Wheat Low Molecular Weight Glutenin Subunits—Structural Relationship to Other Gluten Proteins Analyzed Using Specific Antibodies[1]

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ABSTRACT

The immunological homologies of low molecular weight glutenin subunits (LMW-GS) were compared with the other major wheat gluten polypeptides, high molecular weight glutenin subunits (HMW-GS) and gliadins. Conventional one-dimensional polyacrylamide gel electrophoretic (PAGE) methods were used as well as a two-step, one-dimensional sodium dodecyl sulfate-PAGE technique, together with immunoblotting and enzyme-immunoassay methods. Many antibodies raised to gliadins and HMW-GS bound well to LMW-GS. Antibodies with specificities for similar groups of gliadins bound to similar groups of glutenins; some antibodies bound to each of the major gliadins, LMW-GS, and HMW-GS but not to other grain proteins, suggesting the existence of "common gluten" amino acid sequences or conformations. The solubility and immunochemical similarities as well as the known linkage between the genes for LMW-GS and certain gliadins mean that LMW-GS may be responsible for many biochemical properties and quality effects usually attributed to gliadins.

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The glutenin complex, defined as the disulfide-linked gluten protein fraction, is considered to be the major fraction responsible for dough strength and loaf volumes of leavened (pan) breads. Much effort has been made in characterizing the high molecular weight glutenin subunits (HMW-GS) by genetic, molecular biological, and protein chemical means (Kreis et al 1985). The monomeric gliadins have been similarly studied, and respective differences in bread- and pasta-making qualities of hexaploid and durum wheats of different varieties were related to gliadin and HMW-GS levels and composition (Damidaux et al 1978, Payne et al 1981, Wrigley et al 1982, Branlard and Dardevet 1985, Lawrence et al 1988). However, despite low molecular weight (LMW)-GS being the major portion of glutenin, they have received less attention for two main reasons. On one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), many LMW-GS overlap with gliadins, and until recently, solubility fractionation methods for wheat flour proteins did not yield these subunits free of appreciable contamination with other gluten proteins. Improved solubility fractionation methods have made preparation of purer LMW-GS possible (Graveland et al 1982), and the development of a two-step one-dimensional SDS-PAGE method (Singh and Shepherd 1988) has enabled the study of the genetic control and quality association of these subunits in increasing detail.

Studies to date suggest that the LMW-GS share similarities with both gliadins and HMW-GS. Certain glutenin subunits have been noted to be soluble in aqueous ethanol (Bietz and Wall 1973), whereas size-exclusion chromatographic experiments have identified high molecular weight, aggregating gliadins (Bietz and Wall 1980). Genes for LMW-GS on the short arms of homoeologous group 1 chromosomes have been found to be closely linked to the group 1 gliadin genes (Payne et al 1984, Singh and Shepherd 1988); limited DNA sequencing studies also indicate homology

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between LMW-GS and certain gliadins (Okita et al 1985, Bartels et al 1986).

More recently, Gupta and Shepherd (1987, 1988) analyzed the relationship between LMW-GS composition and dough quality. Biotypes with differing LMW-GS varied appreciably in dough extensibilities (Gupta and Shepherd 1988), whereas analyses of F_5 progeny selected for presence or absence of specific chromosome 1A-encoded glutenin subunits indicated that both HMW-GS and LMW-GS made a similar contribution to dough resistance (Gupta and Shepherd 1987, Payne et al 1987, Gupta et al 1989). Furthermore, relationships between the amount of alcohol-soluble proteins (attributed to gliadins) or chromosome-1 gliadin composition and end-use quality (Wrigley et al 1982, Huebner and Bietz 1986) may actually arise from effects of LMW-GS.

An objective of our laboratory is to use a large library of monoclonal antibodies to gluten proteins to characterize homologies between endosperm proteins from various cereal grains (Skerritt 1988). Furthermore, on the basis of such information, we aim to use certain antibodies in development of simple screening tests to predict dough characteristics in breeders' samples or in grain at delivery to elevators (Skerritt et al 1987). In view of other evidence for relationships between gliadins and LMW-GS and the likely quality effects of these subunits, we have investigated immunological homologies between LMW-GS and other gluten proteins using ELISA (enzyme-linked immunosorbent assay) and immunoblotting methods.

MATERIALS AND METHODS

Grain Samples

Chinese Spring wheat and derivatives that were nullisomic for particular chromosome pairs and tetrasomic for homoeologous chromosomes were obtained from K. W. Shepherd (Waite Institute, Adelaide, South Australia). A triple wheat/rye chromosomal translocation containing the short arm of chromosome 1 of rye (1RS) in place of each of the short arms of chromosomes 1A, 1B, and 1D of wheat was isolated by R. B. Gupta (Waite Institute) by a cross of a Chinese Spring-Hope 1AL-1RS (single translocation) and a double translocation Gabo-Chinese Spring 1BL/1RS-1DL/1RS; 1RS in each case was from Imperial rye (Gupta and Shepherd 1988).

Isolation of High- and Low Molecular Weight Glutenin Subunits

"Gel" protein was prepared from Timgalen flour, and HMW-GS and LMW-GS fractions were prepared using the method of Graveland et al (1985). The fractions were confirmed by SDS-PAGE to have appropriate subunit compositions. Residual SDS was removed by ion-pair extraction with triethylammonium acetate (Henderson et al 1979); SDS was not detectable by the method of Waite and Wang (1976).

Electrophoresis and Immunoblotting

Gradient-gel electrophoresis of 1 *M* urea-extracted gliadins was performed using 5–12% polyacrylamide gradient gels run under acidic buffer conditions (Na lactate, pH 3.1) and immunoblotted as described earlier (Skerritt and Underwood 1986). One-dimensional SDS-PAGE was performed using homogeneous (15% total acrylamides [T], 2% cross-linker [C]) polyacrylamide gels and a discontinuous Tris-glycine-HCl buffer system as described in Skerritt and Underwood (1986). These gels were electroblotted for 3 hr at 36 V to 0.45 μm nitrocellulose (Schleicher and Schull, Dassel, West Germany) using 20 m*M* Tris, 150 m*M* glycine, 0.1% SDS, and 20% methanol in a Gradipore (Pyrmont, NSW, Australia) electroblotting apparatus.

