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Chromosomal control of wheat gliadin: analysis by reversed-phase high-performance liquid chromatography*

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Summary. Gliadin proteins of the hexaploid wheat variety 'Chinese Spring', and of its nullisomic-tetrasomic and ditelocentric aneuploid lines, were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC). Reversed-phase separations were carried out at 70 °C on C_8 and C_{18} columns using a gradient of increasing acetonitrile concentration in the presence of 0.1% trifluoroacetic acid. Thirty-five components were separated and all were found to be controlled by genes on the short arms of group 1 and group 6 chromosomes (the complex *Gli-1* and *Gli-2* loci). Results indicated that gluten polypeptides elute as groups in order of increasing hydrophobicity in the following approximate order: (1) albumins plus globulins, (2) ω-gliadins, (3) high molecular weight (MW) glutenin subunits, (4) α-type gliadins, (5) low MW glutenin subunits, and (6) γ-gliadins. The three distinct protein types coded by genes at the complex *Gli-1* loci (ω-gliadins, γ-gliadins, and low MW glutenin subunits) thus have uniquely different surface hydrophobicities. Similarly, gene locations for hexaploid 'Cheyenne' gliadins and durum gliadin proteins in the varieties 'Langdon', 'Edmore', and 'Kharkovskaya-5' were determined through RP-HPLC analysis of aneuploid lines. All results confirm known locations of genes for gliadin proteins, and demonstrate that RP-HPLC is a powerful new tool for analysis of gliadins in breeding and genetic studies.

Key words: Gliadin – *Triticum* – RP-HPLC – Chromosomes – Coding – Control

Introduction

The endosperm proteins of the wheat kernel are the primary nutritional storage reservoir of proteins and amino acids for the germinating plant. The most abundant of these proteins are gliadins, the prolamin fraction of wheat. Gliadins are characterized by their high content of glutamine and proline, their solubility in neutral solutions containing high concentrations of alcohol, and their great heterogeneity (see Kasarda et al. 1976a; Wall 1979 for reviews). To wheat breeders and geneticists, gliadins are also of much interest because of their nearly invariant expression, making them accurate genotypic indicators (Wrigley et al. 1982).

The development of aneuploid lines of the wheat variety 'Chinese Spring' by Sears (1954) allowed an important new type of gliadin analysis in wheat genetic studies. Boyd and Lee (1967) first showed major changes in starch gel electrophoresis patterns of gliadins in ditelocentric (DT) lines of 'Chinese Spring' involving the 1B and 1D chromosomes; polypeptide bands were thus identified which were coded by genes on specific chromosome arms. Such bands may subsequently be used as reliable markers of the presence or absence of specific genes, since numerous studies (Wrigley et al. 1982) suggest that there is little or no regulation of gliadin expression. Shepherd (1968) extended these studies by examining both compensating nullisomic-tetrasomic (NT) and DT lines of 'Chinese Spring', and was able to relate specific chromosomes with expression of 10 out of 18 major bands separated by starch gel electrophoresis. Expression of these proteins was found to be controlled by genes located on specified chromosomal arms (subsequently termed "short") of homoeologous groups 1 and 6.

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned

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As new analytical methods for fractionating wheat proteins were developed, the heterogeneity of gliadin became more evident. Examination of aneuploid lines by new methods also continued to reveal more detail concerning their chromosomal control. Thus, Wrigley and Shepherd (1973) separated 'Chinese Spring' gliadin into 46 components by two-dimensional electrophoresis, and demonstrated that most major components were controlled by single homoeologous group 1 and 6 chromosomes. More recent studies, utilizing other two-dimensional electrophoresis procedures and using aneuploids or substitution lines involving additional cultivars, have continued to more precisely locate genes controlling expression of wheat endosperm proteins. These studies have confirmed that control of the expression of gliadins by the short arms of homoeologous group 1 and 6 chromosomes is a general phenomenon (Kasarda et al. 1976b; Brown et al. 1979; Brown and Flavell 1981; Brown et al. 1981; Zehatschek et al. 1981; Galili and Feldman 1983; Kasarda et al. 1984). Results of such studies are also summarized in several excellent recent reviews (Payne et al. 1982; Wrigley 1982; Garcia-Olmedo et al. 1982; Porceddu et al. 1983; Payne et al. 1984).

In 1983, Bietz described the application of another new method, reversed-phase high-performance liquid chromatography (RP-HPLC), for analysis of cereal proteins. This method was applicable to all classes of wheat proteins, and was found to be fast, sensitive, reproducible and automatable, and the data were easily quantified. Resolution by RP-HPLC generally equalled or exceeded that of other existing methods, and, most importantly, RP-HPLC complemented other analytical methods. Subsequently, Bietz et al. (1984a) established optimal conditions for extraction and separation of gliadin proteins by RP-HPLC, and showed that the method is an excellent alternative to electrophoresis for identifying wheat cultivars (Burnouf et al. 1983; Bietz et al. 1984b).

These studies also demonstrated the applicability of RP-HPLC to wheat aneuploid analysis (Bietz et al. 1984b). Conditions for extraction, derivitization, and RP-HPLC analysis of wheat glutenin subunits were also optimized (Burnouf and Bietz 1984a), and found to be applicable to aneuploid analysis of glutenin. The present communication presents results of a detailed study localizing genetic control of gliadin expression to specific chromosomal arms in the hexaploid wheat cultivars 'Chinese Spring' and 'Cheyenne', and in durum wheat cultivars using RP-HPLC. A separate manuscript (Burnouf and Bietz 1985) reports results of a similar study concerning aneuploid analysis of wheat glutenin subunits.

Materials and methods

Plant materials

Compensating NT and DT lines of the hexaploid wheat cultivar 'Chinese Spring' were generously provided by E.R. Sears (Univ of Missouri, Columbia, MO, USA). In the NT lines, the absence of a chromosome pair is compensated for by the presence of a homoeologous chromosome pair of the same group from an alternate genome, and in DT lines, a pair of chromosome arms is deleted. For example, N1AT1D indicates the absence of the two 1A chromosomes and the presence of four 1D chromosomes within the genotype, and DT1DL indicates the presence of the long arms of chromosomes 1D, and the implied absence of the short arms of these chromosomes. Thus, comparison of proteins from these aneuploid genotypes to those of euploid 'Chinese Spring' permits assignment of deleted polypeptides to missing chromosomes or chromosome arms. In the present study, all such assignments are based upon agreement between two or more aneuploid lines, plus a lack of conflicting data.

