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Chromosomal control of glutenin subunits in aneuploid lines of wheat: analysis by reversed-phase high-performance liquid chromatography *

T. Burnouf** and J. A. Bietz

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, IL 61604, USA

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Summary. Glutenin subunits from nullisomic-tetrasomic and ditelocentric lines of the hexaploid wheat variety 'Chinese Spring' (CS) and from substitution lines of the durum wheat variety 'Langdon' were fractionated by reversed-phase high-performance liquid chromatography (RP-HPLC) at 70 °C using a gradient of acetonitrile in the presence of 0.1% trifluoroacetic acid. Nineteen subunits were detected in CS. The presence and amounts of four early-eluted subunits were found, through aneuploid analysis, to be controlled by the long arms of chromosomes 1D (1DL) (peaks 1–2) and 1B (1BL) (peaks 3–4). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that these four subunits are the high molecular weight subunits of glutenin, which elute in the order 1Dy, 1Dx, 1By, and 1Bx. Similar amounts of 1DL subunits were present (6.3 and 8.8% of total glutenin), but 1BL subunits differed more in abundance (5.4 and 9.5%, respectively). Results indicate that most late-eluting CS glutenin subunits were coded by structural genes on the short arms of homoeologous group 1 chromosomes: 6 by 1DS, 5 by 1AS, and 4 by 1BS. Glutenin of tetraploid 'Langdon' durum wheat separated into nine major subunits: 6 were coded by genes on 1B chromosomes, and 3 on 1A chromosomes. Gene locations for glutenin subunits in the tetraploid durum varieties 'Edmore' and 'Kharkovskaya-5' are also given. These results should make RP-HPLC a powerful tool for qualitative and quantitative genetic studies of wheat glutenin.

Key words: HMW glutenin subunits – LMW glutenin subunits – *Triticum* – RP-HPLC

Introduction

Glutenin is a high-molecular-weight (HMW) heterogeneous protein aggregate of wheat gluten which is insoluble in water, saline solution, or neutral 70% ethanol. It is formed by interchain disulfide crosslinking of numerous subunits, having at least 15 different apparent MW (Bietz and Wall 1972; see also Wall 1979; Miflin et al. 1983 for reviews). Wheat glutenin subunits play a critical role in determining the technological properties of flour and semolina (Burnouf and Bouriquet 1980; Payne et al. 1981a; Moonen et al. 1982; Payne et al. 1984b). Because of the importance of glutenin, its genetics has been studied extensively in recent years (see Payne et al. 1984a for review).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) permits good separation of subunits according to MW (Bietz and Wall 1972; Payne et al. 1981b; Lawrence and Shepherd 1980); two-dimensional electrophoretic procedures involving separations by charge are, however, necessary to better resolve low MW (LMW) polypeptides (Jackson et al. 1983). Electrophoresis of glutenins from aneuploid lines of the hexaploid variety 'Chinese Spring' has revealed that the long arms of the homoeologous group 1 chromosomes 1D and 1B (Bietz et al. 1975) [and in some varieties 1A (Lawrence and Shepherd 1980)] code HMW subunits, while the short arms of chromosomes 1A, 1B, and 1D bear loci responsible for synthesis of LMW subunits (Jackson et al. 1983; Payne et al. 1984a for a review). It remains possible that expression of genes for gluten polypeptides is affected by other genes (e.g., regulator or suppressor) which, though located at a distance from structural genes, can still affect protein synthesis. This pos-

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** Postdoctoral Research Associate, Department of Agronomy, University of Nebraska, Lincoln (USA). Stationed at the Northern Regional Research Center, Peoria. Present address: Laboratoire Recherche et Développement, CRTS, 19–21 rue Camille-Guérin, F-59012 Lille Cedex, France

sibility, however, seems fairly remote in terms of extensive previous studies of chromosomal location and expression of storage protein genes in wheat (see Payne et al. 1984a for review); we will, therefore, subsequently refer to structural genes for specific polypeptides as being located at those loci indicated by aneuploid analysis.

Recently, reversed-phase high-performance liquid chromatography (RP-HPLC) has been adapted to separation of cereal proteins (Bietz 1983), including glutenin subunits (Burnouf and Bietz 1984b). RP-HPLC fractionates hexaploid wheat glutenin into at least 15–20 components, matching the resolving power of one-dimensional electrophoresis. RP-HPLC separates glutenin subunits, as well as other proteins, primarily on the basis of surface hydrophobicity (Burnouf and Bietz 1984b) and not by charge or MW as in most classical electrophoretic or chromatographic methods. Thus, RP-HPLC furnishes new biochemical information. RP-HPLC is fast, easy to use, reproducible, readily automated, and the results easily quantified. RP-HPLC is a convenient tool for wheat genetic (Burnouf and Bietz 1984c) and breeding studies. For example, use of this technique can allow screening for traits associated with specific glutenin subunits when many samples must be analyzed, as in early generations of bread and durum wheat breeding (Burnouf and Bietz 1984a).

In a companion manuscript (Bietz and Burnouf 1985), we used RP-HPLC to ascertain the chromosomal control of gliadin proteins in hexaploid and tetraploid wheats. We here now report the use of RP-HPLC to determine the coding of glutenin subunits from bread and durum wheats. Results confirmed chromosomes 1A, 1B, and 1D as bearers of genes controlling glutenin subunit biosynthesis, and indicated the arm locations of genes coding the glutenin subunits as they resolve by RP-HPLC. This study also demonstrates that amounts of HMW and LMW glutenin subunits can readily be quantitated by RP-HPLC.