A modification of the two-step SDS-PAGE procedure of Singh and Shepherd (1988) was used to analyze disulfide-bonded proteins. Using the same buffers as for single-dimension SDS-PAGE, gels for two-step SDS-PAGE were run as follows. The initial gel was 10% T, 1% C (stacking gel, 3% T, 2.6% C). Wholemeal samples were extracted in 10 vol sample buffer (25 m*M* Tris-HCl, pH 7.5 containing 4% SDS) for 4 hr at 60°C; in certain experiments this extraction solution was modified. Either 400 μl of clarified extract was loaded across the top of a 19-cm wide × 14-cm high slab, or 20 μl per lane was used. This first dimension gel was run for 175 Vhr until the bromophenol blue front had migrated 6 cm. The cathodic 1-cm strip, which

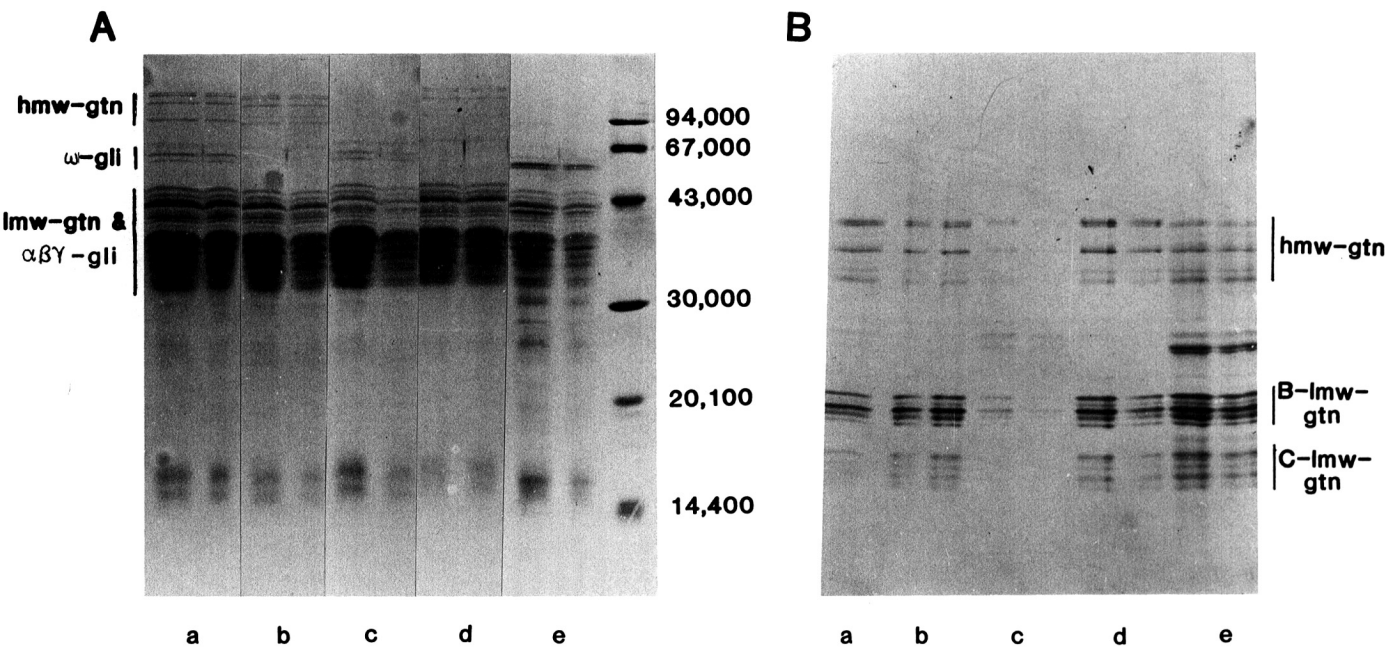

Fig. 1. Analysis of aqueous ethanol extracts (following salt extraction) and urea extracts of defatted (DF) and nondefatted Timgalen flour (NDF) by (**A**) standard and (**B**) two-step, one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. From left: (**a**) NDF 40% ethanol extract, (**b**) NDF 70% ethanol extract, (**c**) DF 40% ethanol extract, (**d**) DF 70% ethanol extract, and (**e**) NDF 1 *M* urea extract. A high and low loading corresponding to (A) 300 and 150 μg and (B) 400 and 200 μg lyophilized extracts, respectively, which were 60–85% protein, is shown for each extract. Molecular weight markers are shown for gel A.

would contain large protein polymers, was cut from the gel and equilibrated 2 hr at 60°C with sample buffer containing 2% (v/v) 2-mercaptoethanol (buffer was changed at 1 hr). This strip, bearing reduced proteins, was loaded onto a thicker (1.5-mm thick) gel slab (6% T, 1.9% C stacking gel on a 7.5–15% T separating gel), and electrophoresed for 1,000 Vhr. Proteins on a representative slab were stained using 0.2% Coomassie Blue R-250 in 40% methanol/10% acetic acid water solvent and destained in the same solvent. The proteins remaining on the slab were transferred to nitrocellulose membranes using the SDS-methanol-Tris-glycine buffer used with homogeneous gels, except that a 15-min equilibration in 0.1% SDS-containing transfer buffer preceded the transfer to 0.1-μm nitrocellulose. A 6-hr blotting time was used with 0.01% SDS in the buffer.