Disomic substitution lines of 'Cheyenne' chromosomes into 'Chinese Spring' (Morris et al. 1966) were generously furnished by R. Morris (Univ. of Nebraska, Lincoln, NE, USA). In these lines, individual 'Cheyenne' chromosome pairs are substituted for their homoeologous chromosomes in the 'Chinese Spring' background.

Additional durum cultivars and aneuploids (Joppa et al. 1978) were kindly provided by L.R. Joppa (USDA, ARS, Fargo, ND, USA). These included the cultivars 'Langdon', 'Edmore', and 'Kharkovskaya-5', substitution lines of 'Kharkovskaya-5' and 'Edmore' 1B chromosomes for 'Langdon' 1B, disomic addition lines of 'Chinese Spring' chromosomes 1D and 6D to 'Langdon', and the disomic 'Langdon' ('Chinese Spring') substitution lines 1A(1D), 1B(1D), 6A(6D), and 6B(6D).

Gliadin extraction

Single wheat kernels were crushed with a mortar and pestle, and, for the 'Chinese Spring' aneuploid series, initially extracted with 0.04 M NaCl (2×5 ml) to remove albumins and globulins (Bietz et al. 1984a). Gliadins of disomic 'Cheyenne' substitutions into 'Chinese Spring' and of 'Langdon' durum aneuploids were extracted directly, without pre-removal of albumins and globulins (Bietz et al. 1984a). Gliadins were then extracted with 70% ethanol (2.0 ml/50 mg), centrifuged (24,000 g, 10 min), and extracts (50 µl) directly analyzed by RP-HPLC. Gliadin extraction conditions have previously been described in more detail (Bietz 1983; Bietz et al. 1984a, b).

RP-HPLC analysis

Extracted gliadins were analyzed on a Waters HPLC system as described previously (Bietz 1983) using either a Brownlee Aquapore C_8 column (number 4416, 300 Å pore size, 10 µm particle size, end-capped, 250×4.1 mm) or a SynChropak RP-P (C_{18}, 300 Å pore size, 6.5 µm particle size, end-capped, 250×4.1 mm) column at 70 °C. The chromatographic solvent gradient used 0.1% trifluoroacetic acid (TFA) (Pierce) in deionized distilled water as solvent A, and 0.1% TFA in acetonitrile (ACN) (MCB, Omnisolv grade) as solvent B. Proteins were eluted with a linear ACN gradient (28–50% ACN during 55 min for the Brownlee column and 25–50% ACN during 55 min for the SynChropak column) with a total run time of 65 min, and detected at 210 nm (0.2 AUFS). Raw analog data were saved using a ModComp computer system for subsequent integration, comparison, and replotting. In replotting the data, the solvent peak (eluting at ca. 2 min) is not shown and ordinates are normalized so that the largest peaks in different chromatograms have the same height. Elution times of peaks are highly reproducible (Bietz 1983). In fact, replicate chromatograms are superimposable, permitting ready identification of corresponding peaks between chromatograms.

Results and discussion

Coding of gliadin proteins in 'Chinese Spring'

RP-HPLC of 'Chinese Spring' gliadin. The pattern resulting from RP-HPLC of 'Chinese Spring' gliadin is

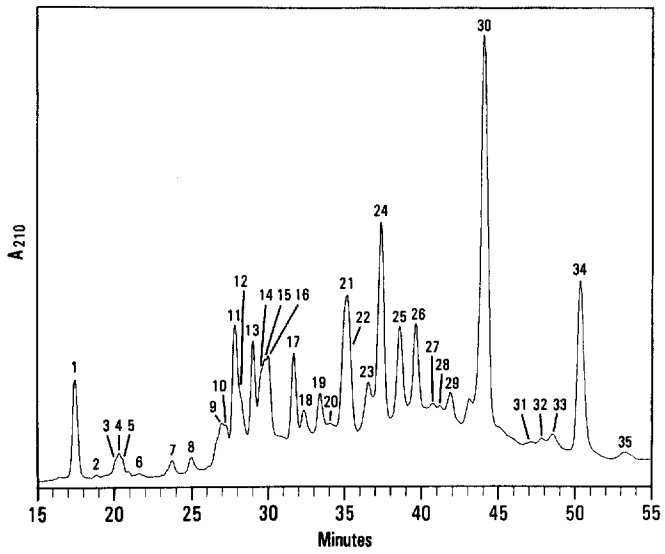

Fig. 1. RP-HPLC of gliadin proteins from the cultivar 'Chinese Spring', separated on a Brownlee Aquapore C_8 column at 70 °C using a gradient from 28 to 50% ACN over 55 min in the presence of 0.1% TFA. See "Materials and methods" for further details. Peaks are numbered in order of increasing hydrophobicity

Table 1. Chromosomal location of genes coding gliadin proteins in the wheat cultivar 'Chinese Spring', as determined by RP-HPLC

Peak[a]	Chromosome[b]	Peak[a]	Chromosome[b]	Peak[a]	Chromosome[b]
1	1D	7	6B	20	1D
2	1B	8	6D	21	1B
3	1A	9	6B	22	1B
4	1A	10	6B	23	1B
5	1A	11	6B	24	1D
6	1D	12	6A	25	1D
		13	6B + 6D[c]	26	1B
		14	6D	27	1A
		15	6D	28	1A
		16	6D	29	1A
		17	6A	30	1A
		18	6A	31	1B
		19	6A	32	1D
				33	1B
				34	1B
				35	1B

[a] Peak number refers to peaks in Fig. 1
[b] All genes listed were found to be located on the short arms of the indicated chromosomes
[c] Polygenic control was indicated for protein components of this peak, indicating that it contained gliadins coded by genes on both chromosomes 6B and 6D

shown in Fig. 1. Thirty-five distinct chromatographic features varying in surface hydrophobicity were observed, including some minor or poorly resolved peaks and shoulders. Peaks are numbered in order of increasing surface hydrophobicity as indicated in the figure; these numbers are for purposes of discussion and comparison in the text and table only, and are not meant as a definitive nomenclature.

