Wheat Dough Extensibility Screening Using a Two-Site Enzyme-Linked Immunosorbent Assay (ELISA) with Antibodies to Low Molecular Weight Glutenin Subunits[1]

J. L. ANDREWS[2] and J. H. SKERRITT[2,3]

ABSTRACT

The relationships between total glutenin subunit content, low molecular weight glutenin subunit (LMW-GS) content, and dough extensibility were examined with several sets of flours, in which the overall correlations between flour protein content and extensibility were poor. The potential for monoclonal antibodies with specificities for various LMW-GS groups to screen for differences in dough extensibility was assessed using a two-site (sandwich) enzyme-linked immunosorbent assay (ELISA), following extraction of LMW-GS using 60% ethanol and 10m*M* dithiothreitol. A range of solid-phase bound (immobilized) antibodies with specificities for different LMW-GS were combined with two labeled (detection) antibodies: 21817, which bound a cluster of B-LMW-GS, and 23724, which bound both low molecular weight glutenin subunits (HMW-GS) and B-LMW-GS. Significant correlations were obtained with each flour set using the more specific detection antibody, 21817, although the specificity of the immobilized antibody was less important. Binding of the antibody combinations that related to extensibility correlated with LMW-GS content measured using reversed-phase high-performance liquid chromatography (RP-HPLC). The antibody binding, RP-HPLC, and extensibility correlations were of the order $r = 0.4$–0.7, which suggested that variation in total LMW-GS content accounted for only part of the variation in extensibility between flours. It is possible that interactions between several factors, including allelic composition of HMW-GS and LMW-GS as well as total LMW-GS content may determine dough extensibility.

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Of the two major protein fractions present in gluten, the glutenin complex provides the major viscoelastic properties of wheat flour dough. These properties occur through the disulfide bonding of the polypeptide subunits of the glutenin complex. The glutenin subunits fall into two groups determined by subunit size, genetic control, and sequence characteristics, the high molecular weight A glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The LMW-GS are further divided into three groups, termed D, B, and C subunits on the basis of decreasing apparent molecular weight; of these the B subunits are the most abundant (Payne and Corfield 1979, Jackson et al 1985, Masci et al 1991). Dough strength characteristics (e.g., extensigraph maximum resistance, farinograph development time) have been predominantly correlated with HMW-GS content (Kolster et al 1993, Andrews et al 1994) and HMW-GS composition (Payne et al 1979, Branlard and Dardevet 1985, Payne 1987). Knowledge of the relationship between the LMW-GS and dough properties is much more limited, even though these subunits represent the majority of the protein present in the glutenin complex.

The molecular determinants of dough extensibility are less well defined, and they remain an important research objective since improvement in extensibility has become a major breeding priority in many countries. Extensibility generally has a high correlation with flour protein content and total glutenin content (Gupta et al 1993, Gianibelli et al 1994). Recent studies have shown a positive relationship between extensibility and total glutenin content (Gupta et al 1993) with contributions from LMW-GS to both dough extensibility and strength in bread wheats (Gupta et al 1989, 1991; Metakovsky et al 1990; Andrews et al 1994). These correlations between LMW-GS and extensibility could be due to cause and effect or alternatively, these subunit polypeptides may also only be acting as a measure of the total protein or glutenin content because LMW-GS form the major component of glutenin and, thus, a significant fraction of the total protein. Knowledge of the role of LMW-GS alleles, compared to that of HMW-GS alleles, in dough rheology is limited. Studies of allelic variation at the major LMW-GS encoding locus (*Glu-3*) in relation to dough properties (Jackson 1983; Gupta et al 1989, 1991, 1994; Metakovsky et al 1990; Masci et al 1991; Khelifi and Branlard 1992; Cornish et al 1993; Lafiandra et al 1994) have produced trends rather than a firm ranking that would be applicable across environments and genetic backgrounds. The main reason for the lower level of certainty is the greater number of LMW-GS alleles and the larger number of polypeptides encoded by most of these loci when compared with the HMW-GS (Gupta and Shepherd 1990). Binding of one antibody, specific for chromosome 1DS B-LMW-GS, has been correlated with loaf volume using a single set of flours (Brett et al 1993) but not with individual dough properties.

The aim of the current study was to further investigate the relationship between the LMW-GS and extensibility and to determine whether a small-scale test for dough extensibility could be developed using antibodies specific for either total LMW-GS or individual groups of subunits, such as the B-LMW-GS. We selected the two-site or double antibody sandwich enzyme-linked immunosorbent assay (ELISA) method in these experiments, as this format has been shown to be robust, and also formed the basis for the dough strength assay (Skerritt 1991a,b; Andrews et al 1993; Hay 1993) that is in use by a number of breeding programs.

MATERIALS AND METHODS

Flour Samples

Samples of cultivars Cook and Halberd were used in the initial analysis of the abilities of different LMW-GS binding monoclonal antibodies (MAbs) to discriminate differences in extensibility using the two-site ELISA format. The wheats were grown at Wongan Hills, Western Australia (1989-90 season) and Turretfield, South Australia (1990-91 season), respectively, as part of the Australian Interstate Wheat Variety Trials (IWVT). Their straight-run flours displayed extreme differences in extensibility (28.8 and 18.5 cm, respectively) but similar dough strength (R_{max} of 320 and 300 BU) and protein contents (12.7 and 12.5%).