Materials and methods

Plant material

Compensating nulli(N)somic-tetra(T)somic and ditelocentric (DT) lines of the hexaploid wheat variety 'Chinese Spring' (CS) were kindly provided by E. R. Sears (University of Missouri, Columbia, MO, USA). Tetraploid 'Langdon' durum D-genome substitution lines, each having a different CS D-genome chromosome pair substituted for a homoeologous A- or B-genome chromosome pair, and the tetraploid substitution lines 'Langdon' 1B ('Edmore' 1B) and 'Langdon' 1B ('Karskovskaya-5' 1B) were a gift from L. R. Joppa (USDA, ARS, Fargo, ND, USA).

Glutenin extraction

Glutenin was prepared from ground single wheat kernels (Bietz et al. 1975). Briefly, undefatted meal (ca. 25 mg) was successively extracted with 5 ml 0.04 M NaCl (twice) and 5 ml 70% ethanol (twice) to remove albumins, globulins, and gliadins. Pellets were suspended in 2 ml 0.7% acetic acid; 5.6 ml 95% ethanol was added and pH was adjusted to 6.8–7.5 to precipitate glutenin (14 h; –20 °C). Glutenin extracts were centrifuged (24,000 g; 10 min), freeze-dried, and stored at room temperature until analyzed. A larger sample of 'Chinese Spring' glutenin was prepared from 120 kernels, using the same procedure, for preparative RP-HPLC.

Preparation of glutenin for RP-HPLC

Glutenin was prepared for analytical RP-HPLC as described previously (Burnouf and Bietz 1984b). Pellets from single kernels were suspended in 750 µl of 0.05 M Tris(hydroxymethyl)aminomethane (Bio Rad)-HCl buffer, containing 8 M urea (ultrapure Serva). Disulfide groups were reduced with 5% 2-mercaptoethanol (Eastman Kodak) for 2 h at room temperature and alkylated with 60 µl freshly distilled 4-vinylpyridine (Pierce) for 2 h at room temperature to yield S-pyridylethyl (PE)-derivatives. Glacial acetic acid was added to terminate each alkylation reaction. Extracts were centrifuged (35,000 g; 15 min) and 40 to 60 µl of clear supernatants were analyzed by RP-HPLC.

For preparative RP-HPLC (Huebner and Bietz 1984), glutenin was prepared by a modified extraction procedure in which all non-glutenin proteins are first extracted with 95% DMSO and with 70% ethanol, and in which reduction is performed with dithiothreitol (Burnouf and Bietz, in preparation). Samples of 2.0 ml of the final clear supernatant were analyzed by preparative RP-HPLC, resulting in resolution similar to that obtained on analytical RP-HPLC columns.

RP-HPLC analysis

The Waters HPLC system used was described previously (Bietz 1983). A 250×4.1 mm Brownlee Aquapore RP-300 column (number 4416; C8; 300 Å pore size; 10 µm particle size, end-capped) was used for all analytical separations. Chromatography was carried out using as solvents 0.1% trifluoroacetic acid (TFA) (Pierce) in deionized, distilled water (solvent A) and 0.1% TFA in acetonitrile (ACN) (MCB, omnisolv grade) (solvent B). Proteins were eluted at 1 ml/min, with a linear 55 min gradient (21 to 48% ACN) and monitored at 210 nm using 0.2 absorbance unit full scale (AUFS). The column was maintained at 70 °C in an aluminum block connected to a constant-temperature water-bath. Preparative RP-HPLC of 'Chinese Spring' PE-glutenin was carried out on a 250×10 mm SynChropak RP-P (C_{18}) column at 70° using the above gradient with a flow rate of 3.0 ml/min; the column was monitored at 280 nm using 0.2 AUFS. Results were processed by a Modcomp computer for quantitation, replotting, and comparison. The solvent peak, eluting ca. 2 min after sample injection, was not plotted. PE-glutenin peaks were designated by number (for CS) or letter (for durum), respectively, on the basis of elution time. In the future, however, when correspondence between RP-HPLC and SDS-PAGE separations is better established, and when aneuploid lines involving other cultivars are examined, glutenin peak designation by the proposed nomenclature based on coding alleles (Payne and Lawrence 1983) will be preferable. Elution times of peaks were highly reproducible: for example, elution times were 19.49 ± 0.05 (SD; N = 14) for peak 1, 35.82±0.02 (N = 13) for peak 10, and 66.32 ± 0.04 (N = 15) for peak 18.

Electrophoresis

Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of PE-glutenin subunits was carried out on 10% gels as described by Laemmli (1970).

Results

Figure 1 shows the RP-HPLC pattern of PE-glutenin from CS. At least 19 major peaks or shoulders were separated. [An early peak (eluted by ca. 29.5% ACN)

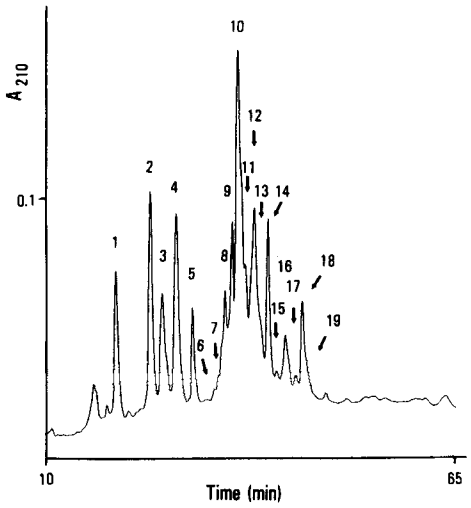

Fig. 1. RP-HPLC separation of PE-glutenin from 'Chinese Spring'. Glutenin were extracted (Section 2.2), suspended in 0.05 M Tris-8 M urea HCl buffer, pH 7.5, reduced with 2-mercaptoethanol (2 h, room temperature), and alkylated with 4-vinyl pyridine (2 h, room temperature). Proteins were eluted with a linear gradient from 21 to 48% ACN, in the presence of 0.1% TFA, during 55 min at 1 ml/min. Absorbance of the column effluent was monitored at 210 nm. The solvent peak, typically eluted at 2.0 min, is not plotted. Chromosomal coding of peaks 1–19 is given in Table 1