Analysis of group 1 aneuploids. Chromosomal locations of genes coding gliadin proteins separated by RP-HPLC were determined by comparison of euploid 'Chinese Spring' to all available NT and DT lines. Figure 2 shows results obtained for six of the major aneuploid lines involving chromosome group 1, namely N1ATIB (a), N1BT1D (b), N1DT1A (c), DT 1AL (d), DT 1BL (e), and DT 1DL (f). Major differences among RP-HPLC patterns were obvious, and were primarily of two types: some protein peaks were missing due to nullisomy or due to absence of chromosome arms containing those genes which control expression of those proteins; other peaks in the NT lines were significantly increased relative to 'Chinese Spring' due to tetrasomy of those chromosomes having genes which control expression of those proteins. Specific peaks in the aneuploids exhibiting such changes, as compared to 'Chinese Spring' (Fig. 1), are numbered (Fig. 2), and chromosomal control of their proteins, as deduced from analysis of these aneuploids, is summarized in Table 1. Single chromosome control of gliadins in each group 1 peak was observed.

Data from RP-HPLC analysis of the other three possible chromosome group 1 NT lines, namely N1AT1D, N1BT1A, and N1DT1B, fully agreed with the data presented in Fig. 2. Furthermore, analysis of DT lines lacking the long arms of group 1 (or group 6; see below) chromosomes gave RP-HPLC patterns (data not shown) essentially identical to that obtained for 'Chinese Spring', independently confirming that genes controlling expression of gliadin proteins in 22 peaks are located entirely on the short arms of group 1 chromosomes (Payne et al. 1984).

It is especially interesting to note that all gliadins coded by genes on group 1 chromosomes elute either in early or late portions of the chromatogram (i.e., peaks 1–6 and 20–35). Furthermore, there are several instances of proteins in at least three adjacent peaks controlled by genes on the same chromosome (peaks 3–5, 21–23, 27–30, and 33–35). These data may suggest that multiple genes from the different gliadin loci code polypeptides that are very similar in hydrophobicity. This would be expected from their homologous structures (Bietz et al. 1977); it is also likely that gliadins eluting in adjacent peaks which have been assigned to

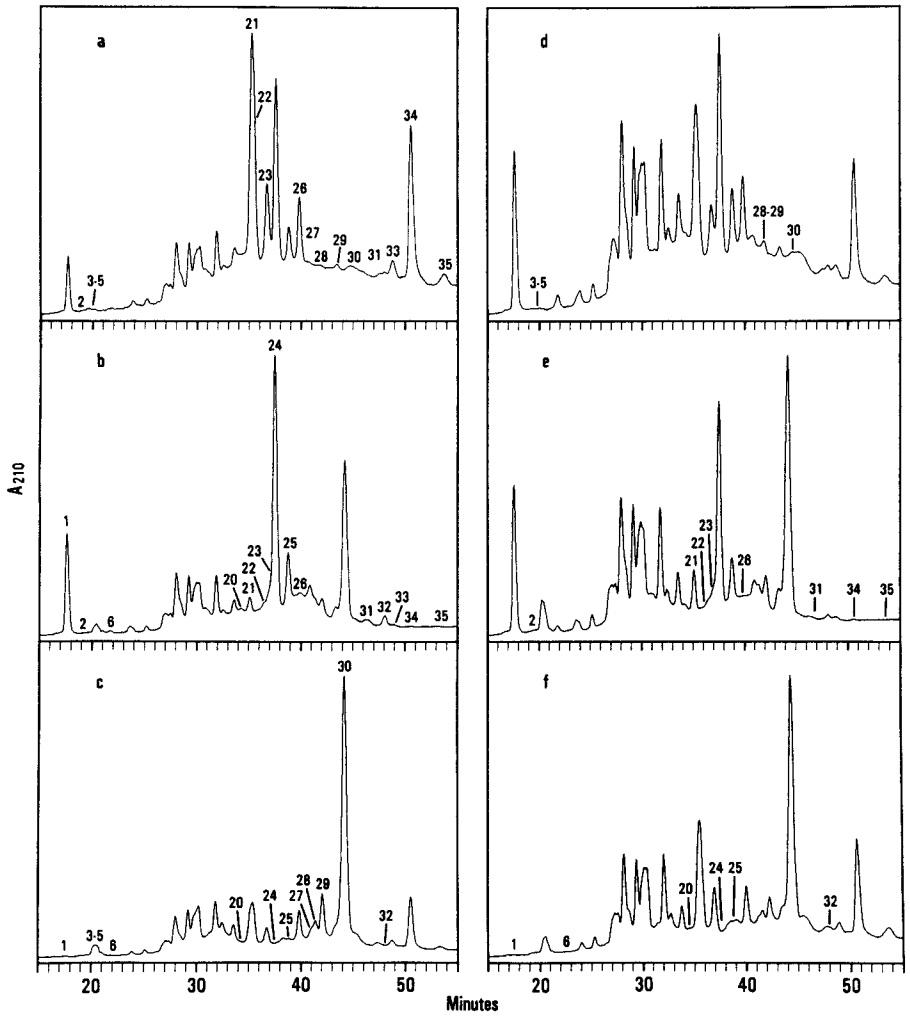

Fig. 2. RP-HPLC analysis of gliadin proteins from the 'Chinese Spring' group 1 aneuploid lines (**a**) N1AT1B (**b**) N1BT1D, (**c**) N1DT1A, (**d**) DT 1AL, (**e**) DT 1BL, and (**f**) DT 1DL. Positions of peaks affected by altered gene dosage, indicated by numerals, correspond to numbering system of Fig. 1. Other details are as in Fig. 1

the same chromosome are coded by genes of the same loci (specifically, *Gli-A1, Gli-B1,* and *Gli-D1*) (see Payne et al. 1984).