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[2]CSIRO Division of Plant Industry, North Ryde, NSW 2113 and Canberra, ACT 2601, Australia.

[3]Corresponding author. CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601 Australia. Fax + 61 6 246 5351/ 5000; Phone + 61 6 246 5350; E-mail: skerritt@pi.csiro.au

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Flour samples from the Australian IWVT (32 breeders' lines from the 1991-92 season) were used for testing correlations between antibody response and HPLC data with dough properties. To minimize the complicating effect of variation in total protein content affecting extensibility, the sites studied in most detail were those for which the samples displayed a weak or statistically non-significant relationship between total flour protein content and extensibility: Wongan Hills, Western Australia, and Turretfield, South Australia. The same set of lines, grown at Wagga Wagga, New South Wales, Australia, was analyzed in confirmatory experiments. In addition, a set of 38 wheat cultivars from various countries were examined, after being grown at a single site, Strathalbyn, South Australia (Campbell et al 1987, Skerritt 1991b).

Comparison of Sample Extraction Methods for ELISA and HPLC

Three glutenin extraction procedures were evaluated with the two-site ELISA methods. In each case, 50 mg of flour was extracted using 1 ml of solvent.

Procedure 1. 2.5% (w/v) sodium dodecyl sulfate (SDS) and 10m*M* dithiothreitol (DTT) extracts were prepared by extracting flour by intermittent vortex mixing for 16–20 hr at 37°C, following 5 min of preextraction using 0.5% (w/v) SDS in water. This method has also been used routinely for sample preparation in the dough strength ELISA described earlier (Andrews et al 1993).

Procedure 2. 50% (v/v) aqueous propan-2-ol and 10m*M* DTT extracts (Kruger et al 1988) were prepared (extraction 16–20 hr at 37°C) after preextraction (30 min at room temp) with 50% propan-2-ol. Glutenin subunits for RP-HPLC were prepared similarly, except that the solvents were HPLC grade (Mallinckrodt, Paris, KY), the residue was washed once with the preextraction solvent and 0.5% (w/v) DTT was used for reduction (65°C for 60 min with mixing at 15-min intervals).

Procedure 3. 60% (v/v) ethanol and 10m*M* DTT (Wieser et al 1989) with flour that had been preextracted by shaking 30 min with neat dimethyl sulfoxide (DMSO) to remove monomeric proteins (Burnouf and Bietz 1989).

All extracts were prepared in 2-ml microcentrifuge tubes and were clarified by centrifugation (5 min at, 15,600 × *g*) before analysis.

RP-HPLC

The procedure was similar to that of Marchylo et al (1992) using a Zorbax C8 column (Dionex, Sunnyvale, CA) (4.6 × 150 mm) eluted at 50°C with a gradient of acetonitrile (30–50%, v/v, over 50 min) in 0.1% (v/v) aqueous trifluoroacetic acid (TFA) (Pierce, Rockford, IL). Aliquots (40 µl) were injected and peak elution was monitored at 210 nm. The relative levels of HMW-GS and LMW-GS in the samples were determined by cumulative integration using the respective time windows of 10–23.5 min and 23.5–55 min. These parameters were established following collection of the peaks and their analysis by SDS polyacrylamide gel electrophoresis (PAGE) and silver staining (data not shown). To prevent the possibility of reformation of disulfide bonds during chromatographic analysis, in other experiments, glutenin subunit preparations were alkylated by addition of 4-vinylpyridine to the 50% propan-2-ol and 10m*M* DTT extracts (Sigma) at a five-fold molar excess over DTT, and incubation at 65°C for 30 min (Burnouf and Bietz 1989). Chromatography of these samples was performed as for the reduced, unalkylated samples except that the acetonitrile gradient was from 23 to 43% in 50 min and the respective HMW-GS and LMW-GS time windows for integration were: 12–27.5 and 27.5–50 min. All samples were filtered through 0.45 µm polyvinylidine difluoride membranes (Gelman, Ann Arbor, MI) before analysis. Data acquisition and analysis, and system control was through Millenium software (Waters, Milford, MA) using Waters model 510 pumps, a model 712 automatic sampler, and model 481 detector.

Antibodies

Some LMW-GS and binding monoclonal antibodies (MAbs; clones 21817, 22205, 22722, 23724, 24311) were selected from an earlier characterized library of MAbs prepared from mice immunized with various gluten protein fractions (Skerritt and Underwood 1986; Skerritt and Robson 1990). Two new antibodies to LMW-GS (82123 5D5 (IgG3) and 82208 20F6 (IgG1)) were prepared by polyethylene glycol fusion of mouse myeloma cells with splenocytes from a mouse immunized with LMW-GS. The immunogen was the glutenin BC fraction (LMW-GS) prepared from cv. Cook using the method of Graveland et al (1985). The immunization and fusion protocols have been described before (Skerritt et al 1984).

The glutenin subunit specificities on blots of SDS-PAGE gels of glutenin subunits of the MAbs tested were, in order of decreasing specificities: 21817 7D7, narrow group of B-subunits bound (three major, two minor); 22722 12H12, bound mainly C-subunits, high concentrations required; 82123 5D5, group of six major B-subunits; 22205 9F9, broad range of 8-10 B-subunits; 82208 20F6, all major (≈12) B-subunits; 23724 4H11, A (HMW-GS) subunits, also bound 8-10 B-subunits and 2–3 C-subunits; 24311 7C10, bound all A-, B-, and C-subunits.