Probing of Immunoblots

Nonspecific antibody binding sites were blocked by 16-hr incubation at 37°C with 3% (w/v) bovine serum albumin (BSA) in 50 m*M* sodium phosphate/150 m*M* NaCl, pH 7.2 (PBS). Tissue culture supernatants or purified monoclonal antibodies were diluted in 3% BSA in PBS and incubated with 7-mm-wide strips cut from the immunoblots for 90 min at 20°C, with gentle rocking. Antibodies yielding a high background on nitrocellulose were diluted in 5% nonfat dried milk powder in PBS containing 1*M* NaCl. After washing with PBS/0.05% Tween 20, strips were incubated with alkaline phosphatase-labeled sheep-anti-mouse immunoglobulins (Promega, Madison, WI) in 2% BSA in Tris-buffered saline-Tween (TBST, 10 m*M* Tris-HCl, pH 8.0, containing 150 m*M* NaCl and 0.05% Tween 20) for 60 min at 20°C. After four 5-min washes in TBST, substrate (330 μg/ml Nitroblue tetrazolium [NBT, Bio-Rad, Richmond, CA] and 165 μg/ml 5-bromo-1-chloro-3-indolyl phosphate [BCIP Boehringer-Mannheim, FRG] in 100 m*M* Tris-HCL, pH 9.5, containing 100 m*M* NaCl/5 m*M* $MgCl_2$) was added. Binding of the antibodies was revealed by the appearance of purple zones within 5–60 min.

Monoclonal and Polyclonal Antibodies

Monoclonal antibodies used were prepared from mice immunized with various gluten protein fractions as follows: 122/24, total gliadin; 218/17, 221/23, 222/5, 227/22, and 230/9, β/γ-gliadin; 401/16, 401/21, 403/8, 404/6, 405/7, 412/11, and 412/23, ω-gliadin; 236/9, 237/24, 246/21, 243/11, 243/12, 245/4, and 245/15, pyridylethylated total glutenin; and 304/13, HMW-GS. Antibodies under study were divided into four classes on the basis of gliadin and HMW-GS specificity, namely 1) high-mobility (α-, β-, and γ-) gliadin binding, 2) γ- and ω-gliadin and HMW-GS binding, 3) ω-gliadin and HMW-GS binding, and 4) broad-specificity. Four to six monoclonal antibodies from each group were tested in immunoblotting, and half of the antibodies in each of these groups were chosen for ELISA analysis with HMW-GS, LMW-GS, and gliadins. Rabbit antisera to gliadin were prepared by three immunizations (half of the dose was subcutaneous and half intramuscular) at monthly intervals with 1 mg of gliadin (prepared by extraction with 10 volumes of 1*M* urea of the residue from extraction with 10% (w/v) NaCl); rabbits were bled 10 days after the last injections.

ELISA Analysis

Protein fractions were dissolved by sonication (10 min) at 500 μg/ml in 170 m*M* KOH. Antigens were then immediately diluted 1:24 or 1:240 in 50 m*M* sodium carbonate buffer, pH 9.6, to yield 2 and 0.2 μg/100 μl protein solutions, respectively. These proteins were coated passively onto microwells of Immulon-B polystyrene plates (Dynatech, Denkendorf, FRG). After two washes with PBS/0.05% (v/v) Tween 20, nonspecific antibody binding was blocked by incubation with 1% BSA in PBS for 60 min at 37°C. Following three washes, microwell-bound antigen was incubated with 100 μl of monoclonal antibodies diluted in 1% BSA in PBS (90 min, 37°C), washed four times, then incubated (30 min, 37°C) with horseradish peroxidase-labeled rabbit anti-mouse immunoglobulins (100 μl, DAKO, Denmark) diluted 1:5,000 in 1% BSA in PBS-Tween. After five washes, 150 μl of substrate (2 m*M* 2,2′-azino-bis-3-ethylbenzthiazoline sulfonic acid; Sigma, St. Louis, MO) in 0.1*M* sodium citrate buffer, pH 4.5, containing 0.003% hydrogen peroxide, was added and plates incubated 20 min at 20°C before termination of enzyme reaction by acidification. Absorbance values were measured at 414 nm.

Rabbit antisera to gliadin were titrated against the different antigens using the protocol above, except that primary antibody binding was assessed by incubation with alkaline-phosphatase-labeled goat-anti-rabbit immunoglobulins (Promega, Madison, WI) diluted 1:5,000 in 2% BSA in 10 m*M* Tris/HCl/150 m*M* NaCl, pH 8.0, containing 0.05% Tween 20. The substrate used was *p*-nitrophenyl phosphate; absorbance values were measured at 405 nm (Skerritt et al 1987). The antibody titer was determined for each antibody and cereal antigen preparation by linear regres-

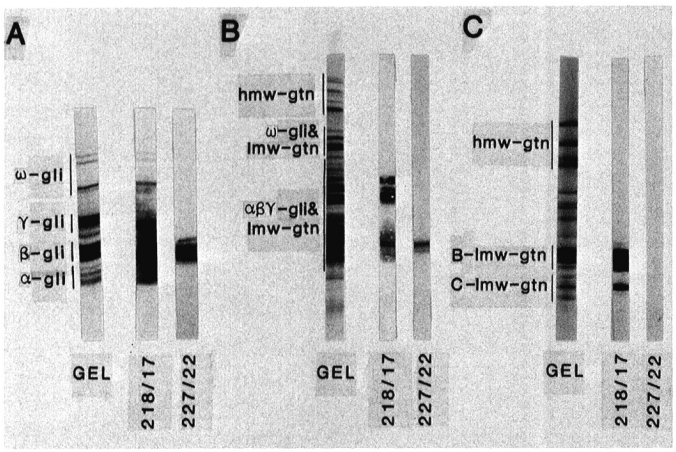

Fig. 2. Comparison of immunoblotting results using antibodies 218/17 subclone 7E8 tissue culture supernatant (S) 1:50 dilution, and 227/22 12H12 S 1:500 from: (**A**) gliadin analysis (acidic buffer polyacrylamide gel electrophoresis [PAGE], 1*M* urea extracts of Timgalen flour); (**B**) total endosperm protein analysis (one-dimensional sodium dodecyl sulfate [SDS]-PAGE, SDS-mercaptoethanol extracts, Timgalen flour); and (**C**) disulfide-bonded endosperm protein analysis (two-step SDS-PAGE, SDS extracts, Chinese Spring flour).