Bietz (1983) Previously noted that ω-gliadins have lower apparent surface hydrophobicities than do α, β, and γ-gliadins, and characteristically elute first upon RP-HPLC. The expression of ω-gliadins is known to be controlled by the complex gene loci *Gli-A1, Gli-B1,* and *Gli-D1.* These gene loci also control expression of γ-gliadins (as well as low MW subunits of glutenin) (Payne et al. 1984). Thus, it is highly likely that peaks 1–6 (Fig. 1) represent ω-gliadins while peaks 20–35 contain primarily γ-gliadins, since expression of these proteins correlates with chromosomes carrying the *Gli-1* loci. This latter conclusion is fully supported by the studies of Burnouf and Bietz (1984b), who demonstrated that the quality-associated durum gliadin bands "42" and "45" (Damidaux et al. 1978), which are γ-gliadins, elute late upon RP-HPLC and apparently have high surface hydrophobicities.

Analysis of group 6 aneuploids. RP-HPLC analyses of gliadins extracted from representative chromosome group 6 aneuploid lines are shown in Fig. 3. Major differences occurred in the lines N6AT6D (Fig. 3a), N6BT6A (Fig. 3b), N6DT6B (Fig. 3c), DT6AL (Fig. 3d), DT6BL (Fig. 3e), and DT6DL (Fig. 3e) (as well as in N6AT6B and N6DT6A, data not shown), which, through comparison to euploid 'Chinese Spring' (Fig. 1), permitted assignment of gliadins in peaks 7–19 to the short arms of the three group 6 chromosomes. Specific aneuploid peaks differing from 'Chinese Spring' are numbered in Fig. 3, and their relation with specific chromosomes is summarized in Table 1. Single-chromosome control was noted for gliadins of all peaks except peak 13, which seemed to contain polypeptides controlled by loci on both 6B and 6D. Indeed, it is surprising that single-chromosome control is so often found, since there may be sufficient heterogeneity at the *Gli-1* and *Gli-2* loci to allow expression of more than 100 gliadins within a single variety (R. Thompson, personal communication). Presumably, gliadins having similar surface hydrophobicities, coded by genes from the same complex locus, may still differ significantly in ionic character and separate by electrophoresis (Bietz

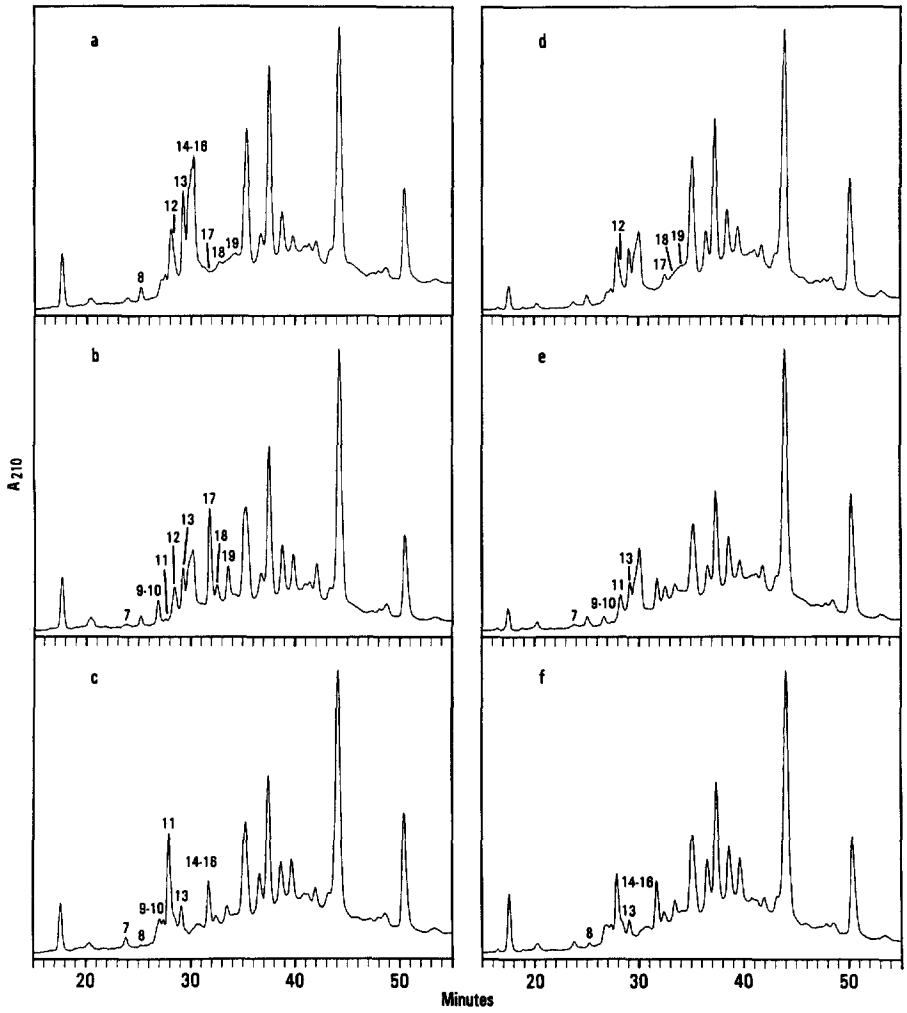

Fig. 3. RP-HPLC analysis of gliadin proteins from the 'Chinese Spring' group 6 aneuploid lines (**a**) N6AT6D, (**b**) N6BT6A, (**c**) N6DT6B, (**d**) DT 6AL, (**e**) DT 6BL, and (**f**) DT 6DL. Positions of peaks affected by altered gene dosage, indicated by numerals, correspond to numbering system of Fig. 1. Other details are as in Fig. 1

1983; Burnouf and Bietz 1984b). Minor gene products may also co-elute with major peaks, as sometimes observed in NT and DT lines.

As noted with group 1 chromosomes (Fig. 2), gliadins controlled by genes on the short arms of chromosomes 6A, 6B, and 6D (loci *Gli-A2, Gli-B2,* and *Gli-D2,* see Payne et al. 1984) have similar surface hydrophobicities, and elute within a fairly narrow range of hydrophobicity as peaks 7–19. These gene loci control expression primarily of α- and β-gliadins (Payne et al. 1984), which are highly homologous (Bietz et al. 1977). As with the group 1 chromosome loci, adjacent RP-HPLC peaks (Fig. 2) frequently contain proteins controlled by the same group 6 chromosome loci (e.g., peaks 9–11, 6B; peaks 14–16, 6D; and peaks 17–19, 6A), suggesting that variation in surface hydrophobicity among gene products of individual complex loci is very slight. This may be expected since group 6 coded α-type gliadins may differ primarily in length of internal polyglutamine sequence regions (domains II and IV) (Kasarda et al. 1984b).