The antibodies had differing specificities for linear epitopes within the LMW-GS (Skerritt and Andrews, *unpublished*). Each antibody had different specificities: 21817 bound to four spatially separate epitopes within the repetitive domain; 22205 bound to two cysteine containing epitopes in the center of the molecule; 24311 bound to sequences at either end of the repetitive domain and one sequence (common to 22205) in the center of the molecule; 23724 also bound to sequences at either end of the repetitive domain and recognized a third sequence about 40 amino acids in from the C-terminus; 82123 bound to three sequences in the repeat region and one in the C-domain; 82208 recognized a single sequence in LMW-GS subunit, near the end of the repetitive domain; 22722 appeared to recognize conformational epitopes, such that its specificity could not be determined using this method. Horseradish peroxidase (HRP) (Boehringer Mannheim, Germany) was conjugated to the MAbs after activation with sodium periodate (modified from Nakane and Kawoi 1974).

SDS-PAGE and Semi-Dry Blotting

For analysis of the subunit composition, flour extracts (see below) were mixed with an equal volume of sample buffer (250m*M* Tris-HCl [pH 7.5], 4% [w/v] SDS, 20% glycerol, with 4% [v/v] mercaptoethanol). For analysis of glutenin subunit specificity by immunoblotting, glutenin was prepared from flour (cv. Suneca) by three preextractions in 1.5 ml of 50% (v/v) propan-1-ol, extraction of the residue for 30 min at 37°C with 200 µl of 50% *n*-propanol and 125m*M* Tris-HCl (pH 7.5) 1% dithiothreitol (DTT) for 30 min, then alkylated by addition of 4-vinylpyridine to a final concentration of 1.4% (v/v) (Singh et al 1991). After 30 min at 60°C, the extract was added to an equal volume of sample buffer. Samples were fractionated on a 12% T, 2% C polyacrylamide gel (15 cm × 17 cm × 1.5 mm), run at constant voltage for 1600 Vhr. Fractionated proteins were either stained using colloidal Coomassie G-250 in methanol-ammonium sulfate and phosphoric acid (Neuhoff et al 1991) or blotted to nitrocellulose (0.2 µm pore size, Schleicher and Schuell, Dassel, Germany). Semidry blotting was performed as follows. The gel was equilibrated for 20 min in 25m*M* Tris and 192m*M* glycine and 0.02% (w/v) SDS and 10% methanol, before transfer of proteins to the membrane (250 mA constant current, 6 hr) using 40m*M* 6-aminocaproic acid and methanol (20%), buffered with Tris to pH 7.5 as the cathode buffer, and 25m*M* Tris and 300m*M* Tris in 20% methanol as the inner and outer anode buffers, respectively. After blotting, nitrocellulose sheets were washed, fixed, blocked, and probed with antibodies, with specific antibody binding revealed by use of an alkaline phosphatase-labeled second antibody (Skerritt and Lew 1990).

ELISA

The two-site (sandwich) ELISA procedure was essentially that described by Hill and Skerritt (1989) with the following exceptions. The extracts for the two-site ELISA were diluted in 0.1% (w/v) fish skin gelatin (Sigma, St. Louis, MO) and 10m*M* sodium phosphate and 140m*M* NaCl (pH 7.2) containing 0.05% (v/v) Tween 20. Sample aliquots (100 µl) were incubated in duplicate with the solid phase MAb for 1 hr at room temperature. The incubation with enzyme-labeled MAb and the color development time were also of 1 hr duration.

Although a number of antibodies and flour extractants were examined (see below), the optimal conditions for extensibility discrimination of flours utilized sample extraction Procedure 3, with dilution of the flour 1:100 before analysis, and antibody 21817 as both the immobilized and enzyme-labeled species.

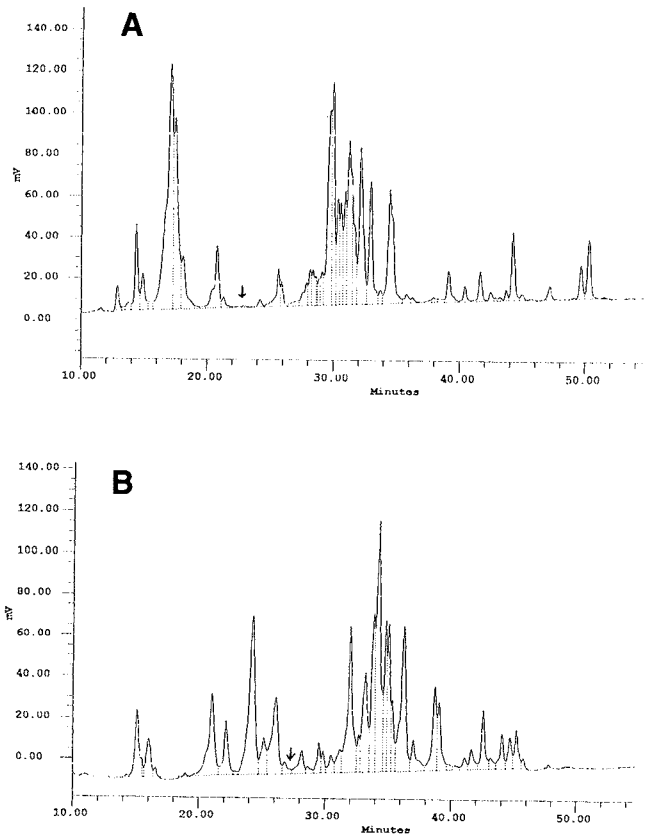

Fig. 1. Reversed-phase high-performance liquid chromatography analysis of reduced **(A)** and reduced and alkylated **(B)** glutenin subunits from cultivar Cook grown at Wongan Hills. Peaks to the left of the arrow contain high molecular weight glutenin subunits, peaks to the right of the arrow contain low molecular weight glutenin subunits.