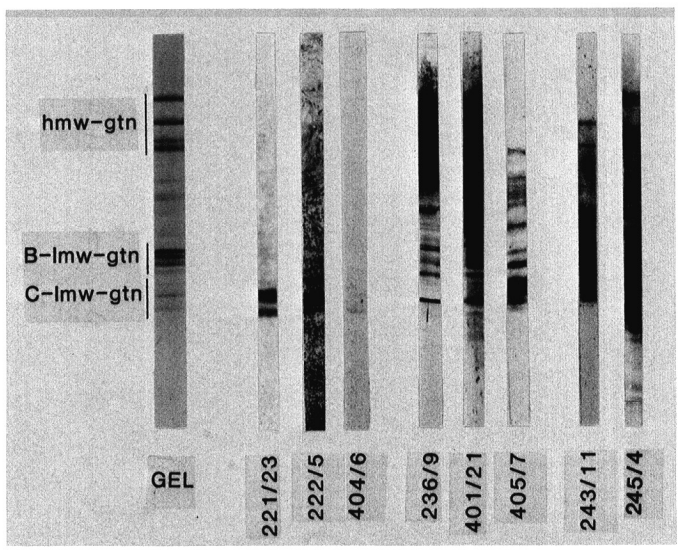

Fig. 3. Specificities of selected antibodies determined by two-step-1D SDS-PAGE analysis of Chinese Spring wheat flour extracts and immunoblotting. High-mobility gliadin-binding (221/23 8D11 purified [2 μg/ml], 222/5 9F9 S [supernatant] 1:1,000, 404/6 1A2 S 1:150), γ- and/or ω-gliadin-binding (236/9 13C6 S 1:10, 401/21 2B7 S 1:1,000, 405/7 3F10 S [neat]) and broad-specificity monoclonal antibodies (243/11 7C10 S 1:100, 245/4 4A10 S 1:100) are depicted.

sion of absorbance points on the descending limb of the absorbance versus (log) antibody concentration curve, and was defined as the antibody concentration yielding an absorbance of 0.100 under the assay conditions used.

The ELISA response to seven protein fractions was measured: fractions 1 and 2 were HMW-GS and LMW-GS (prepared as described above), respectively, and 3–7 were various aqueous ethanol extracts prepared following 10% NaCl treatment. Fraction 3 was prepared from defatted flour extracted with 40% ethanol. Preparations 4–7 were treated with 1.5% (w/v) SDS, then the SDS was removed such that they were comparable to glutenin fractions 1 and 2 and effects of SDS treatment on antigenicity could be controlled for. They were prepared as follows, after 10% NaCl treatment: fraction 4 from nondefatted flour extracted with 70% ethanol; fraction 5 from defatted flour extracted with 70% ethanol; fraction 6 from nondefatted flour, extracted with 40% ethanol; and fraction 7 from defatted flour, extracted with 40% ethanol.

RESULTS

Extraction of Glutenin Fractions for ELISA and Immunoblot Analysis

Extraction of gluten proteins from flour or whole meal for the first step of two-step one-dimensional SDS-PAGE requires the absence of a reducing agent. Preliminary comparison of a variety of extraction solvents indicated that best results were obtained using 4% SDS in Tris-HCl buffer. Addition of concentrated urea or use of an acidic rather than buffered extractant dissolved less glutenin. The classical Osborne (1907) fractionation scheme for wheat flour proteins defined gliadins as the protein extracted with 70% (v/v) ethanol following prior extraction of albumins and globulins. However, several groups have noted the presence of high molecular weight or aggregated polypeptides in these 70% ethanol extracts (Beckwith et al 1966, Bietz and Wall 1980, Shewry et al 1983).

TABLE I
Cross-Reaction of Monoclonal Antibodies with Gliadins and Glutenins—Immunoblotting Results

Gliadin Specificity	Glutenins[a] HMW (A)	LMW B	LMW C
$\alpha\beta\gamma$ (high-mobility)-Gliadin binding			
221/23 ($\alpha>$)[b]	−	−	++
230/9 ($\alpha\beta$)	−	+	+
403/8 ($\alpha\beta>\gamma$)	−	−	+
227/22 (β)	−	−	−
404/6 ($\beta>\gamma$)	−	−	±
222/5 ($\alpha\beta\gamma$)	−	−	++
$\gamma\omega$-Gliadin binding			
218/17	±	++	+
236/9	+	+	±
237/24	+	+	±
246/21	+	+	±
401/16	+	++	++
ω-Gliadin binding			
122/24 (ω slow)[b]	+	+	+
304/13 (ω fast)	++	−	−
401/21 (ω slow)	+	+	+
405/7 (ω slow)	++	++	++
Broad specificity			
243/11	±	+	+
243/12	+	+	+
245/4	+	+	+
245/15	±	+	+
412/11	+	+	+
412/23	+	+	−

[a] No reaction (−), rather weak reaction (±), weak-moderate reaction (+), very strong reaction (++). HMW and LMW = high and low molecular weight, respectively.
[b] Specificity determined by acidic-buffer PAGE and immunoblotting (Skerritt and Underwood 1986).

A further complication is the use of defatted flours by some workers for gliadin extraction studies (Meredith 1965, Bietz and Wall 1973, Jackson et al 1983). Others claim that lower concentrations of ethanol (40–60%) or 1 *M* urea are superior to 70% ethanol as gliadin extractants (Meredith 1965, Lee 1968). Analysis of these solvents and treatments (Fig. 1) showed that relatively little ω-gliadin was noted by acidic-buffer PAGE in the 70% ethanol extracts compared with the 40% ethanol or 1 *M* urea extracts, while the other gliadins were present in equivalent amounts. Differences in HMW-GS content were seen on one-dimensional analysis (Fig. 1A). Nondefatted 70% ethanol extracts had the highest HMW-GS content. After defatting, HMW-GS were observed in the 70% ethanol extract but not the 40% ethanol extract. Only trace amounts of HMW-GS were seen in the 1 *M* urea extracts. Two-step SDS-PAGE (Fig. 1B) revealed that significant amounts of LMW-GS were present in the ethanol extracts, except for the defatted 40% ethanol extract. The "B" cluster of LMW-GS was more prominent than the "C" cluster. One molar urea also extracted considerable LMW-GS and some additional disulfide-bonded albumins (Gupta and Shepherd 1987).