and additional minor components were not numbered since the location of their coding gene(s) could not be determined.] Glutenin subunits were eluted under widely different ACN concentrations (ca. 30–45%), indicating large variation in surface hydrophobicity. Early subunits, eluted from the column by 30–35% ACN thus have lower surface hydrophobicities than late-eluting subunits (35–45% acetonitrile).

Chromosomal locations of genes coding these RP-HPLC glutenin subunits peaks were determined by analysis of aneuploid CS NT and DT lines.

Nullisomic-tetrasomic lines of 'Chinese Spring'

Figure 2 shows RP-HPLC elution profiles of PE-glutenin from the 6 CS compensating nullisomic-tetrasomic lines involving homoeologous group 1 chromosomes [(A) N1DT1A; (B) N1DT1B; (C) N1BT1A; (D) N1BT1D; (E) N1AT1B; and (F) N1AT1D]. For the compensating nullisomic-tetrasomic lines, nullisomy is present for the first indicated chromosome, and tetrasomy for the second; thus, e.g., N1DT1A indicates zero 1D, four 1A, and two of all other CS chromosomes. Differences between patterns are obvious. For instance, peaks 1, 2, 5, 7, 10, 12, 14, and 18 are either absent or reduced in nullisomic lines of chromosomes 1D (Fig. 2A, B) but are increased in tetrasomic 1D lines (Fig. 2D, F). Thus, these peaks contain glutenin subunits whose synthesis is regulated (coded) by genes on chromosomes 1D. Similarly, peaks 3, 4, 6, 10, 11, and 16 contain subunits coded by genes on chromosomes 1B, and peaks 9, 10, 13, 17, and 19 contain subunits coded by genes on chromosomes 1A. Peak 10 was heterogeneous as evidenced by the shoulder in Fig. 2F, and contained subunits coded by genes on chromosomes 1D, 1B, and 1A. Elution profiles of PE-glutenin from NT lines of homoeologous group 6 chromosomes did not differ significantly from that of CS PE-glutenin, except that nullisomic 6D lines had a much smaller peak 8 (results not shown); thus most components of peak 8 are coded by genes on chromosomes 6D. Analysis of NT lines of homoeologous group 2, 3, 4, 5, and 7 chromosomes revealed no differences from CS, indicating that they have no glutenin controlling genes.

'Chinese Spring' DT lines

DT lines, which lack specific chromosome arms, permit a finer location of genes controlling glutenin subunits. The nomenclature for DT lines specifies those arms which are present; thus, e.g., DT 1BS has both short arms of the 1B chromosomes, but lacks the long 1B chromosome arms. Results for most representative lines are shown in Fig. 3. Comparison of these chromatograms to euploid CS (Fig. 1) indicates that the long arms of chromosomes 1D are responsible for coding subunits of peaks 1 and 2, which were absent in nullisomic 1D lines (Fig. 2A, B) but present in DT 1DL (Fig. 3A), which lacks the short arms of chromosomes 1D. Similarly, the short arms of chromosomes 1D code for all other 1D-coded subunits (as determined by analysis of NT lines), namely subunits in peaks 5, 7, 10, 12, 14, and 18, since these peaks are absent or decreased in size (for peak 10) in DT 1DL (Fig. 3A). Peaks 3 and 4 contain subunits coded by genes on the long arms of chromosomes 1B, since they are absent in DT 1BS (Fig. 3C) which lacks the long 1B chromosomal arms, but present in DT 1BL (Fig. 3B). Similarly, subunits in peaks 10, 11, and 16 were found to be coded by 1BS (present in DT 1BL, Fig. 3B), and subunits in peaks 9, 10, 13, 17, and 19 by 1AS (not detected in DT 1AL, Fig. 3D). Subunits in peak 8 are controlled by genes on chromosome 6DS (results not shown).

Table 1 summarizes locations of genes controlling CS glutenin subunits, as they resolve by RP-HPLC (Fig. 1), as well as ACN concentrations at which subunits elute.

SDS-PAGE of RP-HPLC glutenin subunit peaks

Peaks 1–4 contained subunits coded by genes of chromosome arms 1DL and 1BL (Table 1); these chromosome arms are known to contain those genes coding for