RESULTS AND DISCUSSION

RP-HPLC

Glutenin polypeptides from 32 wheats grown at each of two sites were analyzed by RP- HPLC. The quantities of both total GS and LMW-GS, as analyzed by RP-HPLC showed consistent and significant correlations with dough extensibility (Table I), in agreement with earlier results using other samples and harvest years (Andrews et al 1994). These correlations held, despite the extensibilities for these sets of samples not being correlated with grain protein. In contrast, the extensibility and HMW-GS content correlations were lower and were only significant at one site. Correlations were observed with farinograph development time and GS content, but they were slightly lower than the extensibility correlations. Maximal resistance and loaf parameters only correlated with most of the glutenin subunit parameters at the Turretfield site, in the range $r = 0.41–0.54$, for reduced GS. Correlations of extensibility with the alkylated samples were slightly lower than for the unalkylated subunits. This may relate to the clearer separation of HMW-GS and LMW-GS in the chromatograms of the unalkylated samples and the fact that the alkylated samples produced a simpler pattern of peaks (Fig. 1).

Assessment of Extraction Conditions by SDS-PAGE and Immunoassay

Initial analyses in the two-site format using flour extracts prepared using 2.5% SDS and 10m*M* DTT extraction after preextraction with 0.5% SDS, as used previously for dough strength prediction (Andrews et al 1993), gave very low absorbance responses with most of the LMW-GS binding antibodies (Fig. 2). This may either indicate that the SDS in these extracts masked epitopes on LMW-GS, or that several antibody combinations were unable to form an appropriate antibody-antigen sandwich. Several antibody combinations were also unable to form a molecular sandwich with LMW-GS in reduced aqueous alcohol extracts.

TABLE I
Correlation Coefficients Between Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Measures of Glutenin Subunit Content[a] and Dough Properties[b] for 32 Advanced Breeding Lines Grown at Each of Two Sites

Site		Dough Property			
		Grain Protein	R_{max}	Ext	DT
Wongan Hills					
	Grain protein		−0.40	0.33	−0.05
	Reduced HMW-GS	0.52**[c]	0.18	0.29	0.42*
	Reduced LMW-GS	0.34	0.26	0.61***	0.55**
	Reduced total GS	0.45*	0.25	0.55**	0.56**
	Alkylated HMW-GS	0.47**	0.26	0.18	0.28
	Alkylated LMW-GS	0.34	0.30	0.53**	0.50**
	Alkylated total GS	0.42*	0.32	0.48**	0.48**
Turretfield					
	Grain protein		−0.26	0.31	0.12
	Reduced HMW-GS	−0.02	0.66***	0.55**	0.49**
	Reduced LMW-GS	0.13	0.72***	0.73***	0.57***
	Reduced total GS	0.07	0.77***	0.72***	0.60***
	Alkylated HMW-GS	−0.05	0.64***	0.47**	0.46**
	Alkylated LMW-GS	0.33	0.59***	0.65***	0.50**
	Alkylated total GS	0.23	0.68***	0.66***	0.55**

[a] HMW-GS = high molecular weight glutenin subunit; LMW-GS = low molecular weight glutenin subunit.
[b] R_{max} = extensograph maximum resistance (BU); Ext = extensograph extensibility (cm); DT = farinograph development time (min).
[c] Levels of significance: * ** *** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