Cross-reaction of Antibodies with Gliadins, LMW-GS, and HMW-GS

An example of the ambiguity of immunoblotting results that can be obtained using only a single electrophoretic system is shown in Figure 2. On gliadin blots (Fig. 2A) 218/17 bound α-, β-, and γ-gliadins and 227/22 bound β-gliadins, whereas on SDS-PAGE blots (Fig. 2B) both antibodies bound polypeptides in the α-, β-, or γ-gliadin and LMW-GS region. Further immunoblotting analysis following two-step SDS-PAGE (Fig. 2C), showed that 227/22 was β-gliadin-specific even at high concentrations, whereas 218/17 bound both gliadins and LMW-GS.

High mobility gliadin-binding antibodies. Most of these bound to small clusters of α- and β-gliadins, although some such as 222/5, 403/8, and 404/6 bound certain γ-gliadins (Skerritt and Underwood 1986, and *unpublished*). These antibodies did not bind HMW-GS in blotting (Fig. 3, Table I) or ELISA assays (Table II) but at concentrations 2- to 10-fold above those used to probe gliadin immunoblots, each antibody bound to the (higher mobility) C-LMW-GS (Fig. 3). In the ELISA assays, the antibodies had very high gliadin titers, but binding to LMW-GS was detectable for only one antibody (404/6) at high antigen coatings. These results could be due to the C-LMW-GS interaction being of much lower affinity than gliadin binding. Unlike the other antibodies, 227/22 did not bind LMW-GS even at very high antibody concentrations (Fig. 2, Table II). Other data indicate that this clone recognizes an unusual subclass of β-gliadin-like proteins (Donovan et al 1989).

γ- and/or ω-Gliadin-binding antibodies. Each of these also bound HMW-GS and LMW-GS (Fig. 3). Antibody 218/17 bound especially well to LMW-GS (Fig. 2), whereas 236/9, 237/24, and 246/21, which had similar gliadin specificities, also had similar glutenin specificities, binding principally to HMW-GS and B-LMW-GS (Table I). ELISA results with 401/16 and 218/17 reflected the immunoblotting results, with less binding to HMW-GS than LMW-GS. Greater binding to HMW-GS was seen in both ELISA and blotting assays with the ω- (but not γ-) gliadin-binding antibodies (Fig. 3, Table II).

Broad gliadin-specificity antibodies. Each of these bound well to B- and C-LMW-GS, with the exception of 412/23 (B-LMW-GS specific). Differences in the degree of HMW-GS reactivity were seen in both blots and ELISA assays. A rabbit antiserum to gliadin showed similar cross-reactivity to certain of the broad-gliadin specificity antibodies (Table II), binding well to gliadin and LMW-glutenin, and significantly less well to HMW glutenin.

Homologies Between LMW-GS Encoded by Different Genomes

To investigate immunological homologies between different LMW-GS, analyses were made of 1) grain from a triple translocation plant bred with the short arms of each of the homoeologous group 1 chromosomes deleted and replaced with short arms

of chromosome 1R (Fig. 4); 2) extracts of Chinese Spring wheat; and 3) the following lines with deletions of specific chromosome pairs, according to Sears (1954): nullisomic 1A-tetrasomic 1D, nullisomic 1B-tetrasomic 1A, and nullisomic 1D-tetrasomic 1A (Fig. 5). Comparison of the stained triple translocation gel (Fig. 4) with euploid Chinese Spring (Fig. 5) revealed that the former had a greater number of HMW-GS (contributed by each of the two parental wheat cultivars). Several polypeptides of lower molecular mass were noted, although the triplet protein subunits (Singh and Shepherd 1988) and the major B-LMW-GS were absent; most of the polypeptides still present in the latter mobility range (three doublets) corresponded in mobility to disulfide-bonded albumins thought to be encoded by genes on long arms of certain chromosomes 4 and 5 (Gupta and Shepherd 1987). Several polypeptides were also missing in the C-LMW-GS.

Binding of different high-mobility gliadin-specific antibodies was affected differently by deletion of the short arms of the wheat chromosomes 1. Whereas C-LMW-GS binding by 221/23 and 230/9 was abolished by the translocation, 222/5, 403/8, and 404/6, which demonstrated different C-LMW-GS specificities, were not affected. The immunoblot patterns of B-LMW-GS binding antibodies were also altered. Antibodies 218/17, 243/11, 243/12, 401/16, 405/7, and 412/11 now bound only to certain C-LMW-GS. As expected, antibody binding to HMW-GS by, for example, 122/24, 236/9, 237/24, 246/21, 304/13, and 405/7 was unaffected by the translocation. However, high concentrations of these antibodies and the very broad-specificity antibody, 245/4, clearly bound polypeptides of B-LMW-GS mobility.

Two-step SDS-PAGE analysis of chromosome 1 nullisomic-tetrasomic stocks (Fig. 5) was able to identify the presence of structural genes for many of the B-LMW-GS (Gupta and Shepherd 1988) and some of the C-LMW-GS. However, the protein products appeared rather homologous, since deletion of a particular pair of short arms of chromosome 1 did not abolish binding of most antibodies. Immunoblot patterns altered as certain polypeptides were removed; in many cases the examination of the simpler immunoblot pattern (with only a few bands labeled, compared with the stained gel) facilitated assignment of chromosome arms bearing genes for particular LMW-GS. For example, B-LMW-GS encoded by chromosome 1BS and 1DS and C-LMW-GS encoded by 1-PS can be readily identified by examination of immunoblots probed with 122/24 and 230/9, respectively. The failure of certain C-LMW-GS to be encoded by short arms of group 1 chromosomes identified in the triple translocation blots was confirmed in these experiments.