The results of RP-HPLC analysis of group 6 aneuploids (Fig. 3), when considered along with those for group 1 aneuploid lines (Fig. 2), suggest that most gliadins are eluted upon RP-HPLC in the relative order ω, $\alpha+\beta$, and γ, in order of increasing surface hydrophobicity. These results also demonstrate that RP-HPLC has the potential of predicting chromosomal control of gliadins in other genotypes on the basis of relative surface hydrophobicities. Similar elution characteristics have been noted for ω-gliadin from another hexaploid wheat cultivar ('Ponca') (Bietz 1983), for γ-gliadins from two tetraploid durum cultivars (Burnouf and Bietz 1984b), and in the present study for 'Chinese Spring', suggesting that the same relative elution order of gliadins will be a general characteristic of all *Triticum* cultivars and species. Thus, RP-HPLC is an attractive alternative method to more difficult, time consuming

electrophoresis methods for differentiating translation products of the complex *Gli-1* loci.

Elution positions of gliadins in terms of absolute hydrophobicity allow comparison to elution positions of other wheat endosperm proteins upon RP-HPLC. Gliadins in peaks 1–6 (controlled by the *Gli-1* loci on group 1 chromosomes) elute from 34.0–36.0% ACN; those in peaks 7–19 (controlled by the *Gli-2* loci on group 6 chromosomes) elute from 36.4–40.8% ACN; and those in peaks 20–35 (controlled by the *Gli-1* loci) elute from 41.2–48.8% ACN. Results from the companion study to this manuscript (Burnouf and Bietz 1985) on glutenin subunits indicate that pyridylethylated high MW polypeptides controlled by the *Glu-1* loci on the long arms of group 1 chromosomes elute from approximately 30.6–34.5% ACN, and the majority of pyridylethylated lower-MW glutenin subunits elute from 37.7–43.0% ACN. Thus, pyridylethylated high MW glutenin subunits have surface hydrophobicities similar to ω-gliadins (from the complex *Gli-1* loci), and pyridylethylated lower-MW glutenin subunits would largely co-elute with the group 6 controlled α and β gliadins (from the *Gli-2* loci), but would elute before the group 1 (*Gli-1* loci) controlled γ-gliadins. The complex *Gli-1* loci are known to contain at least three separate families of genes primarily controlling expression of ω-gliadins, γ-gliadins, and low MW glutenin subunits (Payne et al. 1984). Our results suggest that translation products of these three gene types differ so significantly in structure, and therefore in resultant surface hydrophobicity, that they have very different, largely non-overlapping, elution characteristics upon RP-HPLC. Predictions concerning structure are in agreement with numerous studies showing significantly different amino-terminal sequences for γ- and ω-gliadins, and for low-MW glutenin subunits (Bietz et al. 1977; Kasarda et al. 1983; Bietz and Wall 1980).

One difficulty in comparing the present results to those for glutenin (Burnouf and Bietz 1985) is that native gliadins are being compared to S-pyridylethylated glutenin subunits. Data of Burnouf and Bietz (1984a), however, as well as unpublished observations, suggest that most S-pyridylethylated gluten polypeptides have surface hydrophobicities somewhat lower than those of native proteins or subunits which are reduced but not alkylated. Thus, comparison of elution positions of individual gluten protein types to reduced and alkylated pyridylethylated whole gluten (data not shown) indicates that each major gluten polypeptide type differs sufficiently in surface hydrophobicity so that their RP-HPLC elution positions are relatively distinct. Specifically, polypeptides elute in the relative order (1) albumins plus globulins, (2) ω-gliadins, (3) high-MW glutenin subunits, (4) α-type gliadins, (5) low-MW glutenin subunits, and (6) γ-gliadins. Although some overlap in elution positions of adjacent protein types may occur, such as between α-type gliadins and low-MW glutenin subunits, such overlap is generally minimal. Consequently, a hypothesis is suggested which indicates that, among wheat gluten polypeptides, protein type can be largely predicted from elution position during RP-HPLC. Further studies will be necessary to confirm the general validity of this hypothesis.

Control of gliadin proteins in 'Cheyenne'. To extend our results for 'Chinese Spring' to a broader genetic base among hexaploid wheats, we also used RP-HPLC to examine chromosomal control of gliadin proteins in the wheat cultivar 'Cheyenne' by examining disomic substitution lines of individual 'Cheyenne' chromosomes for their homoeologous chromosome pair in 'Chinese Spring'. A summary of results obtained, along with typical RP-HPLC data, are presented in Fig. 4.

RP-HPLC patterns for 'Cheyenne' (Fig. 4A) and 'Chinese Spring' (Fig. 4B) differed significantly in numerous peaks, as expected (Bietz et al. 1984b), but also shared many components of comparable elution times. Assuming identity and similar chromosomal control for these latter components, examination of substitution lines would obviously not reveal coding of all components, as was possible in 'Chinese Spring' through comparison of euploid and aneuploid lines (Figs. 2 and 3); nevertheless, examination of these substitution lines did permit determination of chromosomal control for many 'Cheyenne' gliadins, as well as confirm control of several 'Chinese Spring' gliadins.

Figure 4B and Fig. 1 both present RP-HPLC results for 'Chinese Spring', but there are two major differences. First, the columns used differed slightly in selectivity, and gradients differed slightly to optimize resolution; consequently, patterns could be expected to differ slightly. Secondly, 'Chinese Spring', as analyzed in Fig. 4B, was not pre-extracted with 0.04 M NaCl to remove albumins and globulins; this modification of the method significantly reduces sample preparation time and is acceptable for many purposes, since additional albumins and globulins extracted are primarily minor components which are less hydrophobic than gliadins and elute earlier upon RP-HPLC (ca. 20–30 min in Fig. 4B) (Bietz 1983).