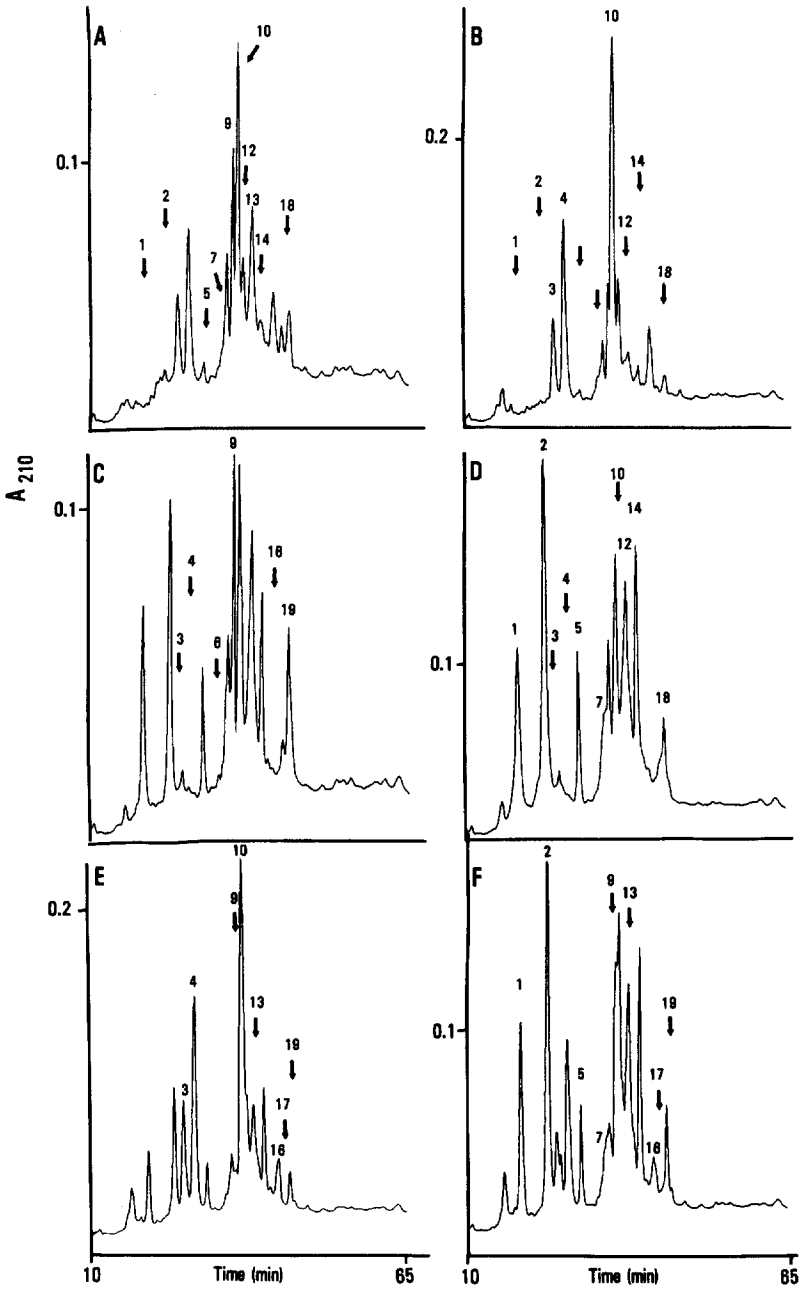

Fig. 2. RP-HPLC separation of PE-glutenin from homeologous group 1 chromosome nullitetrasomic lines N1DT1A (**A**); N1DT1B (**B**); N1BT1A (**C**); N1BT1D (**D**); N1AT1B (**E**); and N1AT1D (**F**). Experimental conditions are as in Fig. 1

HMW glutenin subunits (Bietz et al. 1975). Peaks 1–4 were therefore isolated by preparative RP-HPLC (results, nearly equivalent in resolution to those in Fig. 1, are not shown) and examined by SDS-PAGE to determine the type and MW of glutenin subunits which they contain.

Results for individual peaks (Fig. 4), compared to SDS-PAGE patterns of total protein (TP) extracts, reveal that major subunits in peaks 1–4 indeed correspond to the four HMW subunits of 'Chinese Spring'. Peak 1 contains the faster 1DL-coded subunit (subunit 12 or 1Dy according to nomenclature of Payne et al. 1981b), peak 2 contains the slower 1DL component (subunit 2 or 1Dx), peak 3 (which partially overlapped peak 2 in the preparative separation) contained primarily the faster 1BL subunit (subunit 8 or 1By), and peak 4 contained the slower 1BL subunit (subunit 7 or 1Bx). Order of elution of HMW subunits, based on increasing surface hydrophobicity, was thus 1Dy, 1Dx, 1By, and 1Bx. Traces of other subunits were observed in all fractions, suggesting either some association with major components during preparative RP-HPLC, or the presence of minor polypeptides differing in MW but of equivalent surface hydrophobicities.

Table 1. Chromosomal location of genes coding glutenin subunits in 'Chinese Spring', 'Langdon', 'Kharkovskaya-5', and 'Edmore' as they separate by RP-HPLC

	'Chinese Spring'																		
Peak	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
ACN %	30.56	32.80	33.59	34.49	35.56	36.93	37.25	37.71	38.18	38.58	39.08	39.63	40.07	40.53	41.08	41.65	42.28	42.76	43.00
Coding	1DL	1DL	1BL	1BL	1DS	1BS	1DS	6DS	1AS	1AS 1BS 1DS	1BS	1DS	1AS	1DS	?	1BS	1AS	1DS	1AS
	Durum[a]																		
Peak	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	
ACN %	32.55	33.45	34.37	34.42	34.95	35.19	35.53	37.09	37.39	37.50	37.93	38.20	38.62	38.68	38.96	40.34	41.66	42.26	
'Langdon' coding	1B (L)	–	1B (L)	–	–	–	–	1A (S)	–	1A (S)	1A (S)	–	–	1B (S)	1B (S)	–	1B (S)	1B (S)	
'Edmore' coding	1B (L)	–	1B (L)	–	–	–	–	?	–	?	–	1B (S)	1B (S)	–	–	–	1B (S)	–	
'Kharkovskaya-5' coding	–	1B (L)	–	1B (L)	?	?	?	–	?	–	–	1B (S)	1B (S)	–	–	1B (S)	1B (S)	–	