Effects of Gliadin and LMW-GS Extraction Conditions on Antibody Bindings

Antibody titers to various SDS-treated aqueous ethanol-extracted fractions were determined relative to "native gliadin," i.e., protein extracted by 40% ethanol from defatted flour and not treated with SDS. The other aqueous ethanol-extracted protein fractions were treated with SDS to enable comparison with glutenin fractions. The binding of several monoclonal antibodies of each specificity class was inhibited by SDS treatment (Table II). The most sensitive antibody, 304/13, was reduced 50% in titer by SDS treatment. Defatting and the use of 40% ethanol rather than 70% ethanol, which decreased the glutenin content of the fractions, had minor effects on antibody titers. The general lack of effect of defatting can likely be attributed to there being at most 10–15% glutenin in the nondefatted preparations (Bietz and Wall 1973).

TABLE II
Monoclonal Antibody Binding to Gliadin and Glutenin Fractions—ELISA[a] Analysis[b]

			Gliadin-rich Preparations in[d]					Glutenin[e]	
			40% Ethanol			70% Ethanol			
Antibody	n[c]	Antigen (μg)	No SDS/DF	SDS/DF	SDS/NDF	SDS/DF	SDS/NDF	LMW	HMW
αβγ-Gliadin binding									
227/22	1	0.2	1	0.73	0.73	0.56	0.56	nd	nd
		2	1	0.70	0.79	0.56	0.70	nd	nd
404/6	3	0.2	1	0.83	0.63	0.52	0.77	nd	nd
		2	1	0.64	0.93	0.89	0.77	0.69	nd
γω-Gliadin binding									
218/17	3	0.2	1	0.58	0.89	0.66	0.52	0.14	0.044
		2	1	0.66	0.67	0.88	0.75	0.18	0.039
401/16	2	0.2	1	0.80	0.80	0.90	0.78	0.35	nd
		2	1	0.73	0.92	0.84	0.93	1.15	0.038
ω-Gliadin binding									
122/24	1	0.2	1	0.94	0.97	1.06	0.61	0.73	0.38
		2	1	1.0	0.90	1.23	0.73	1.8	0.18
304/13	2	0.2	1	0.52	0.54	0.52	0.46	0.41	0.88
		2	1	0.42	0.45	0.50	0.40	0.59	1.6
401/21	3	0.2	1	0.61	0.91	0.94	1.06	0.82	0.76
		2	1	0.91	1.15	0.86	0.78	0.78	0.56
Broad									
243/11	1	0.2	1	0.67	0.76	0.68	0.88	0.64	0.024
		2	1	0.86	0.79	0.86	0.71	0.71	0.26
412/23	1	0.2	1	0.85	1.10	1.35	1.20	1.25	0.35
		2	1	0.96	0.94	2.1	2.1	1.12	1.0
Polyclonal									
rabbit	1	0.2	1	1.9	1.27	0.88	0.96	0.16	0.046
		2	1	0.83	0.79	1.08	1.0	0.50	0.063

[a] ELISA = Enzyme-linked immunosorbent assay.
[b] Data shown are antibody titers, relative to non-SDS treated, defatted 40% ethanol-extracted protein ("control gliadin") = 1.
[c] n = Number of experiments.
[d] SDS = treated with sodium dodecyl sulfate, DF = defatted, NDF = not defatted.
[e] LMW = Low molecular weight, HMW = high molecular weight, nd = not detectable (<0.01).

DISCUSSION

The three chromosome 1 short-arm loci, *Gli-A1*, *Gli-B1*, and *Gli-D1*, encode γ-gliadins, ω-gliadins, and the major LMW-GS, respectively. As these genes are very tightly linked (Payne et al 1984; Singh and Shepherd 1985, 1988), and the gliadins have been more amenable to analysis by single-step electrophoretic analysis, many quality characteristics attributed to allelic variation in "group 1" gliadin composition may actually be due to LMW-GS polymorphism (Wrigley et al 1982, Branlard and Dardevet 1985, Autran et al 1987, Payne et al 1987, Gupta et al 1989). In bread wheats, certain γ- and ω-gliadins have been associated with dough strength (Sozinov and Poperelya 1980, Pogna et al 1982, Wrigley et al 1982). Dal Belin Peruffo et al (1985) found linkages between allelic γ-gliadin bands to be related to both quality and different LMW-GS groups in bread wheats.

Two-step SDS-electrophoretic analysis is a useful means of examining the disulfide-bonded protein composition of wheat grain extracts. The major LMW-GS can be resolved and studies of their genetic control, allelic variation, and quality relationships performed (Gupta and Shepherd 1988, Gupta et al 1989). Slab two-step SDS-PAGE enables either simultaneous examination of many grain samples or else analysis of disulfide-bonded proteins from a single extract with many antibodies. However, it should be noted that the LMW-GS preparations analyzed by two-step SDS-PAGE followed by immunoblotting and those analyzed by ELISA may not be identical in composition. The material analyzed by two-step SDS-PAGE contains subunits of an SDS-soluble aggregate of sufficiently low molecular weight to enter the first polyacrylamide gel, whereas preparation of glutenin fractions for ELISA (Graveland et al 1985) relied on the initial formation of an SDS-insoluble gel.

Strongest cross-reactions were seen between several antibodies binding γ- and ω-gliadins and B-LMW-GS. These results indicate that the genes encoding these proteins are not only tightly linked, but the protein products bear structural relationship to one another, and extend earlier findings of specific monoclonal antibody reactions with individual gliadin blocks encoded for by genes on a particular chromosome arm (Skerrit et al, *unpublished*). LMW-GS and γ-gliadins also are similar in their amino acid compositions (Bietz and Wall 1973), sulfur-containing amino acid contents, electrophoretic mobility under acidic buffer conditions (after reduction and alkylation, Charbonnier [1973]), and three-dimensional conformations (Tatham et al 1987). However γ- and ω-gliadins have differing amino acid compositions and N-terminal sequences, despite their tight genetic linkage (Kreis et al 1985). Nevertheless, there are some similarities between the two prolamin groups as several antibodies cross-react. While the immunochemical results indicate that homologies exist between γ-type gliadins and LMW-glutenins, other data indicate that quite distinct sequences exist in LMW-GS or in "aggregated gliadin" fractions. Shewry et al (1983) found a novel N-terminal sequence very prevalent in an aggregated gliadin fraction; sequences similar to this were found by Okita et al (1985) deduced from cDNA sequencing.