Comparison of substitution lines 1A, 1B, 1D, 6A, 6B, and 6D to 'Cheyenne' and 'Chinese Spring' enabled assignment of control of several major and minor components to specific chromosomes (Figs. 4A, B). For 'Chinese Spring', chromosomal control of several major components, determined by the absence of 'Chinese Spring' peaks in the substitution lines, was comparable to that found using 'Chinese Spring' aneuploids; these components are identified and numbered in Fig. 4B to permit comparison to data in Fig. 1. For 'Cheyenne', chromosomal control was ascertained for 12 specific peaks (Fig. 4A), including early-eluting components which, by analogy to 'Chinese Spring', may be albumins or globulins. For both 'Chinese Spring' and 'Cheyenne', multiple control was observed for some peaks, indicating

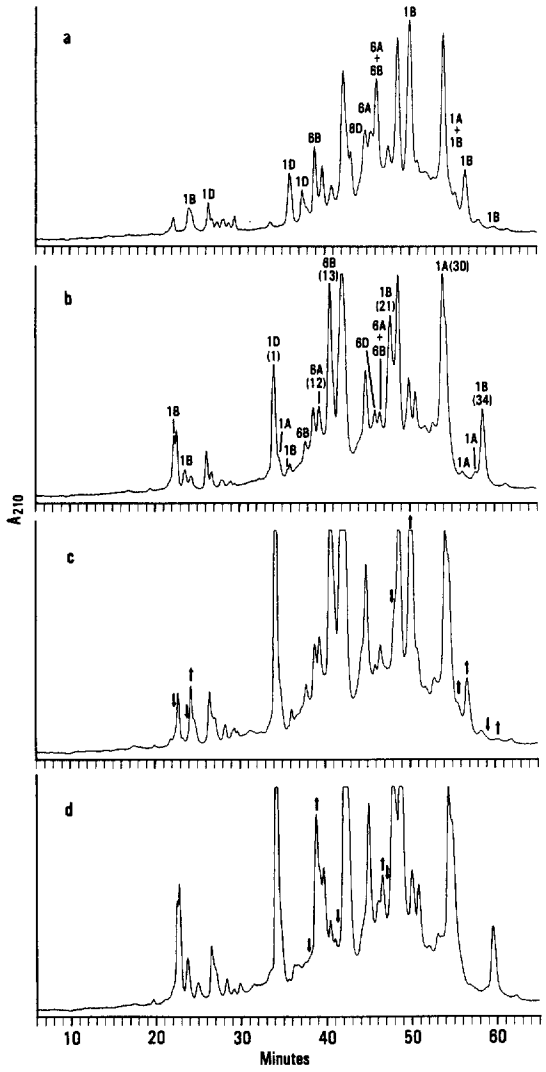

Fig. 4. RP-HPLC of gliadin proteins of the wheat cultivars 'Cheyenne' (**a**) and 'Chinese Spring' (**b**), and of disomic 'Chinese Spring' 1B ('Cheyenne' 1B) (**c**) and 'Chinese Spring' 6B ('Cheyenne' 6B) (**d**) substitution lines. Proteins were separated on a SynChropak C_{18} column at 70 °C using a gradient from 25–50% ACN in the presence of 0.1% TFA during 55 min, with a final 10 min hold at 50% ACN. See "Materials and methods" for additional details. Chromosome designations in (**a**) and (**b**) denote chromosomal control determined through comparison to substitution lines. Numbers in parentheses in (**b**) refer to peak numbers for 'Chinese Spring' gliadins in Fig. 1. Ascending and descending *arrows* in (**c**) and (**d**) denote elution positions of components exhibiting major increases or decreases, respectively, as compared to 'Chinese Spring' (**b**)

that they contain proteins controlled by genes on more than one chromosome. The assignments for 'Cheyenne' were apparent from noting specific 'Cheyenne' peaks in the substitution lines which were absent in 'Chinese Spring'. Only representative data for substitution lines are presented in Fig. 4; examination of the 1A, 1D, 6A, and 6D lines also clearly revealed control of components (indicated in Figs. 4A, B). Examination of substitution lines involving chromosome groups 2, 3, 4, 5, or 7 revealed no significant differences from 'Chinese Spring', confirming control of gliadins by genes on group 1 and 6 chromosomes. Those results also confirmed previous analyses of these substitution lines using one-dimensional electrophoresis (Kasarda et al. 1976b).

Assignment of gliadin genes to specific chromosome arms is impossible using whole-chromosome substitution lines, but through analogy to the data of Fig. 3, and on the basis of numerous previous studies (see Payne et al. 1984), control of 'Cheyenne' gliadins can be assumed to reside on the short arms of these chromosomes.

The most significant observation from Fig. 4A is that control of 'Cheyenne' gliadins separated by RP-HPLC follows the same pattern observed for 'Chinese Spring': group 1-coded ω-gliadins (*Gli-1* loci) appear to elute first, followed by group 6-coded α- and β-gliadins (*Gli-2* loci), followed finally by group 1-coded γ-gliadins (*Gli-1* loci). The same relative RP-HPLC elution order for gliadins in these two hexaploid cultivars suggests that marked differences in surface hydrophobicity of different classes of gluten polypeptides is a general phenomenon for all hexaploid wheats.

Coding of gliadin proteins in durum cultivars

Coding of 'Langdon' gliadins. A typical RP-HPLC chromatogram of gliadin from the durum cultivar 'Langdon' is shown in Fig. 5a. Approximately 33 peaks (including small ones and partially resolved shoulders) were observed; the pattern is slightly simpler than that observed for 'Chinese Spring' (Fig. 1), undoubtedly due to the lack of polypeptides coded by D-genome chromosomes. All gliadins from durum lines were examined by RP-HPLC on both C_{18} SynChropak and C_8 Aquapore columns, with nearly identical results; only the data for the C_{18} column are shown.