[a] Chromosome arm designations in parentheses are inferred from CS results since only coding chromosomes can be positively identified from these durum whole chromosome substitution lines

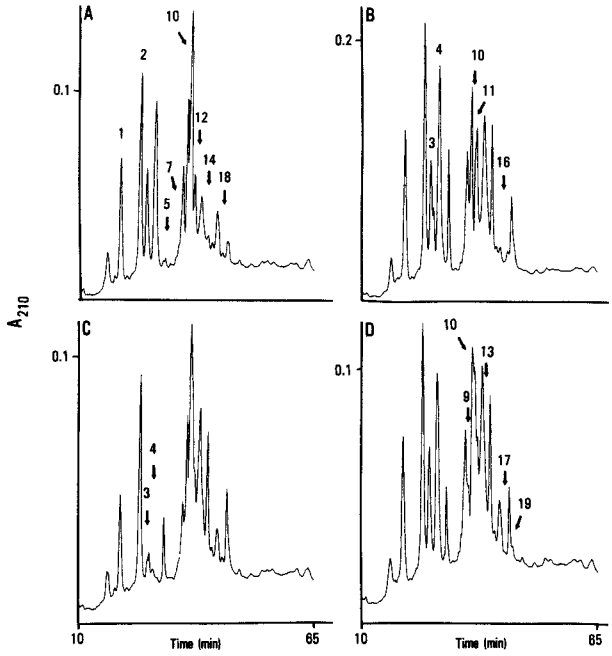

Fig. 3. RP-HPLC separation of PE-glutenin from the ditelocentric lines of CS: 1DL (**A**); 1BL (**B**); 1BS (**C**); and 1AL (**D**). Experimental conditions are as in Fig. 1

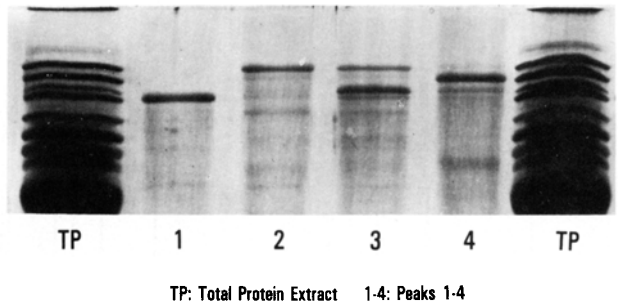

Fig. 4. SDS-PAGE of HMW PE-glutenin subunits in total protein (TP) extracts of 'Chinese Spring' and in peaks 1–4 (see Fig. 1) separated by preparative RP-HPLC. Origin is at top of figure, and subunits of lowest mobilities have highest apparent MW

Quantitation of RP-HPLC peaks

Computer integration of RP-HPLC patterns permits quantitation of eluted proteins based on absorbance of peptide bonds at 210 nm; such quantitation is more accurate than that based on densitometry of stained electrophoresis gels, where amount of dye which complexes to proteins varies with differences in amino acid composition.

The ratio of HMW glutenin subunits (peaks 1–4) to total glutenin was found to be 30% in CS. However, it varied from as low as 17.7% (for glutenin extracted

