Gliadin High-Performance Liquid Chromatography and Polyacrylamide Gel Electrophoresis Patterns of Wheats Grown with Fertilizer Treatments in the United States and Australia on Sulfur-Deficient Soils

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ABSTRACT

Gliadin protein patterns were determined by polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC) for wheats grown with various fertilizer treatments on sulfur-deficient soils in Washington state (USA) and in Australia. The patterns of those samples, grown on sulfur-deficient soil to which nitrogen was added, exhibited higher relative intensities of their 73 relative mobility band than the relative intensity of that band in corresponding samples from unfertilized soil. The samples from Australia, which differed in their response to a semiquantitative sulfur deficiency test, also differed in PAGE and HPLC patterns. Wheat grown on sulfur-deficient soil, which had been fertilized with sulfur, showed more intense high mobility PAGE bands and less intense low mobility bands than wheat grown on unfertilized sulfur-deficient soil. Similarly, the peaks eluting in the 20–25 min range from the HPLC column were smaller and those eluting in the 43–55 min range were larger from the Australian wheat grown on sulfur-fertilized than on unfertilized soil.

Several studies in Australia and England have indicated the desirability of providing adequate sulfur to increase the yield and improve the quality of wheat (Archer 1974, Byers and Bolton 1979, Spencer and Freney 1980, Wrigley et al 1980, Moss et al 1981, Randall et al 1981, Timms et al 1981, Wrigley et al 1984). Some of the investigations were conducted on plants grown in pots, some on sand cultures, and some on sulfur-deficient experimental field plots. Grain containing sulfur below 0.12% and nitrogen-sulfur ratios above 17 was considered to be sulfur-deficient (Moss et al 1981). Wrigley et al (1980) found that restriction of sulfur in wheat increased the proportion of low-mobility gliadins at the expense of high-mobility gliadins. Timms et al (1981) found that on some soils in England late application of a nitrogenous fertilizer, without sulfur, can render the nitrogen-sulfur ratio so high that insufficient sulfur is available for "normal" grain development. Considerable modification of biochemical characteristics of the wheat proteins occurred.

In parts of Idaho and Washington in the United States, sulfur is deficient for the growth of legumes. Some of those soils showed significant responses in wheat yields to sulfur fertilization. The prevalence of sulfur deficiency and need for sulfur fertilization were attributed to an overall decline in soil organic matter, use of concentrated pure nitrogen fertilizers, and use of high nitrogen rates on high yielding varieties that depleted reserve soil sulfur (Harder and Thiessen 1971).

This study was conducted to determine the effects of nitrogen and sulfur fertilization on the high-performance liquid chromatography (HPLC) and polyacrylamide gel electrophoresis (PAGE) gliadin patterns of wheats grown on soil that showed a yield response to such fertilization. Characteristics of wheat protein patterns (HPLC and PAGE) were compared for an Australian soft wheat and a Washington state soft wheat grown on sulfur-deficient soil. Functional (breadmaking) characteristics of those wheats were reported elsewhere (Shogren et al 1984).

MATERIALS AND METHODS

Materials

Two sets of samples were used in this study. One set was the soft wheat cultivar Olympic grown in Australia on sulfur-deficient soils and treated with fertilizer (N_0S_0, N_0S_{81}, and $N_{100}S_0$ kg/ha). The other set was a soft white spring wheat, Dirkwin, from a 1983 experimental site (of presumably sulfur-deficient soils) in Washington state. The fertilization was $N_0P_0S_0$, $N_{100}P_{30}S_{20}$, and $N_{100}P_{30}S_0$ kg/ha with average yields of 0.94, 2.96, and 2.02 tonnes/ha, respectively. The Washington state site (Dye) had been in a winter wheat-spring wheat cropping system with no-till (direct drilling) system for two years (F. E. Kohler, *personal communication*).

Methods

The wheat gliadin proteins were characterized with slight modifications to the PAGE method of Lookhart et al (1982) and the HPLC method of Bietz (1983). The changes in the PAGE method were 10° C for 6 hr vs. 21° C for 5 hr. The HPLC method changes were: solvents A and B were 20 and 80% acetonitrile, respectively, with 0.1% TFA (trifluoroacetic acid); temperature was 23° C; and the gradient was as shown in Table I.

The test to detect sulfur deficiency in wheat was described by Moss et al (1982).