The C-subunits of LMW-GS remain poorly characterized. The genes encoding the quantitatively major B-LMW-GS have been located in the *Gli-1* loci, and many of the rather basic C-subunit polypeptides resolve separately from other grain proteins in two-dimensional electrophoresis. However, some C-LMW-GS migrated similarly to α- and β-gliadins on two-dimensional electrophoresis (Jackson et al 1983). Neither these workers nor Gupta and Shepherd (1987) were able to locate the genes encoding all of the C-subunits. The electrophoresis and immunoblotting results

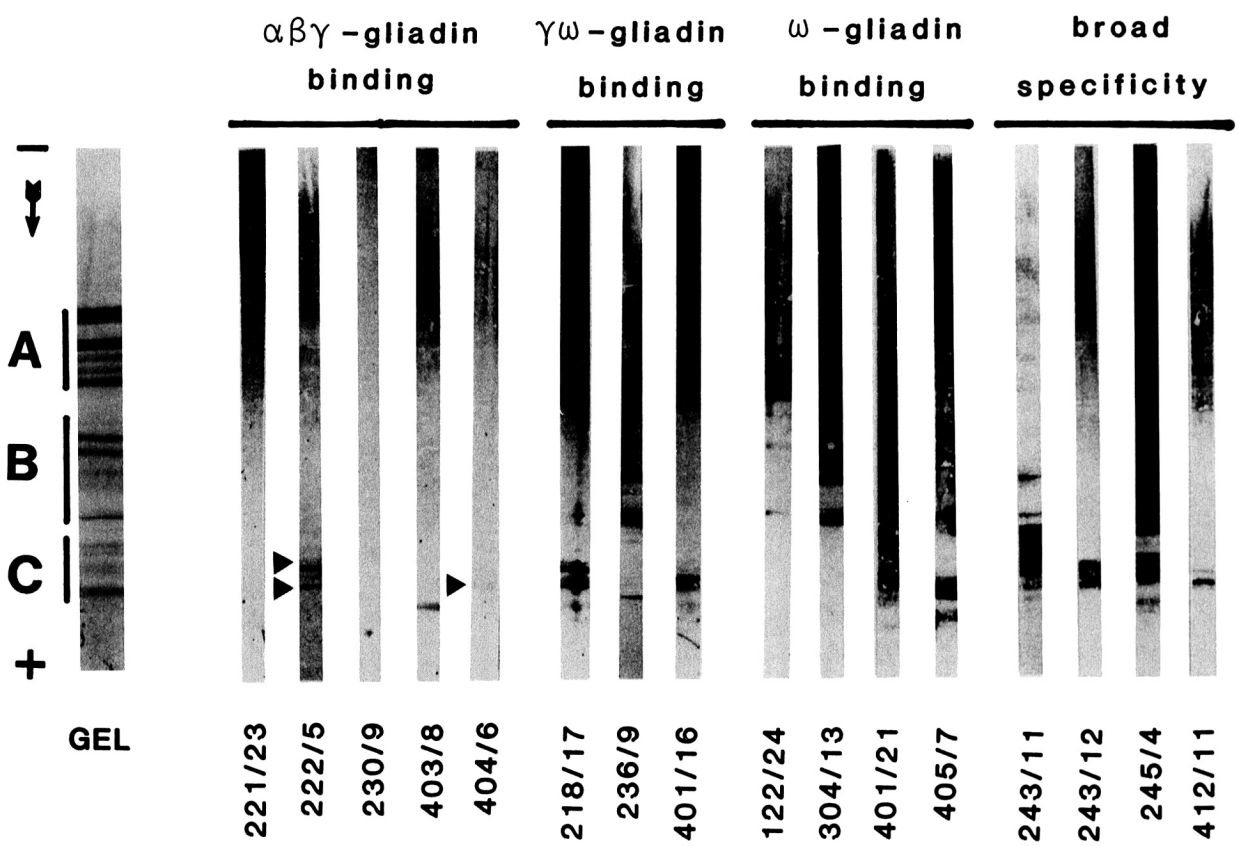

Fig. 4. Specificities of selected antibodies determined by two-step, one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of disulfide-bonded endosperm proteins from a triple (chromosomes 1 short arm) translocation line. High-mobility gliadin binding (221/23 8D11 [2 μg/ml], 222/5 9F9 S [supernatant] 1:1,000, 230/9 1A5 [5 μg/ml], 403/8 8G1 S [neat], and 404/6 1A2 S 1:20), γ- and ω-gliadin-binding (218/17 7E8 [10 μg/ml], 236/9 13C6 S 1:10, 401/16 1C9 S 1:25), ω-gliadin binding (122/24 ascites 1:50, 304/13 1B2 S 1:200, 401/21 2B7 S 1:00), and broad-specificity antibodies (243/11 7C10 S 1:100, 243/12 13H6 S 1:10, 245/4 4A10 [20 μg/ml], 412/11 2G10 S 1:10) are depicted.

indicate that all of the major B-LMW-GS are encoded by genes on the short arms of these chromosomes, but several C-LMW-GS subunits are not, in agreement with earlier studies (Jackson et al 1983, 1985). Several antibodies that bound α- and β-gliadins bound to C-LMW-GS but not to other glutenin subclasses. N-terminal sequencing of several C-LMW-GS after fractionation by two-dimensional electrophoresis has revealed both α-gliadin and γ-gliadin-type sequences (Tao and Kasarda, *personal communication*). This raises the possibility that the C-LMW-GS are derived from gliadins but that they formed inter- rather than intra-molecular disulfide bonds, possibly arising from the polypeptides having an odd number of cysteine residues. Indeed, Kasarda et al (1988) noted that certain α- and γ-type gliadins corresponding to known DNA sequences (Okita et al 1985, Sugiyama et al 1986) have odd numbers of cysteine residues and proposed that they may actually be glutenin subunits.