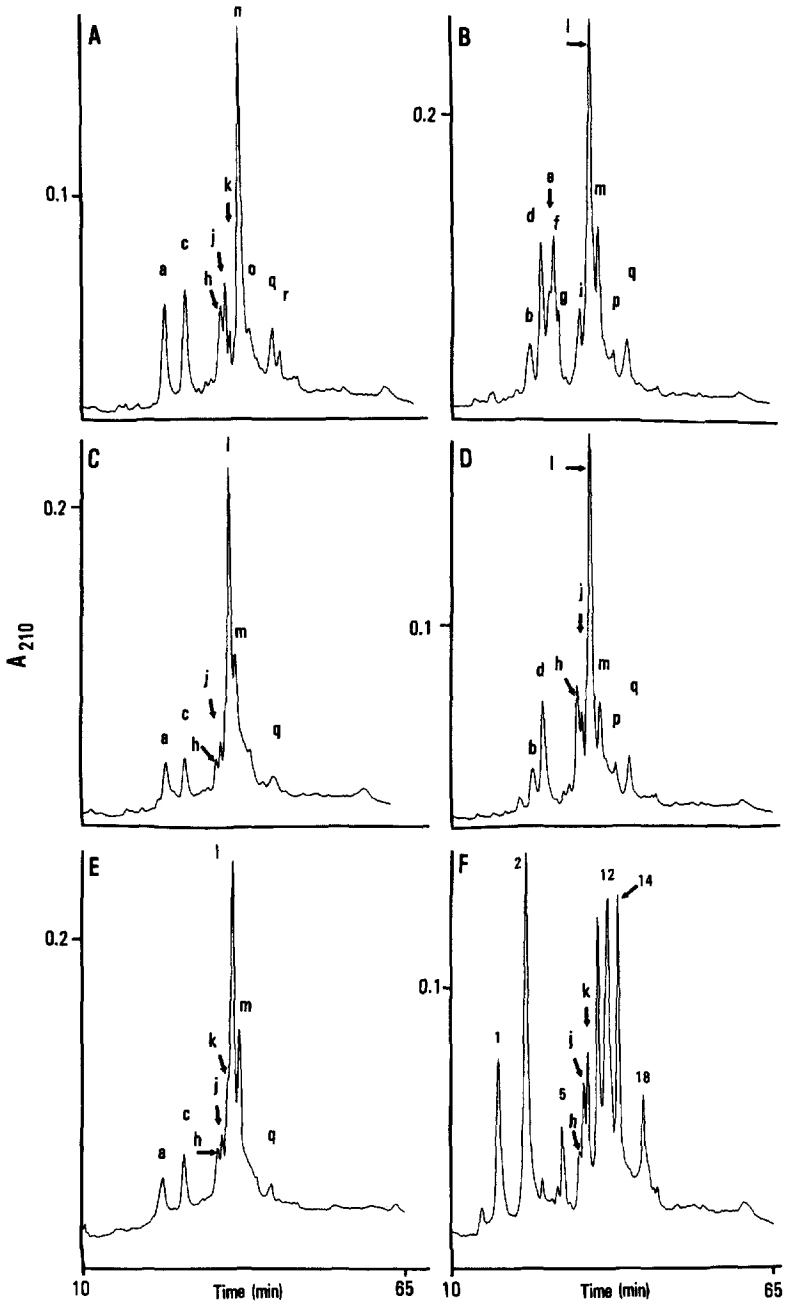

Fig. 5. RP-HPLC separation of PE-glutenin from durum wheat (*Triticum turgidum*) varieties 'Langdon' (**A**); 'Kharkovskaya-5' (**B**); and 'Edmore' (**C**) and from the substitution lines 'Langdon 1B' ('Kharkovskaya-5' 1B) (**D**); 'Langdon' 1B ('Edmore' 1B) (**E**); and Langdon 1B ('Chinese Spring' 1D) (**F**). Chromosomal coding of peaks *a–r* is given in Table 1. Experimental conditions are as in Fig. 1

from DT 1BS), 18.5% (N1DT1A), or 19.5% (N1BT1A) to as much as 32.9% (N1AT1B), 35.0% (DT 1BL), 35.2% (N1AT1D), or 35.5% (DT 1DL). Such variation reflects the contributions of missing or duplicated chromosomal arms in coding HMW or LMW glutenin subunits, but may also result from differences in promoter regions that affect the frequency with which polymerases transcribe the gene DNA, or from other possibilities. The relative importance of these possibilities in determining the different amounts of protein expressed is not known at present.

CS chromatographic peaks 1–4 (Fig. 1) were found to represent 6.3, 8.8, 5.4, and 9.5%, respectively, of total glutenin. Thus, relative abundance of 1D and 1B subunits are: peak 4 (1B) > 2 (1D) > 1 (1D) > 3 (1B). So, large difference in amounts of 1B peaks 3 and 4 were found (peak 3 represents only 58% of peak 4) while similar amounts of 1D subunits (peaks 1 and 2) are synthesized. This may indicate quite different copy numbers of genes on 1BL coding subunits in peaks 3 and 4, while copy numbers of genes on 1DL coding subunits in peaks 1 and 2 are similar. Interestingly, the

ratio of 1DL and 1BL subunits to total glutenin was 15.1 and 14.9%, respectively; these similar amounts suggest an equivalent number of gene copies on 1DL and 1BL coding for HMW glutenin subunits. Alternatively, if very different numbers of structural genes are present, their regulatory expression may differ significantly.

Aneuploid lines of durum 'Langdon'

Figure 5 shows RP-HPLC patterns of PE-glutenin from durum varieties 'Langdon' (A), 'Kharkovskaya-5' (B), and 'Edmore' (C) and from substitution lines 'Langdon' 1B ('Kharkoskaya-5' 1B) (D), 'Langdon' 1B ('Edmore' 1B) (E), and 'Langdon' 1B (CS 1D) (F). Durum glutenins are less complex than those of hexaploid varieties because of the absence of the D-genome: thus, durum wheats posess 28 chromosomes, whereas 42 are present in hexaploid wheats. Eight to 12 subunits were detected in each durum variety (Fig. 5). Only peak *q* was detected in all three varieties. Peaks *a, c, h,* and *j* were present in 'Langdon' and 'Edmore', peaks *b, d, e, f, g, i,* and *p* were present only in 'Kharkovskaya-5', peaks *l* and *m* were only in 'Kharkovskaya-5' and 'Edmore', and peaks *k, n, o,* and *r* were only in 'Langdon'.

Analysis of the substitution line between chromosomes 1B of 'Langdon' and 'Kharkovskaya-5' (Fig. 5 D) shows that peaks *a, c, n, o* and *r* (present in 'Langdon' but absent in the substitution line) are coded by genes on 'Langdon' chromosomes 1B. Similarly, peaks *b, d, l, m,* and *p* (present in 'Kharkovskaya-5' and in the 1B substituion line but absent in 'Langdon') are coded by 'Kharkovskaya-5' chromosomes 1B.

Analysis of the 'Langdon' 1B ('Edmore' 1B) substitution line (Fig. 5 E) confirms that 'Langdon' chromosomes 1B genes code glutenin peaks *n, o,* and *r*, and indicates that 'Edmore' 1B genes code glutenin peaks *l* and *m*. Through analogy with 'Langdon' glutenin (Fig. 5 A), it is likely that 'Edmore' peaks *a* and *c* are also coded by genes on chromosomes 1B. Chromosomal location of genes coding 'Langdon' peaks *h, j,* and *k* could be determined by analysis of glutenin from 'Langdon' 1A ('Chinese Spring' 1D) (results not shown), which lacked these three peaks, indicating that they are coded by 'Langdon' 1A genes. Analysis of 'Langdon' 1B ('Chinese Spring' 1D) (Fig. 5 F) confirms that peaks *a, c, q,* and *r* are coded by chromosomes 1B; as expected, glutenin from this line contains subunit peaks 1, 2, 5, 10, 12, 14, and 18, coded by 'Chinese Spring' chromosomes 1D. Finally, since *q* is absent in 'Langdon' 1B ('CS' 1D) but present in 'Langdon' 1B ('Kharkovskaya-5' 1B) and 'Langdon' 1B ('Edmore' 1B), its coding by the 1B chromosomes of 'Edmore' and 'Kharkovskaya-5' may also be inferred. Table 1 summarizes also information from the aneuploid analysis of 'Langdon' durum.