RESULTS AND DISCUSSION

PAGE

There were no differences in the relative mobilities of the bands in the gliadin electrophoretic patterns of the wheat samples from Washington state (Fig. 1) treated with different fertilizers. One minor variation was found in the relative intensity of a band at 73 relative mobility (RM) in Dye sample 20. That band is darker (more intense) than the corresponding bands in Dye 15 or Dye 19, even with respect to relative intensity (RI). The Dye samples were grown on soil that gave a positive yield effect on nitrogen and sulfur fertilization. Dye 20 was grown with added nitrogen (and phosphorus) but no sulfur ($N_{100}P_{30}S_0$), which is similar to the Australian sample 4713 ($N_{100}S_0$). In both of those samples, RIs were higher for the band at 73 RM units than for the same RM band in the corresponding samples. The 73 RM band contains protein(s) that are increased in conditions of high nitrogen and low sulfur.

The effect of sulfur fertilization on electrophoretic patterns of the Australian wheats is seen by comparing the patterns (Fig. 2) of 4706 (N_0S_0) with 4708 (N_0S_{81}). The RMs of the bands in both samples are nearly identical. Because the intensities of the bands in the RM

TABLE I
Gliadin Analysis Gradient

Solvent[a] (%)	Time (min)				
	0	20	50	55	57
A	28	41	46	75	28
B	72	59	54	25	72

[a] A = Acetonitrile plus 0.1% trifluoroacetic acid (TFA). B = Water plus 0.1% TFA.

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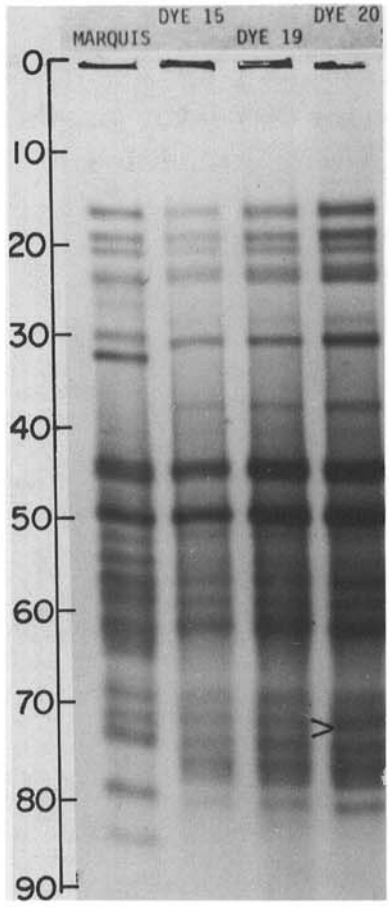

Fig. 1. Electrophoregrams of gliadins in wheat samples from Washington state (USA). Dye 15 ($N_{100}P_{30}S_{20}$), Dye 19 ($N_0P_0S_0$), Dye 20 ($N_{100}P_{30}S_0$). Relative mobility scale at left.

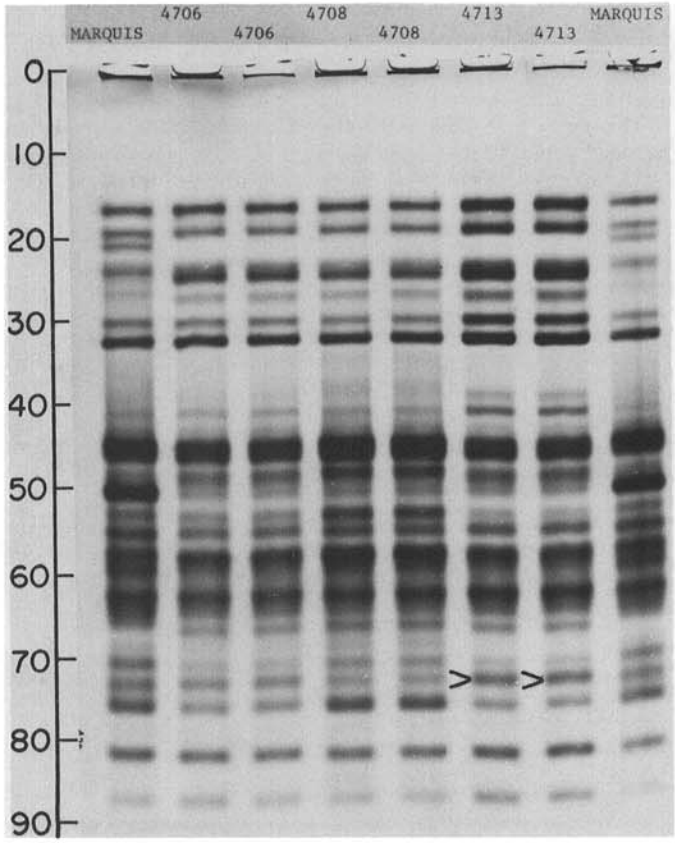

Fig. 2. Electrophoregrams of gliadins in wheat samples from Australia. 4706 (N_0S_0), 4708 (N_0S_{81}), and 4713 ($N_{100}S_0$). Relative mobility scale at left.