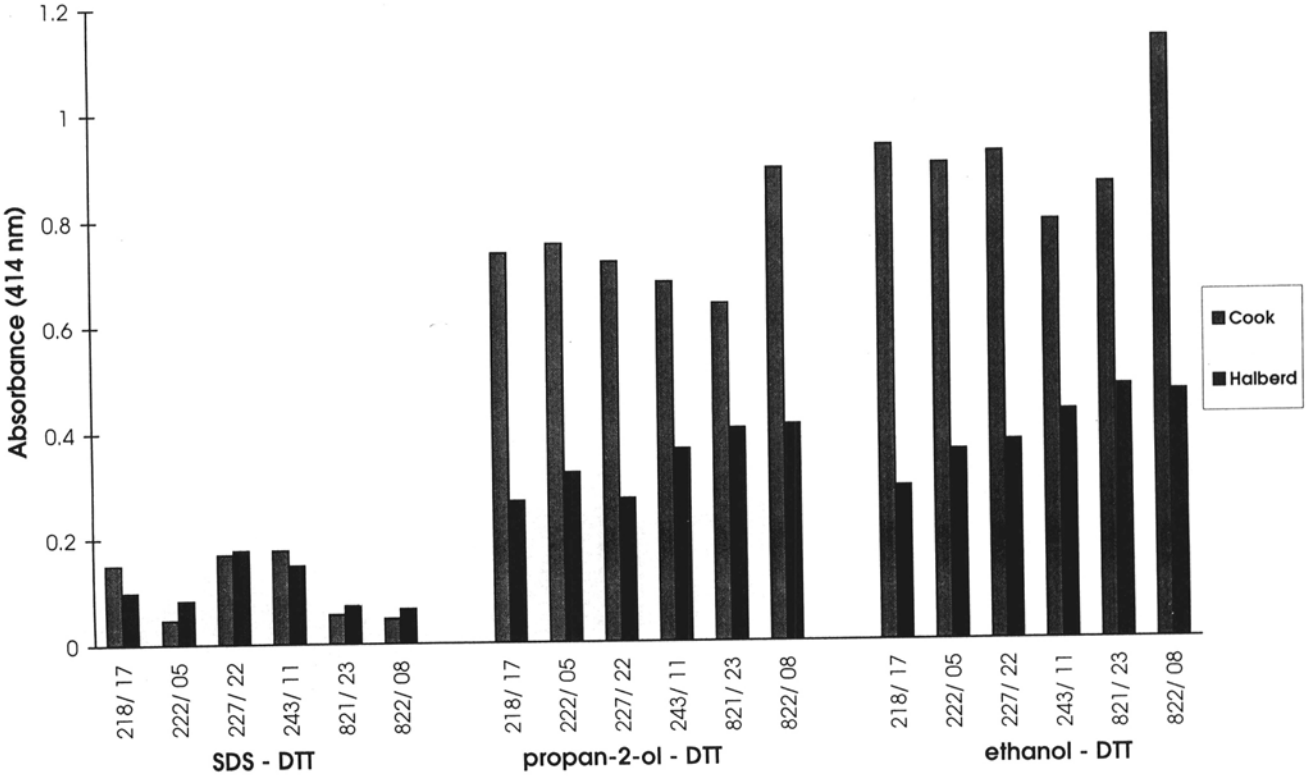

Fig. 2. Differentiation of an extensible (Cook) and inextensible wheat flour (Halberd) in the two-site enzyme-linked immunosorbent assay using different extraction protocols. Different solid-phase antibodies were used (illustrated); the detection antibody used throughout was 21817. Extracts diluted 1/100 for the 60% ethanol and 10 m*M* dithiothreitol (DTT) treatment, 1/200 for other treatments. SDS = sodium dodecyl sulfate.