Discussion

RP-HPLC has now been used to determine chromosomal locations of genes controlling glutenin's HMW and LMW subunits. RP-HPLC separates hexaploid wheat glutenin into 16–20 subunits, and durum wheat glutenin into 8–12 subunits. Absence of the D-genome in durum wheats accounts for its decreased complexity (Orth and Bushuk 1973), as clearly evidenced in the substitution line 'Langdon' 1B (CS 1D), which possesses CS's 1D chromosomes and contains more subunits. Analyses of aneuploid lines from both hexaploid and tetraploid wheats further indicate that genes on chromosomes 1A, 1B, and 1D code most, if not all, glutenin subunits. A minor component controlled by 6DS of CS is most likely a gliadin or soluble protein entrapped in glutenin during extraction.

These chromosome assignments for glutenin subunits, as well as those obtained by RP-HPLC for gliadin (Bietz and Burnouf 1985), support extensive electrophoretic studies (see Payne et al. 1984a for a review) which have revealed that genes controlling synthesis of most major gluten proteins are located in nine complex loci on homoeologous group 1 and 6 chromosomes. Loci *Gli-A1, Gli-B1,* and *Gli-D1* are located distally on the short arms of chromosomes 1A, 1B, and 1D: they code all ω-gliadins, most γ-gliadins, a few β-gliadins, and the major LMW glutenin subunits. Recent data suggest the existence of an additional gluten protein subunit locus located 25.5 cM units proximally from the *Gli-B1* locus on the short arms of chromosomes 1B (Galili and Feldman 1984). Loci *Gli-A2, Gli-B2,* and *Gli-D2* are located on the short arms of chromosomes 6A, 6B, and 6D, presumably slightly closer to the centromere than are the *Gli-1* loci: they code all α-gliadins, most β-gliadins, and a few γ-gliadins. Loci *Glu-A1, Glu-B1,* and *Glu-D1* are on the long arms of chromosomes 1A, 1B, and 1D, approximately 9.0 cM from the centromere. They code all HMW glutenin subunits. Depending on variety, the *Glu-D1* locus codes for synthesis of 2 HMW glutenin subunits (1Dx and 1Dy), the *Glu-B1* locus for synthesis of 1 or 2 HMW subunits (1Bx and 1By), and the *Glu-A1* locus for synthesis of one (1Ax) or zero HMW glutenin subunits.

Glutenin subunits may be divided into two groups on the basis of RP-HPLC elution time and chromosomal arm coding. Most polypeptides in early RP-HPLC peaks in CS are determined by genes on the long arms of chromosomes 1B and 1D while synthesis of most late-eluting subunits is regulated by genes on the short arms of homoeologous group 1 chromosomes. Other studies (see Payne et al. 1984a) have shown that only genes which code HMW glutenin subunits are located on 1DL and 1BL in CS [no HMW glutenin subunits in CS are coded by genes on chromosomes 1A (Bietz et al. 1975)]. Two HMW subunits are coded by genes on chromosomes 1DL and two by genes on 1BL;

these subunits have been referred to as 1Dx, 1Dy, 1Bx, and 1By, respectively (Payne et al. 1984a). It therefore seemed likely that early-eluting RP-HPLC peaks 1–4 contain HMW glutenin subunits; this was confirmed by SDS-PAGE (Fig. 4).

Similarly, since genes for LMW glutenin subunits are located on the short arms of group 1 chromosomes (Jackson et al. 1983), we conclude that late-eluting RP-HPLC peaks, coded by short arms of group 1 chromosomes, contain LMW subunits. Although incompletely resolved, 15 LMW subunits could be distinguished. In CS, the 1DS chromosome arms control 5 major and 1 minor subunits, 1BS 2 major and 2 minor subunits, and 1AS 2 major and 3 minor subunits (Table 1); these results agree with those of Jackson et al. (1983; see Fig. 6) who detected 14 major LMW subunits by 2-D electrophoresis, 8 coded by 1DS, 5 by 1BS and 1 by 1AS. Slight discrepancies between results may be attributed to mode of glutenin extraction, which may not remove all gliadins, or to differences in resolution of the methods. In RP-HPLC, LMW subunits generally elute later than HMW subunits, under condition of greater hydrophobicity. At least one 1D-coded LMW subunit (in peak 5), however, elutes close to HMW subunits. Differences in surface hydrophobicity is not surprising, since structural and charge heterogeneity among these LMW subunits has already been revealed by isoelectric focusing (Jackson et al. 1983), and since surface hydrophobicity may not always correlate well with predicted over-all hydrophobicity (Popineau and Godon 1982).

Elution order of polypeptides in RP-HPLC generally, however, does reflect their surface hydrophobicity. For glutenin subunits, the present results generally agree with hydrophobicities predicted from amino acid compositions. HMW subunits which elute first are indeed richer in polar amino acids than are LMW subunits (Huebner et al. 1974; Bietz and Wall 1973) and contain fewer hydrophobic amino acids, such as phenylalanine, valine, leucine, isoleucine, and methionine.