We compared 'Langdon' gliadin to gliadins extracted from 'Langdon' ('Chinese Spring') substitution and addition lines to determine chromosomal locations of genes coding specific 'Langdon' gliadins; results for the disomic substitution lines 1A(1D) and 1B(1D) are shown in Figs. 5b and 5c, respectively. Comparison of these chromatograms to that of 'Langdon' reveals, by peaks missing in the substitution lines, that genes controlling two polypeptides, eluted at ca. 27 and 48 min, are located on chromosomes 1A in 'Langdon', and genes for six polypeptides (eluting at ca. 10.3, 11.0, 11.6, 13.3, 28.0, and 46.5 min) are located on 'Langdon' chromosomes 1B; these peaks are indicated in Fig. 5A. Similarly, proteins in peaks at ca. 24.5, 39.7, and 41.3 min (indicated in Figs. 5B, C) in the substitution

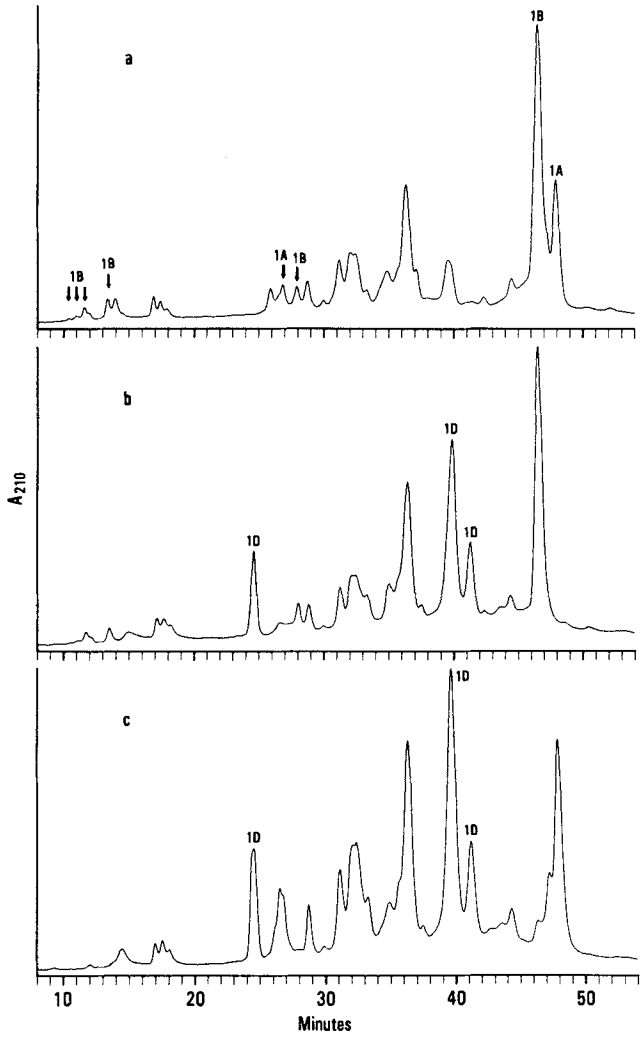

Fig. 5. RP-HPLC of gliadin proteins from the durum wheat cultivar 'Langdon' (**a**) and from 'Langdon' ('Chinese Spring') disomic substitution lines 1A(1D) (**b**) and 1B(1D) (**c**). *Arrows* and *chromosome* designations indicate chromosomal control determined for specific peaks

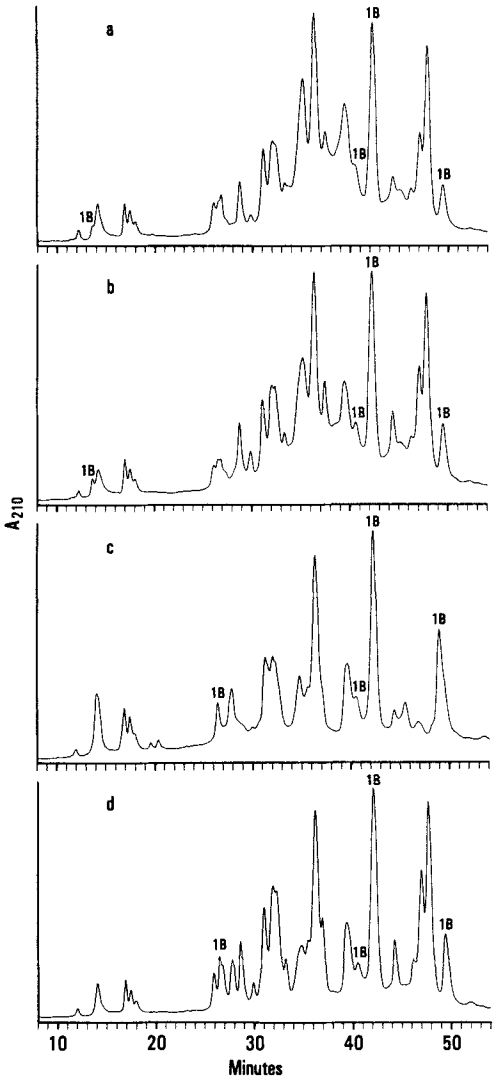

Fig. 6. RP-HPLC of gliadin proteins of the durum wheat cultivars 'Edmore' (**a**) and 'Kharkovskaya-5' (**c**), of the substitution line of 'Edmore' chromosomes 1B into 'Langdon' (**b**), and of the substitution line of 'Kharkovskaya-5' chromosomes 1B into 'Langdon' (**d**). Other details are as in Fig. 5

lines may be attributed to genes on the 1D chromosomes; this also agrees with data for the disomic 'Chinese Spring' 1D addition to 'Langdon' (data not shown). These three major 1D-coded polypeptides correspond to peaks 1, 24, and 25 in Fig. 1; slight differences in elution times are due to slightly different selectivities of the two columns used (also compare Fig. 4 B and Fig. 1), as well as to a slight difference in the gradient.

Comparison of the disomic 6D addition line of 'Chinese Spring' to 'Langdon', as well as disomic 6A(6D) and 6B(6D) substitution lines, did not clearly reveal control of 'Langdon' gliadins (data not shown). Major quantitative effects in these lines occurred for peaks between ca. 29 and 38 min (Fig. 5 a), indicating coding of medium-hydrophobicity polypeptides by genes on group 6 chromosomes in 'Langdon', as in 'Chinese Spring' (Fig. 3); however, in these substitution lines, control of these polypeptides by 'Chinese Spring' group 6 chromosomes is obviously similar to that of 'Langdon' ('Chinese Spring') 6A and 6B chromosomes (as expected from homoeology of the *Gli-2* loci in 'Chinese Spring'), thereby preventing exact location of gene products of the *Gli-2* loci in 'Langdon' ('Chinese Spring') substitution lines. Heterogenous bands coded by group 6 chromosomes were also observed by Joppa et al. (1983) and by du Cros et al. (1983) using one- and two-dimensional electrophoresis.