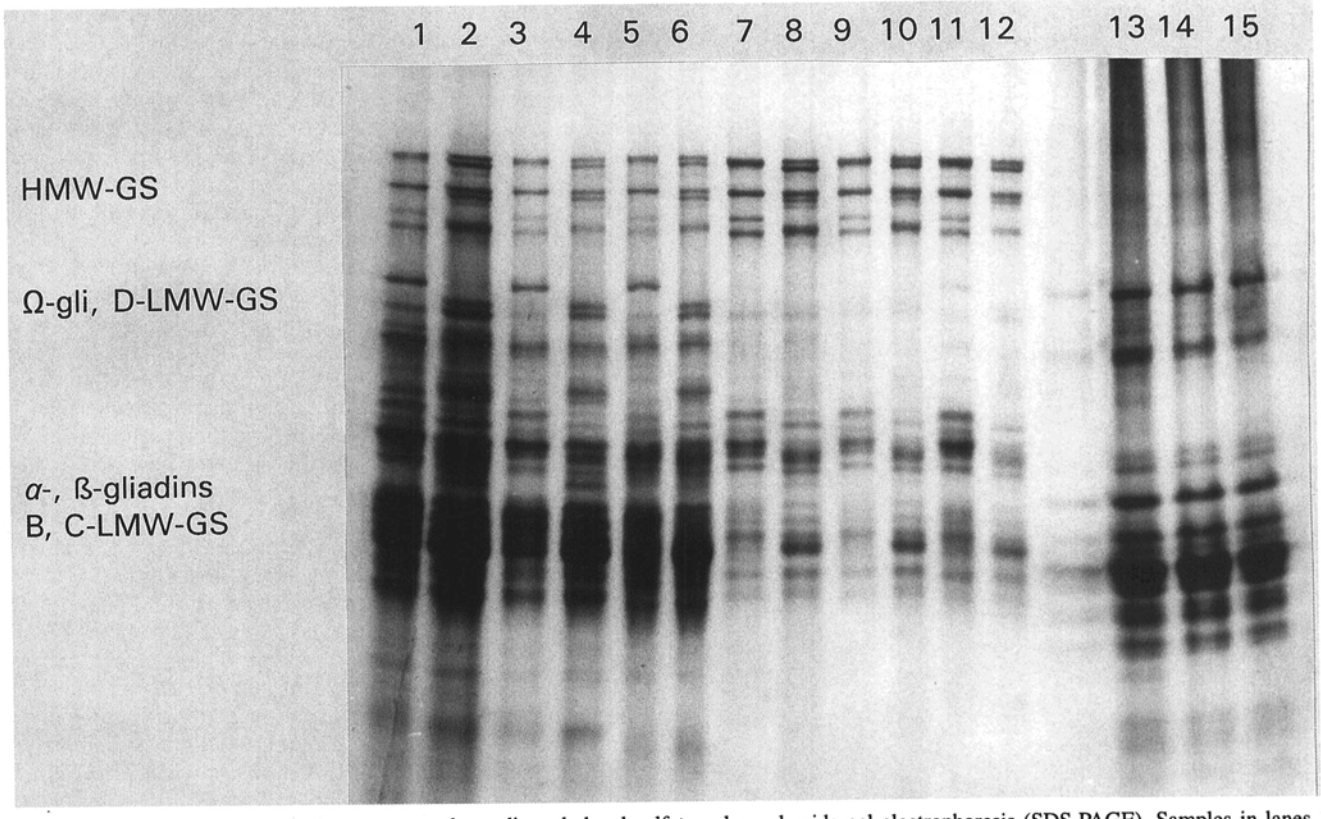

Fig. 3. Analysis of extracts used for immunoassay by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples in lanes 1–12 were reduced using 2% mercaptoethanol before analysis. For samples in lanes 13–15, SDS and glycerol were added to 2 and 20%, respectively, before analysis. SDS preextracts, lanes 1 and 13 (Cook) and 2 (Halberd); 50% propan-2-ol preextract, lanes 3 and 14 (Cook) and 4 (Halberd); dimethyl sulfoxide preextract lanes 5 and 15 (Cook) and 6 (Halberd); SDS and dithiothreitol (DTT) extract, lanes 7 (Cook) and 8 (Halberd); 50% propanol-DTT extract lanes 9 (Cook) and 10 (Halberd); 60% ethanol-DTT extract lanes 11 (Cook) and 12 (Halberd). HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.

The lack of ELISA response can be due to factors such as steric hindrance preventing binding of two antibodies to spatially close epitopes (Skerritt et al 1990), especially if antibodies recognize single, rather than multiple epitope sequences. Of the combinations tested, 21817, with relatively narrow specificity, was the only antibody able to be used as both the solid-phase and detection antibody with the LMW-GS extraction solvents assessed in the current study.

This may be because the antibody recognizes groups of amino acid sequences in spatially separate regions of the LMW-GS sequence, one near the N-terminus and the other in the center of the sequence.

The other antibodies either recognized single linear sequences in LMW-GS, or multiple sequences that were rather close (i.e., within 20 amino acid residues of one another). Earlier work (Skerritt et al 1990) had also shown that 21817 functioned well with a wide range of other antibodies as either an immobilized or labeled partner. In this study, each of the LMW-GS MAbs tested was effective as a solid phase MAb for the discrimination of the two flours which differed in extensibility, when either 21817 (narrow B-subunit specificity) or 23724 (LMW-GS and HMW-GS binding) were used as the detection MAb.

Two MAbs, 21817 and 23724, when used as the detection antibodies, consistently gave high color responses using aqueous reduced propan-2-ol extracts (Fig. 2).

Although these extracts produced reasonable discrimination between the Cook and Halberd samples, the reduced 60% ethanol extracts gave greater discrimination (Fig. 2). Extracts produced using the three methods were analyzed by SDS-PAGE after reduction (Fig. 3). The LMW-GS and gliadin composition of the preextracts appeared similar, but the HMW-GS were extracted to different extents. DMSO appeared to have extracted more glutenin, especially *x*-type HMW-GS, than either 50% propan-2-ol or 0.5% SDS. Analysis of the extracts used for immunoassay demonstrated that the reduced 60% ethanol extracts extracted greater amounts of both B and C LMW-GS than other two solvents. In addition, the 60% ethanol-DTT extract was also almost devoid of *y*-type HMW-GS, in agreement with Wieser et al (1989). Thus, this solvent was used for extraction of LMW-GS in further experiments described herein.

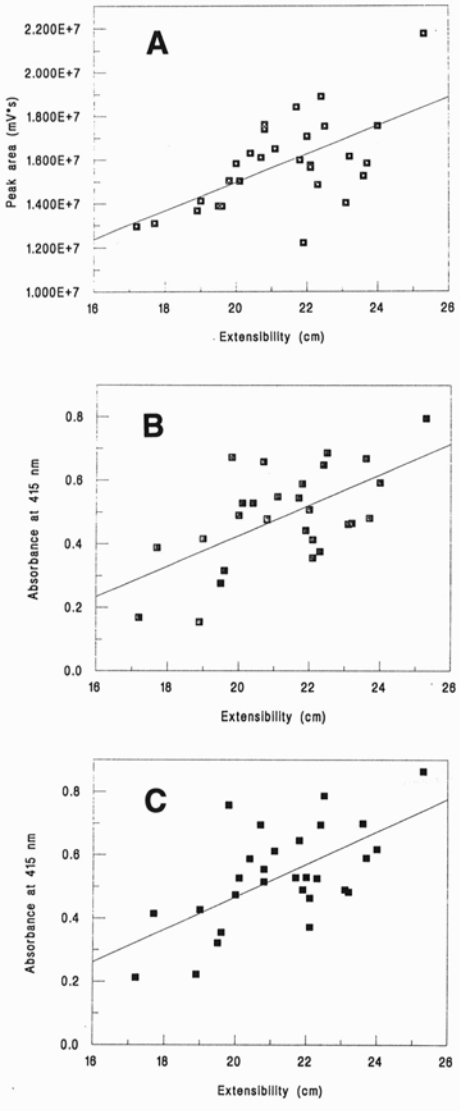

Fig. 4. Relationship between extensibility (for 29 flours milled from wheats grown at the Wongan Hills site) and: **A,** areas of reversed-phase high-performance liquid chromatography peaks (unalkylated extracts) corresponding to low molecular weight glutenin subunits ($r = 0.61$, $P < 0.001$); **B,** binding of labeled antibody 21817 and solid phase 21817 ($r = 0.61$, $P < 0.001$); **C,** binding of labeled antibody 22205 and solid phase 21817 ($r = 0.63$, $P < 0.001$), to 60% ethanol and 10 m*M* dithiothreitol flour extracts.