The two 1DL-coded HMW glutenin subunits (peaks 1 and 2) elute earlier than their respective counterparts, the 1BL-coded HMW subunits (peaks 3 and 4). Thus, although these subunits are probably homoeoallelic (Payne et al. 1981b) and homologous (Shewry et al. 1984), they differ in surface hydrophobicity, as well as MW (Bietz et al. 1975), isoelectric points (Holt et al. 1983), and amino acid compositions (Shewry et al. 1984), emphasizing the unique structure of each HMW subunit. Such structural uniqueness may explain why some HMW subunits have different effects on the physical properties of flour (Payne et al. 1981a; Burnouf and Bouriquet 1980; Moonen et al. 1982).

In addition, the two 1D subunits (peaks 1 and 2) differ in surface hydrophobicity. Recently, amino acid compositions of HMW glutenin subunits, including the 1D subunits 2 and 12 (following the nomenclature of Payne and Lawrence 1983) present in CS, have been reported (Shewry et al. 1984). The 1Dx subunit 2 is generally more polar than the 1Dy subunit 12 (it contains less valine, isoleucine, and phenylalanine), which might indicate that glutenin subunit 2 elutes in RP-HPLC peak 1, while subunit 12 elutes in peak 2. SDS-PAGE results (Fig. 4), however, showed the opposite to be true: i.e., 1Dy subunit 12 elutes in peak 1, while 1Dx subunit 2 elutes in peak 2. Such apparently contradictory results may most likely be explained by the fact that there is not necessarily any correlation between overall hydrophobicity, as predicted from amino acid compositions, and surface hydrophobicity, as predicted by hydrophobic interaction chromatography of gliadin components (Popineau and Godon 1982).

Quantitation based on peptide bond absorbance at 210 nm should fairly accurately indicate protein content. Our results show differences in amounts of each HMW subunit. The 1BL subunit eluted in peak 4 is most abundant. The 1DL subunits (peaks 1 and 2) occur in similar amounts, with slightly more peak 1 than peak 2 subunit. Finally, the second 1BL subunit (peak 3) exists in much lower amount than either the 1DL subunits or the other 1BL subunit. Similar results were obtained by Payne et al. (1981b) and Galili and Feldman (1983) by quantitation of stained electrophoretic gels: 1By subunit 8 was produced in lower quantity than the other HMW subunits. According to Payne et al. (1981b) relative intensities of CS HMW subunits are 1Bx > 1Dx = 1Dy > 1By; our RP-HPLC results show the quantitative order to be: 1B-peak 4 > 1D-peak 1 > 1D-peak 2 > 1B-peak 3. If RP-HPLC and electrophoretic quantitation are equivalent, it is thus likely that CS subunit 1Bx elutes in RP-HPLC peak 4, and subunit 1By in peak 3. This prediction was confirmed by SDS-PAGE (Fig. 4), which also indicated that, based on RP-HPLC elution, the 1Dx and 1Bx HMW subunits have higher surface hydrophobicities than their respective y counterparts.

Differences in subunit abundance may indicate variation in the respective number of structural genes (Payne et al. 1981b; Burnouf et al. 1981), although number of gene copies for HMW subunits is probably low (Thompson et al. 1983). However, we noted that the total amount of 1DL subunits equals that of 1BL subunits, suggesting (if transcription rates are similar) that total gene copy numbers for HMW glutenin subunits on 1DL and 1BL are quite similar in CS, which may also be likely since these two chromosomes are homoeologous. Since the origin of the B genome is probably polyphyletic (Kimber and Athwal 1972), creating variation in the set of 1BL subunits synthesized (e.g. some varieties have only one 1BL subunit) equivalence in amounts of 1DL and 1BL subunits might not be found in all varieties.

The present RP-HPLC analyses were done using a column maintained at 70 °C. Compared to analyses performed using the same column at room temperature (Burnouf and Bietz 1984b), glutenin subunits eluted sooner and peak shape and resolution are improved. For instance, peak 5 could be resolved at 70 °C, but not at room temperature. In general, a dramatic improvement in resolution of cereal proteins by RP-HPLC takes place at high temperature (Bietz and Cobb 1984). For other proteins, effects of temperature in RP-HPLC resolution differ (Cohen et al. 1984). For cereal proteins, beneficial effects of increased temperature on RP-HPLC resolution may result from modification of non-covalent interactions between polypeptides (Bietz and Cobb 1985). Cole et al. (1983) noted disaggregation of A-gliadin at increased temperature, indicating that hydrogen bonding may be the most significant contributor to gluten protein aggregation (although ionic and hydrophobic bonding may also occur).

In conclusion, RP-HPLC of aneuploid lines has confirmed that glutenin subunit biosynthesis in hexaploid and tetraploid wheats is determined by genes residing on homeologous group 1 chromosomes. RP-HPLC can satisfactorily and simultaneously separate both HMW and LMW glutenin subunits. Separations are rapid, and base-line resolution of HMW subunits can be achieved, permitting accurate quantitation. At present RP-HPLC technology does not resolve LMW subunits as well. The combined sensitivity, speed, and resolution of RP-HPLC should, however, make this technique the method of choice for many genetic analyses of glutenin requiring rapid screening of large numbers of samples for specific components. RP-HPLC should be particularly useful for determining technological quality of flour or semolina by screening for specific HMW or LMW subunits linked to breadmaking (Payne et al. 1981a; Burnouf and Bouriquet 1980; Moonen et al. 1982) or pastamaking (Payne et al. 1984b) qualities.

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