# Characterization of the 1B-Type ω-Gliadins from *Triticum aestivum* Cultivar Butte

Frances M. DuPont,[1,2] William H. Vensel,[2] Ronald Chan,[2] and Donald D. Kasarda[2]

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**ABSTRACT**

ω-Gliadins were purified from wheat (*Triticum aestivum* L. 'Butte') flour and characterized. Although reversed-phase HPLC (RP-HPLC) separated the 1B-encoded ω-gliadins into two fractions, 1B1 and 1B2, these fractions had nearly identical amino acid compositions, three similar protein bands in SDS-PAGE, 10 similar spots in two-dimensional PAGE, and two similar N-terminal amino acid sequences. The main components had a range in mass of 48,900–51,500 when estimated by mass spectrometry, significantly less than the mass estimated by SDS-PAGE. The 1B fractions were digested with thermolysin, the peptides were separated by RP-HPLC, the peptide mass was determined, and nine peptides from each fraction were sequenced with nearly identical results for the 1B1 and 1B2 digests. A possible consensus sequence of the 1B-encoded ω-gliadin internal repeat was QQQXP, where X was F, I, or L in descending order of occurrence. The 1D-encoded ω-gliadins were purified by RP-HPLC as a single fraction that had one band in SDS-PAGE, two spots in two-dimensional PAGE, two components with mass of 41,923 and 42,770 detected by mass spectrometry, and two N-terminal sequences. Circular dichroism (CD) spectra for the 1B and 1D ω-gliadins were similar and were suggestive of mainly flexible random structure with a significant amount of the left-handed polyproline II helical conformation in the 1D components.

The ω-gliadin components of wheat flour are sulfur-poor prolamins with no cysteine. Closely related components that contain cysteine are referred to as D-glutenin subunits (Masci et al 1998). The ω-gliadins are composed almost entirely of high-glutamine, high-proline repeats and are related to the rye ω-secalins and the barley C-hordeins (Tatham and Shewry 1995). Several distinct ω-gliadins were identified in wheat based on electrophoretic patterns and N-terminal sequences, and their genes were localized to the *Gli-B1* and *Gli-D1* loci on the short arms of chromosome 1B and 1D, respectively (Kasarda et al 1983, Lafiandra et al 1984, Tatham and Shewry 1995, Zhao et al 1999). Additional ω-gliadin-like proteins, with genes located at the *Gli-A1* locus on the short arm of chromosome 1A are less well characterized. Wheat was estimated to have at least 15–18 copies of ω-gliadin genes (Sabelli and Shewry 1991). In this article, we refer to the *Gli-A1*, *Gli-B1*, and *Gli-D1* encoded ω-gliadins as the 1A, 1B, and 1D ω-gliadins.

Sulfur availability and the ratio of sulfur to nitrogen in the soil affect dough elasticity and resistance and loaf volume, probably because of the effect of sulfur-to-nitrogen ratios on the gluten protein composition of wheat flour (Zhao et al 1999). The relative abundance of the sulfur-poor prolamins, particularly the ω-gliadins, and the intermediate-sulfur high molecular weight glutenin subunits (HMW-GS), increased when wheat plants were depleted of sulfur (Wrigley et al 1984, Zhao et al 1999) but the significance of changes in ω-gliadin amounts to dough quality is unknown. Because the ω-gliadins have no cysteines, they do not form intra- or intermolecular disulfide bonds and do not participate covalently in the formation of gluten polymers. The role of ω-gliadins in dough formation and their significance to flour quality is not well defined (Tatham and Shewry 1995).

To understand the contributions of gluten proteins to the viscoelastic properties of wheat flour dough, it is essential to develop physical models of the individual components. Obtaining gene and protein sequence information is the first step in developing these models. Although the primary structure of many gliadins and glutenins has been determined by extensive protein and gene-sequencing efforts, no sequences of cDNA or genomic clones have been reported for ω-gliadins from wheat. In this study, we purified 1B ω-gliadins from a wheat flour extract and characterized them by N-terminal amino acid sequencing, matrix assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectrometry, and circular dichroism spectroscopy (CD). The 1B proteins were digested using thermolysin, and the resulting peptides were characterized by MALDI-TOF and N-terminal sequence. We also compared the 1B ω-gliadins with the 1D ω-gliadins from the same flour extract by amino acid composition analysis, N-terminal sequencing, mass spectrometry, and CD.