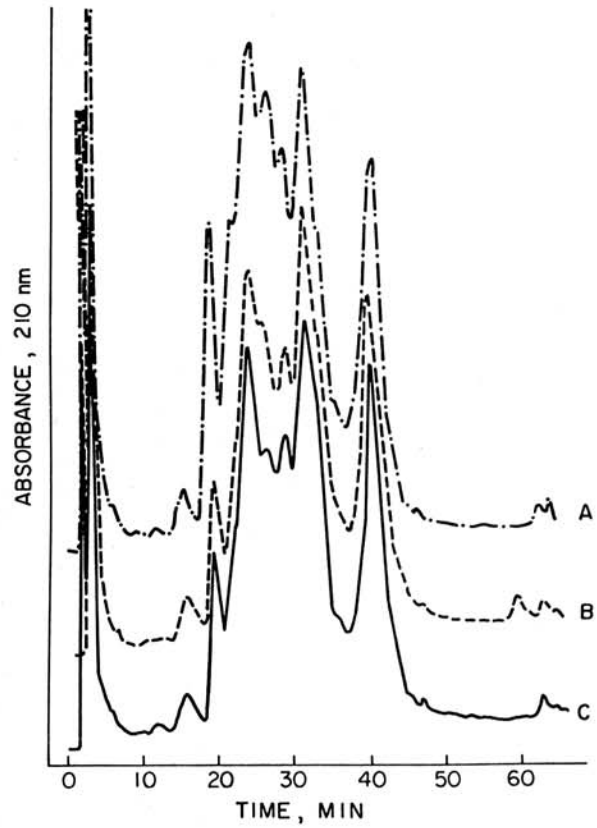

Fig. 3. Chromatograms of gliadins in wheat samples from Washington state (USA). **A,** Dye 15 ($N_{100}P_{30}S_{20}$); **B,** Dye 19 ($N_0P_0S_0$); **C,** Dye 20 ($N_{100}P_{30}S_0$).

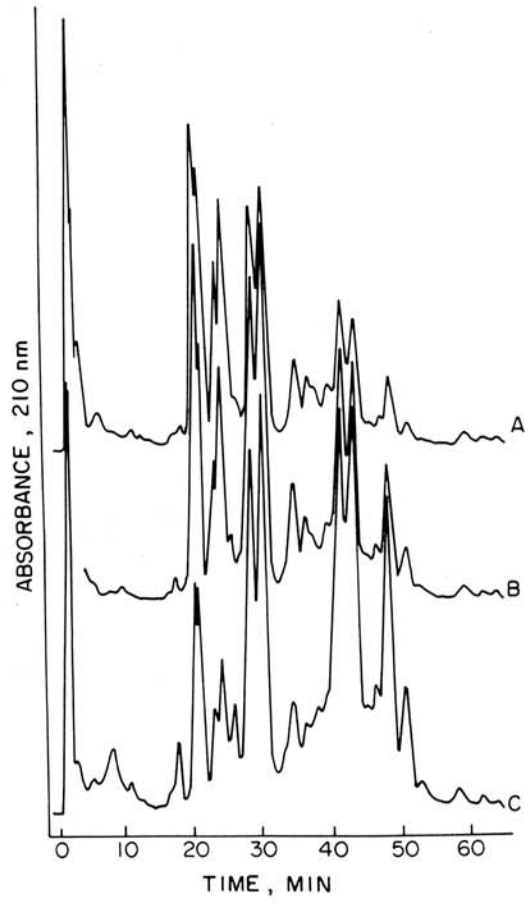

Fig. 4. Chromatograms of gliadins in wheat samples from Australia. **A,** 4713 ($N_{100}S_0$); **B,** 4706 (N_0S_0); **C,** 4708 (N_0S_{81}).

range of 15–32 are the same, intensity changes between bands with the same RM (in the 35–90 range) should be indicative of differences in protein content. Increases in intensity of the bands in 4708 over those in 4706 are most likely due to additional amounts of sulfur-containing proteins. Several bands were found in sample 4708 (sulfur fertilized) that appear to have higher RIs than the corresponding bands in 4706 (no sulfur). Those bands likely consisting of sulfur-containing proteins were found at RMs 45, 47, 53, 55, 57, 65, 71, and 75. An additional sulfur-rich band at 68 RM was found in the electrophoregram of 4708 but not in 4706. Therefore, it appears that samples grown on soil severely deficient in sulfur may exhibit slightly different (atypical) gliadin electrophoregrams.