Coding of gliadins in other durum cultivars. Substitution lines of the 1B chromosomes of 'Kharkovskaya-5' and 'Edmore' for homoeologous 1B 'Langdon' chromosomes were also investigated to determine genetic control of gliadins by 1B chromosomes in these two additional durum cultivars. Results are presented in Fig. 6. Comparison of these chromatograms to that of 'Langdon' (Fig. 5 a) showed that four 'Edmore' gliadins (at ca. 13.6, 40.5, 42.5, and 49.5 min) and four 'Kharkovskaya-5' gliadins (at ca. 26.5, 40.5, 42.5, and 49.5 min) were controlled by genes on chromosomes 1B.

The three late-eluting 1B-coded gliadins in these two varieties were not present in 'Langdon' (Fig. 5 a). This is due to the presence of alternate allelic forms of loci controlling certain major gliadins (bands "42" and "45" by polyacrylamide gel electrophoresis) (Damidaux et al. 1978) and additional closely-linked gliadins in poor- and good-quality durums, respectively (Burnouf and Bietz 1984 b). Thus, the major 1B-controlled gliadin in 'Langdon' (46.5 min in Fig. 5 a) represents band "42", and the major 1B-controlled gliadin in 'Kharkovskaya-5' and 'Edmore' (ca. 42.5 min in Fig. 6) corresponds to gliadin "45".

Conclusions

Our results demonstrate that RP-HPLC is a valuable procedure for genetic analysis of wheat aneuploid lines. Resolution of RP-HPLC is generally equal to or superior to that of one-dimensional electrophoresis procedures, and often nearly equals that of two-dimensional electrophoresis. Its great rapidity and convenience may, however, make RP-HPLC preferable: optimal separations generally require about one h, and many separations can be obtained in 5–15 min (Burnouf and Bietz 1984 b; Bietz and Cobb 1984). High temperatures (70 °C) significantly improve resolution and promote polypeptide dissociation (Bietz and Cobb 1984), thus facilitating rapid separations. RP-HPLC separates proteins on the basis of surface hydrophobicity, complementing most other separation techniques; it thus may achieve separations impossible by other methods. RP-HPLC may also reveal information relating protein hydrophobicity to functionality: through accurate quantitative analysis (Burnouf and Bietz 1985), results may predict quality of bread or durum wheats (Burnouf and Bietz 1984 a, b).

The present study contains the first comprehensive examination of the 'Chinese Spring' aneuploid series by RP-HPLC. These materials represent the most complete series of wheat aneuploid lines available. RP-HPLC separated 'Chinese Spring' gliadin into 35 components (plus a few unnumbered minor chromatographic features), and successfully located genes controlling expression of the protein components for these peaks on specific chromosomes. Single-gene control was observed for proteins of all except one peak, suggesting that the still greater heterogeneity thought to exist for gliadins is probably due to charge variation, which may not affect surface hydrophobicity, within groups of homologous proteins coded by the same loci. All gliadins were found to be controlled by genes on the short arms of the group 1 and group 6 chromosomes (primarily the *Gli-1* and *Gli-2* loci). These results are in total agreement with results obtained by electrophoresis. The successful analysis of 'Chinese Spring' aneuploid materials suggested that RP-HPLC should be applicable to analysis of aneuploid materials of other hexaploid wheat cultivars as well. This was confirmed by analysis of 'Cheyenne'/'Chinese Spring' disomic substitution lines, which revealed that chromosomal control of 'Cheyenne' gliadins is very similar to that in 'Chinese Spring'. Experiments are also now in progress to establish the chromosomal control of gliadin and glutenin polypeptides in 'Wichita'/'Cheyenne' substitution lines.

One of the most surprising observations in the present study is that different classes of polypeptides controlled by the same complex *Gli-1* loci (see Payne et al. 1984 for review) have significantly different surface hydrophobicities, permitting their ready separation as groups by RP-HPLC. Thus, genes of the *Gli-1* loci, on the short arms of group 1 chromosomes, control ω-gliadins of relatively low surface hydrophobicity, ethanol-soluble glutenin subunits of intermediate hydrophobicity, and γ-gliadins of highest hydrophobicity. The *Gli-2* loci, located on the short arms of the group 6 chromosomes, contain genes which control all α- and most β-type gliadins, of intermediate hydrophobicities. And the *Glu-1* loci, located on the long arms of group 1 chromosomes, control high MW glutenin subunits of relatively low hydrophobicity. Each of these polypeptide classes is structurally unique, and now can be seen to differ uniquely in surface hydrophobicity characteristics as well; in addition, it is likely that the same relative elution order of polypeptide classes exists for all hexaploid and tetraploid wheats. Thus, our results suggest the hypothesis that each major gluten polypeptide type has a relatively distinct range of surface hydrophobicities, permitting identification of gluten protein type from RP-HPLC elution characteristics.

We were also able to use RP-HPLC to assign genes controlling specific durum gliadins to specific 1A and 1B chromosomes in the cultivars 'Langdon', 'Edmore', and 'Kharkovskaya-5', and to suggest that control of durum gliadins by genes of the *Gli-2* loci (chromosome group 6) is similar to that in the hexaploid varieties 'Chinese Spring' and 'Cheyenne'. Durum aneuploid analysis has been achieved previously by one- and two-

dimensional electrophoresis (Joppa et al. 1983; du Cros et al. 1983), which also revealed hybrid bands controlled by group 6 chromosomes. Resolution of durum β-gliadins by two-dimensional electrophoresis (du Cros et al. 1983) is superior to that achieved by RP-HPLC in the present study. For γ- and ω-gliadins, however, resolution by the two methods appears similar. Because of its numerous advantages and complementary nature, RP-HPLC will become a valuable tool for durum aneuploid analysis. Our results also confirm that 1B alleles control γ-gliadin polypeptides "42" and "45", which are related to quality. Thus, RP-HPLC should become a valuable general method to the breeder and geneticist for quality evaluation, as well as for selection and for evaluation of early-generation materials through analysis for specific protein markers of chromosome arms.

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