## MATERIALS AND METHODS

### Materials

Wheat plants (*Triticum aestivum* L. 'Butte') were grown in the greenhouse as previously described (Hurkman et al 1998). The plants were moved to a growth chamber after anthesis and exposed to a high temperature regime including 5 hr at 40°C daily for 15 days. Grain was harvested at maturity and ground to flour using a Quadramatic Jr. mill (C. W. Brabender, South Hakensack, NJ). Protein was determined using a nitrogen analyzer (Leco Corporation, St. Joseph, MI) and a protein-to-N ratio of 5.7. The grain from the heat-treated plants was >20% protein. Flour at 16% protein from greenhouse-grown plants was used for some experiments.

### Protein Extraction

*n*-Propanol (10 mL, 50%, v/v) was added to 1.5 g of flour in a 15-mL Falcon polypropylene centrifuge tube with a screw top (Becton Dickinson, Franklin Lakes, NJ), which was rotated for 20 min at 22°C using a hybridization oven (Robbins Scientific, Sunnyvale, CA), then centrifuged in an Eppendorf 5810R desk-top centrifuge (Brinkman Instruments, New York, NY) at 4,500 × *g* for 20 min. The supernatant was saved, the extraction was repeated twice, and the three supernatants were pooled and dried in a freeze-dry system (FreeZone, Labconco, Kansas City, MO) with a –80°C collector coil. Dried samples were stored at –20°C. Protein was determined by N analysis. As reported previously (Fu and Sapirstein 1996), 50% propanol extracted all of the gliadins along with a small amount of HMW-GS and LMW-GS. For large-scale preparation of ω-gliadins with reduced contamination by HMW-GS, 30 g of flour was extracted by stirring at room temperature with 300 mL of 40% ethanol, followed by centrifugation at 5,800 × *g* in a Sorval GSA rotor (Kendro Laboratory Products, Newtown, CT). The supernatant was centrifuged a second time, decanted, mixed with two volumes

[1] Corresponding author. Phone: 510-559-5702. Fax: 510-559-5818. E-mail: fmd@pw.usda.gov

[2] USDA Agricultural Research Service, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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of 1.5*M* NaCl, placed at 4°C overnight, and then centrifuged at 1,500 × *g* to pellet the gliadins. The gliadin-enriched pellet was rinsed with $H_2O$, dissolved in 50 mL of 0.1*M* acetic acid, and freeze-dried. The proteins (100 mg) were resuspended in 6 mL of 0.1*M* acetic acid and separated on a BioGel P-100 (BioRad, Hercules, CA) column with 0.05*M* acetic acid as eluant. Fractions enriched in ω-gliadins were freeze-dried. The large preparations were used mainly for CD.

**RP-HPLC**

Freeze-dried proteins were dissolved at a concentration of 1 mg of protein/mL in 6*M* guanidine HCL adjusted to pH 8.0 with Tris, plus 50 m*M* DTT, and 500 µL was applied to a Vydac (Hesperia, CA) 218TP C18 semipreparative RP-HPLC column (3-nm pore diameter, 10-mm i.d., 250-mm length). HPLC (HP Series 1100) was used to elute the proteins using a gradient of 10–90% (v/v) acetonitrile in 0.05% trifluroacetic acid (TFA) at 1.5 mL/min at 50°C.

**SDS-PAGE**

Freeze-dried 50% propanol extract from the equivalent of 50 µg of flour was suspended in 50 µL of sample buffer (Novex NuPage, Invitrogen, Carlsbad, CA) with Novex NuPAGE antioxidant by vortexing for 1 hr at 22°C in a microtube mixer (MT-360, Tomy Seiko, Tokyo, Japan), then centrifuged for 10 min at 14,000 rpm in a microfuge. The supernatant solution was transferred to a clean tube and 5 µL was loaded onto a Novex NuPAGE 4–12% acrylamide, bis-Tris gel and separated using the Novex MES SDS running buffer. HPLC fractions were freeze-dried, resuspended in 20 µL of sample buffer, and 5 µL was loaded onto the gel. The Mark12 protein standard (Invitrogen) was used. Gels were stained overnight using Brilliant Blue G (Sigma, St. Louis, MO) in 20% methanol and destained with water (Kasarda et al 1998).