ELISA Responses of Flour Sets

The results from analyzes of the IWVT and the World flour sets using immunoassay are given in Table II. Highly significant correlations were observed between extensibility and antibody response using 21817 as the detection antibody in combination with each of the LMW-GS selective antibodies, using flours from the Wongan Hills site. Slightly lower, but still significant correlations between antibody binding in the two-site ELISA and dough extensibilities were obtained with two other sets of the same cultivars grown at Turretfield and Wagga Wagga (correlation coefficients ranged from 0.41 to 0.51).

The abilities of the assay to discriminate flours varying in extensibilities, as determined by the slopes of the ELISA color development and extensibility regression lines, were similar for each site for the IWVT set (data not shown). The ability of the assay to act as a general screen for extensibility was further tested using a set of unrelated genotypes obtained from over a dozen countries.

In this set, significant positive correlations were again seen between antibody binding and extensibility, but not other parameters, such as grain protein, maximal resistance, development time or loaf volume, or score (not shown). In each of the four sets of flours examined, neither dough extensibility nor the antibody responses in assays using 21817 as the detection antibody were correlated with farinograph development times, extensigraph maximal resistances, or grain protein contents.

Several antibodies, differing in specificities for B-LMW-GS, gave similar correlations with extensibility when 21817 was used as detection antibody. These included antibodies which either bound small clusters (21817, 82123) or the wider range (22205, 82208) of B subunits (Fig. 4). Use of 22722, which mainly bound C subunits and only weakly bound B subunits or 24311, which bound each group of glutenin subunits as solid-phase antibody, gave significant correlations with extensibility for the IWVT set of genotypes but not with the World set of genotypes (Campbell et al 1987). Presumably, if groups of polypeptides additional to the cluster of B-LMW-GS recognized by 21817 are immobilized on the microwell surface by a particular solid-phase antibody, they will not contribute to the ELISA response when 21817 is used as the detection antibody. The slopes of the ELISA color and extensibility regressions were similar for different detection antibodies, with a 2-cm increment in extensograph length providing a 0.1 A unit different in ELISA color; the slope of the LMW-GS measurement (by HPLC) and extensibility regression for the same set of flours (Fig. 4A) was also similar.

Use of the broader-specificity antibody (23724) as the detection antibody did not provide significant correlations with extensibility (r = 0.20–0.34), when it was evaluated with the IWVT set at the Wongan Hills and Turretfield sites. In contrast, the ELISA responses with these combinations were significantly correlated with farinograph development time at both sites. The basis for this correlation is not clear; although this antibody detected HMW-GS when used in combination with itself and other antibodies, such as 41201 (Andrews et al 1994), HPLC analysis (Table III) showed little relationship between binding of the other combinations utilizing 23724 as detection antibody and HMW-GS content. In contrast, Hay (1993), using extracts of flours selected for puff pastry baking performance, demonstrated a correlation between the binding of an antibody reactive with HMW-GS (41201) and dough extensibility. However, since there was significant variation in flour protein content in the set of samples evaluated, there may not be a direct cause-and-effect relationship between the binding of this antibody and extensibility. In another study using indirect

TABLE II
Correlation Coefficients Between Low Molecular Weight Glutenin Subunit Monoclonal Antibody (MAb) Binding and Dough Properties[a] Using Various Antibody Combinations and Sites[b]

Solid-Phase Antibody	Site	Grain Protein	R_{max}	Ext	DT
1. Using labeled 21817					
21817	Wongan Hills	0.15	0.10	0.61***[c]	0.12
	Turretfield	0.19	0.25	0.43*	0.15
	Wagga Wagga	0.22	0.36*	0.49**	0.20
	World Set	–0.02	–0.02	0.36*	–0.21
22205	Wongan Hills	0.24	0.03	0.63***	0.07
	Turretfield	0.20	0.31	0.46*	0.26
	Wagga Wagga	0.27	0.32	0.51**	0.28
	World Set	0.19	–0.14	0.34*	–0.38*
22722	Wongan Hills	0.16	0.11	0.59***	0.15
	Turretfield	0.19	0.25	0.45*	0.16
	Wagga Wagga	0.22	0.19	0.44*	0.22
	World Set	0.29	0.14	0.26	0.12
24311	Wongan Hills	0.18	0.15	0.62***	0.12
	Turretfield	0.12	0.32	0.47**	0.15
	Wagga Wagga	0.03	0.20	0.46*	0.07
	World Set	0.12	0.11	0.25	–0.05
82123	Wongan Hills	0.11	0.26	0.60***	0.09
	Turretfield	0.33	0.2	0.47**	0.27
	Wagga Wagga	0.15	0.17	0.42*	–0.07
	World Set	0.24	0.15	0.49**	0.10
82208	Wongan Hills	0.12	0.14	0.56**	0.00
	Turretfield	–0.07	0.39*	0.41*	0.21
	Wagga Wagga	0.18	0.23	0.42*	0.27
	World Set	0.17	0.02	0.41**	–0.28
2. Using labeled 23724					
21817	Wongan Hills	0.07	0.39*	0.37*	0.41*
22205	Wongan Hills	0.07	0.32	0.34	0.41*
	Turretfield	0.06	0.51**	0.42*	0.42*
22722	Wongan Hills	0.13	0.32	0.38*	0.38*
	Turretfield	0.15	0.31	0.31	0.25
24311	Wongan Hills	–0.01	0.41*	0.25	0.43*
	Turretfield	–0.06	0.45*	0.24	0.41*