The simple, semiquantitative test for sulfur deficiency (Moss et al 1982) was positive for one of the three Australian samples (4713, $N_{100}S_0$) but for none of the Washington state wheat samples. The electrophoretic pattern of sample 4713 showed increased band intensities in the 18–35 RM range and in the 73 RM band (Fig. 2) compared with the other Australian samples.

Gliadin electrophoretic patterns are normally not affected by nitrogen fertilization (Zillman and Bushuk 1979). Because the electrophoregrams of sample 4713 showed minor differences, wheat samples grown on sulfur-deficient soil to which nitrogen fertilizer has been added may give atypical results. Those results may preclude identification by PAGE. Fortunately, sulfur-deficient soil is rare.

The gliadin electrophoregrams of wheat grown on sulfur-deficient soil both with (4713) and without (4706) nitrogen fertilizer showed no differences in the RMs of the bands. The RIs between bands at the same RM in samples 4706 and 4713 were very similar. The intensity of each band in 4713 was higher, owing to increased protein content caused by nitrogen fertilization. These overall increases in intensity (not RI) of the electrophoretic patterns do not affect pattern recognition.

HPLC

The gliadin HPLC patterns of wheat samples Dye 15, Dye 19, and Dye 20 from Washington state are shown in Figure 3. The effect of nitrogen fertilization on gliadin chromatograms cannot be determined by any combination of the three curves because no fertilization level was done when nitrogen changed and phosphorus and sulfur were constant. However, to compare the effect of sulfur fertilization on gliadin chromatograms, curves A and C (Fig. 3) need to be considered. Curves A and C are similar, with no differences in peak retention times and only minor differences in peak RI over the major portion of the chromatograms, between 15 and 50 min. The increased heights of peaks eluting at 19, 23, 26, 28, and 31 min and the more pronounced shoulder at 33 min are the apparent results on the gliadin patterns of wheat grown on sulfur-deficient soil to which sulfur had been added. The increases in peak heights on HPLC analysis are more pronounced than the increases in band RI on the PAGE patterns of the same samples, but the effect of increased protein content on specific peaks is analogous (Fig. 2). Curve B is very similar to A except that the RIs are slightly less; this might indicate that phosphorous fertilization has no major effect on gliadin patterns.

The HPLC patterns of the Australian wheats (Fig. 4) show effects similar to their PAGE patterns. The effect of nitrogen fertilization is determined by comparing the chromatogram curves A (4713) and B (4706). Those curves are similar; all of the major peaks possess the same retention times and similar peak heights. Nearly every peak in B (low nitrogen) is larger than in A, most likely owing to more protein being injected on the column. If only one or two peaks were different, a selective effect could be postulated.

The effects of sulfur on gliadin HPLC patterns of the Australian wheats can be determined by comparing curves B (N_0S_0) and C (N_0S_{81}). Some differences are seen in the first 20 min of the chromatograms, but the most important differences are those of the major peaks. The early eluting major peaks (20–25 min) of curve C were less intense (lower peak heights) and the late eluting peaks (43–55 min) were more intense (higher peak heights) than the corresponding peaks of curve B. Because, in hydrophobic interaction chromatography, large molecular weight proteins are expected to elute slower than small molecular weight proteins, this work corroborates the findings of Wrigley et al (1980) who found increases in higher molecular weight proteins (elution time >25 min) resulting from addition of sulfur to wheat grown on soil severely deficient in sulfur.

CONCLUSIONS

The conclusions of this study are that alterations in gliadin PAGE and HPLC patterns of wheat resulted from sulfur fertilization of soils severely deficient in sulfur. As stated by Moss et al (1981), some Australian wheat soils are potentially sulfur-deficient; sulfur deficiency, however, is not common in wheat crops. Therefore, wheat must have a minimal requirement for sulfur, which is easily furnished in most soil conditions. Changes in the gliadin electrophoretic patterns of bulk field-grown samples with sulfur fertilization have only been reported in the samples described in this article. Some changes in mobility and more likely intensity changes are possible in the gliadin patterns of wheat grown on soil severely deficient in sulfur versus wheat grown on the same soil with sulfur fertilization. Because gliadin PAGE and HPLC patterns varied with the addition of nitrogen or sulfur to soil severely deficient in sulfur, it may be possible to differentiate wheat grown on those soils by their PAGE and HPLC patterns.

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