and 90,000-mol wt subunits.

These results suggest that the A and B genomes of this durum cultivar are more like these genomes of the hexaploid Chinese Spring than the hard red spring hexaploids used in this study (*e.g.*, Thatcher).

Results of this study indicate that glutenin subunit composition, as determined by SDS-PAGE, depends strongly on the pH of the buffer used for the electrophoresis. Some wheat cultivars may give incomplete resolution at one pH, while others will show similar behavior at another pH. Accordingly, subunit compositions by this technique should be interpreted with caution unless determined at several different pH values. Perhaps one-dimensional SDS-PAGE is not the best criterion for defining subunit composition, establishing complexity, or comparing varieties, since it is likely that individual bands may themselves be heterogeneous by other techniques.

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[Received June 28, 1976. Accepted November 8, 1976]