[a] R_{max} = extensograph maximum resistance (BU); Ext = extensograph extensibility (cm); DT = farinograph development time (min).
[b] 38 Samples analyzed in each breeders set, 32 samples in World Set.
[c] Levels of significance: * ** *** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

TABLE III
Correlation Coefficients Between Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)-Quantified Glutenin Fractions and Enzyme-Linked Immunosorbent Assay (ELISA) Response

Solid phase MAb:		21817			23724	
Labeled MAb:	21817	22205	24311	21817	22205	24311
Wongan Hills[a]						
Grain protein	0.15	0.24	0.18	–0.07	0.07	–0.01
Reduced HMW-GS	0.29	0.28	0.29	0.16	0.18	0.10
Reduced LMW-GS	0.70***[b]	0.68***	0.68**	0.49**	0.50**	0.41**
Reduced total GS	0.62***	0.60***	0.60**	0.41*	0.43*	0.33*
Alkylated HMW-GS	0.25	0.28	0.30	0.22	0.26	0.21
Alkylated LMW-GS	0.68***	0.68***	0.69**	0.59***	0.61***	0.54***
Alkylated total GS	0.62***	0.62***	0.63**	0.53**	0.56**	0.49***
Turretfield						
Grain protein	0.19	0.20	0.12	nd	0.06	–0.06
Reduced HMW-GS	0.04	0.03	0.01	nd	0.15	0.09
Reduced LMW-GS	0.55**	0.59***	0.55**	nd	0.46*	0.25
Reduced total GS	0.36*	0.40*	0.35	nd	0.36	0.20
Alkylated HMW-GS	0.00	0.02	0.08	nd	0.24	0.11
Alkylated LMW-GS	0.35	0.41*	0.43*	nd	0.48**	0.30
Alkylated total GS	0.26	0.32	0.34	nd	0.45*	0.27

[a] 29 samples analyzed at Wongan Hills and Turretfield.
[b] Levels of significance: * ** *** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

ELISA (Brett et al 1993), correlations were found between LMW-GS binding antibody response and loaf volumes. This study had similar levels of significance for correlations using 23724 as detection antibody in a two-site format. Correlations were higher when a broad specificity MAb (24311) was immobilized for subunit capture.

Relationships Between Antibody Binding and RP-HPLC Data

The antibody responses obtained using 21817 as the detection antibody correlated significantly with LMW-GS contents determined by RP-HPLC (Table III). Correlation coefficients were higher and more consistent for LMW-GS content compared with total glutenin subunit contents, and also when unalkylated subunits were quantified. Correlations between LMW-GS content and antibody 21817 binding were high ($r = 0.55–0.70$) but there are two factors that probably limit the correlations. First, the relationship between antigen (i.e., LMW-GS) content and ELISA response is logarithmic, while HPLC peak areas should be linearly related to subunit content. Second, the antibody specificity data suggest that the antibodies were measuring a subpopulation of LMW-GS that is not present in a constant proportion within the glutenin complex of each sample. Several combinations involving antibody 23724 also exhibited correlations between antibody response and LMW-GS content measured by RP-HPLC, but the correlations were lower than for combinations that used 21817.

CONCLUSIONS

The typically strong dependence of extensibility on total protein content complicates development of an understanding of the chemical basis of variation in extensibility and therefore the development of simple screening tests. However, in the sets of samples evaluated in this and other studies (Andrews et al 1994), LMW-GS content correlated with dough extensibility even when total protein and extensibility did not correlate. We have demonstrated that the two-site ELISA can quantify major groups of LMW-GS. Significant correlations with dough extensibility and LMW-GS content can be obtained by ELISA analysis of flour sets. The strength of the correlations were determined by the specificity of the detection MAb even though the specificity of the immobilized MAb determines the range of LMW-GS to be available for detection. The superior correlations provided by the 21817 detection antibody suggest that extensibility relates more to the content of B-LMW-GS (bound by 21817) than total (A-, B- and C-) GS bound by 23724. However, irrespective of whether ELISA or HPLC methods are used to quantify total LMW-GS or the major groups of LMW-GS, the predictive value of these correlations is lower than would be ideal for most breeding situations. This may suggest that variation in LMW-GS content only contributes part of the variation in dough extensibility and that other flour components need to be determined in combination with LMW-GS to allow a good predictive method for extensibility.

Two additional approaches have been suggested. First, it has been demonstrated that biotypes of cultivars expressing chromosome 1D-encoded HMW-GS 2+12 exhibit greater extensibilities than those expressing 5+10, possibly because the former subunits may limit development of very large glutenin polymers (MacRitchie 1992). Second, several studies have suggested that allelic variation in B-LMW-GS composition correlates with differences in extensibility. Using some recently developed monoclonal antibodies with specificities for small clusters of LMW-GS (Hill, Andrews and Skerritt, unpublished), we are currently evaluating the ability of a direct ELISA format to monitor allelic variation in LMW-GS composition as it may relate to variation in extensibility.

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