Glutenins encoded by genes on homoeologous chromosomes bore considerably greater structural homology to one another than do gliadins encoded by the chromosome 1 or 6 short arms in each genome. For example, 218/17 bound equally well to chromosome 1A-, 1B-, and 1D-encoded B-LMW-GS but bound specifically to chromosome 1B-encoded γ/ω-gliadins (Skerritt, *unpublished*). Antibody 122/24, however, demonstrated similar trends in binding to gliadins and LMW-GS, binding most strongly to 1DS products and less strongly to 1BS products. Some cross-reaction patterns could not be predicted; for example, 230/9 bound chromosome 6A gliadins but 1DS C-LMW glutenins. Nevertheless, antibody probes enable the better identification of products encoded by different chromosomes, and in some cases identify "blocks" of related components. These homologies also suggest that the role of LMW-GS in the toxicity of aqueous alcohol or urea extracts of flour or gluten in celiac disease (Skerritt 1988) requires closer investigation. Antibodies specific for groups of LMW-GS should be useful in understanding the roles of different LMW-GS groups in quality and therefore in the development of quality tests. While each of the monoclonal antibodies that

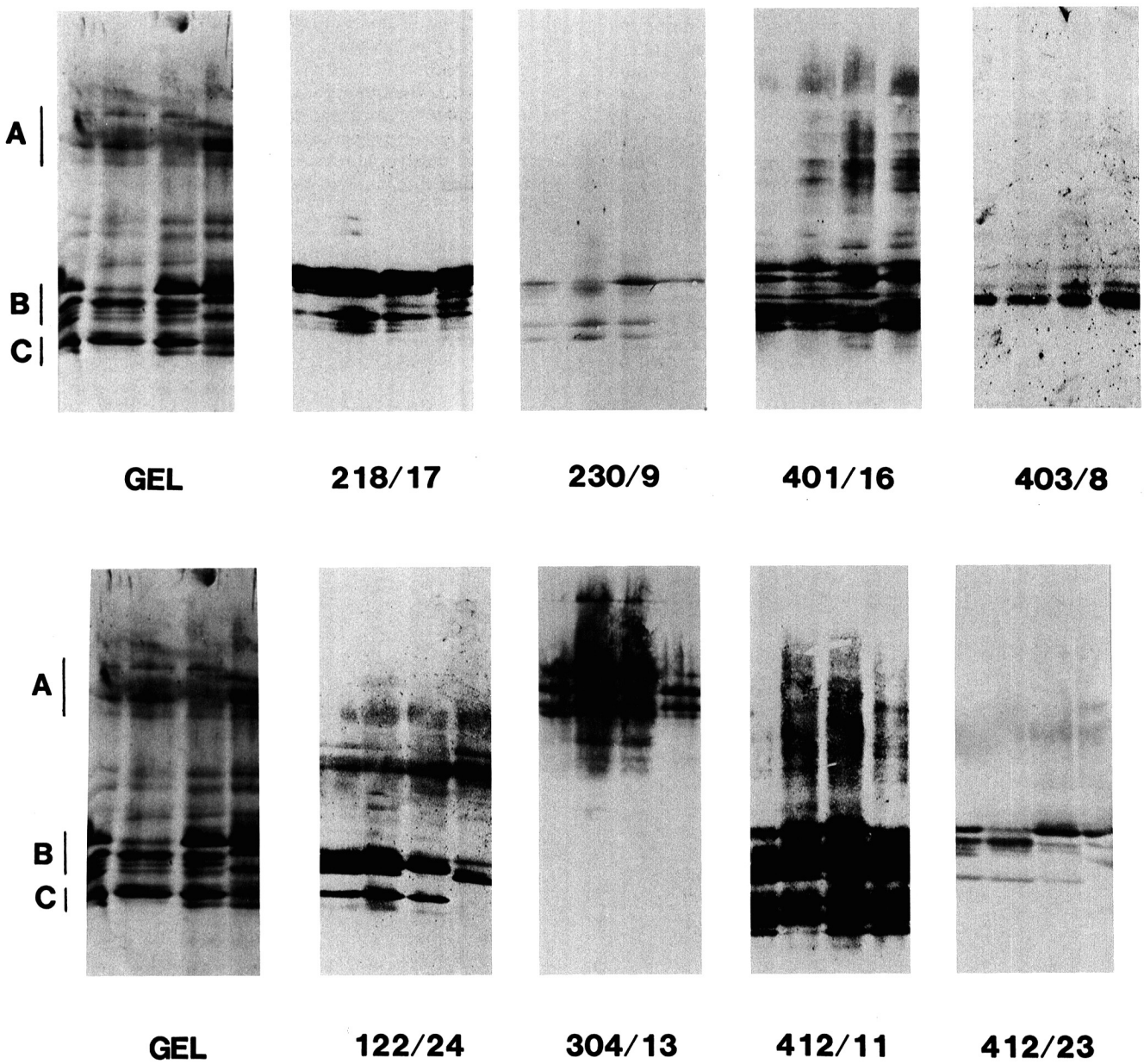

Fig. 5. Specificity of antibodies for low molecular weight glutenin subunits encoded by homoeologous chromosomes 1. **Top,** $\alpha\beta\gamma$-Gliadin- and $\gamma\omega$-gliadin-binding antibodies (218/17 7E8 [100 g/ml], 230/9 1A5 [5 g/ml], 401/16 1C9 S [supernatant] 1:1,500, and 403/8 8G1 S 1:10). **Bottom,** ω-Gliadin and broad-specificity antibodies (122/24 ascites 1:100, 304/13 1B2 S 1:200, 412/11 2G10 S 1:50, and 412/23 2D6 S 1:500). On the gel and each blot from left: Chinese Spring, Chinese Spring nullisomic (n) 1A-tetrasomic (t) 1P, n1B-t1A, n1D-t1A. Glutenin subunit clusters A (high molecular weight) and B and C (low molecular weight) are indicated.

bound LMW-GS in the current study also bound gliadin or HMW-GS, this cross-reactivity arose because the antibodies used were initially screened for reaction to gliadin or HMW-GS. Presence of unique N-terminal (Shewry et al 1983, Okita et al 1985, Kasarda et al 1988) and C-terminal sequences (P. R. Shewry, *personal communication*) in certain LMW-GS may enable the development of the necessary LMW-GS-specific antibodies.

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