**Two-Dimensional PAGE**

Samples from HPLC-purified fractions were separated by two-dimensional PAGE (Hurkman and Tanaka 1986) with the following modifications: a 3–10% ampholyte mixture was used, the isoelectric focusing gel was run for only 1 hr, giving nonequilibrium conditions, and the second dimension gel was a Novex 4-12% acrylamide, bis-Tris gel.

**Amino Acid Composition**

Amino acid composition was determined by the University of California-Davis Protein Structure Laboratory using ion-exchange chromatography followed by a postcolumn ninhydrin reaction to detect the amino acids (Ozols 1990).

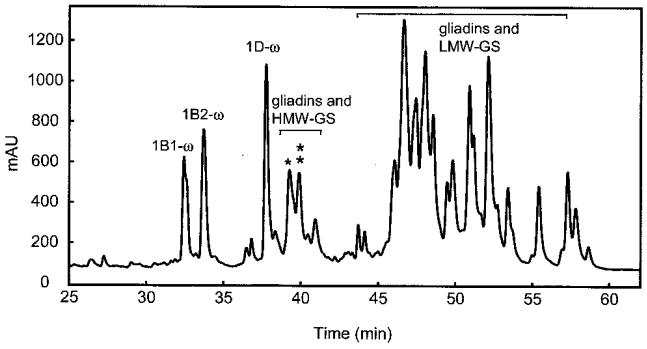


**Fig. 1.** Reverse-phase HPLC elution pattern of proteins extracted from Butte flour with 50% propanol. Proteins were reduced before being applied to HPLC column. Position of 1B and 1D ω-gliadin peaks, other gliadins, and small amounts of HMW-GS and LMW-GS are indicated. * = Peak containing a putative 1A-code ω-gliadin; ** = peak containing another unknown that coelutes with the HMW-GS.

**Thermolysin Digestion**

Freeze-dried protein (83 µg) was suspended in 1.0 mL of 50 m*M* Tris, pH 6.8, 4*M* urea, 2 m*M* $CaCl_2$ by stirring for 3 hr using a small stir bar in a microfuge tube, then 1.7 µg of thermolysin (cat. 161586, Boehringer Mannheim, Indianapolis, IN) was added and the protein was digested overnight with constant stirring at room temperature (≈22°C). The digest was acidified by adding 20 µL of glacial acetic acid, filtered, and injected onto the HPLC. Peptides were eluted as described above.

**MALDI-TOF Mass Spectrometry**

Protein samples, purified by RP-HPLC and dried in a centrifugal evaporator (Savant Speed-Vac, GMI, St. Paul, MN), were dissolved in 50 µL of 30% (v/v) acetonitrile containing 0.1% TFA, and mixed with an equal volume of saturated matrix (3,5-dimethoxy-4-hydroxycinnamic acid) dissolved in 30% acetonitrile, 0.1% TFA. Samples (≈0.5 µL) were spotted onto an MALDI target, allowed to dry at room temperature, and analyzed. HPLC-purified peptides from the thermolysin digestion were spotted onto dried matrix rather than being mixed with it. For the peptides, the matrix was a mixture of 20 g/L α-cyano-4-hydroxy-trans-cinnamic acid (Aldrich, Milwaukee, WI) and 5 g/L nitrocellulose dissolved in acetone-propanol (1:1) (Schevchenko et al 1996). The target surface for the peptides was prepared using ≈0.5 µL of matrix solution deposited on the MALDI target to produce a thin film of matrix after rapid evaporation of the solvent. Small portions of the sample (typically 0.5 µL) were then deposited onto the dried matrix and allowed to dry. The dried spots were washed by applying 10 µL of 0.1% TFA. After ≈10 sec, the TFA solution was removed by vacuum aspiration. Spectra were taken in the positive ion mode using a MALDI mass spectrometer (Reflex II, Bruker Daltonics, Billerica, MA). The instrument was operated in the linear mode for proteins, but the delayed extraction and reflectron mode was used for peptides. External calibration for proteins was performed using both the double- and single-charged ion of bovine serum albumin. For peptides, the matrix ion (α-cyano-4-hydroxy-*trans*-cinnamic acid), adrenocorticotropic hormone (clip 18–31) and insulin were used as calibrants.

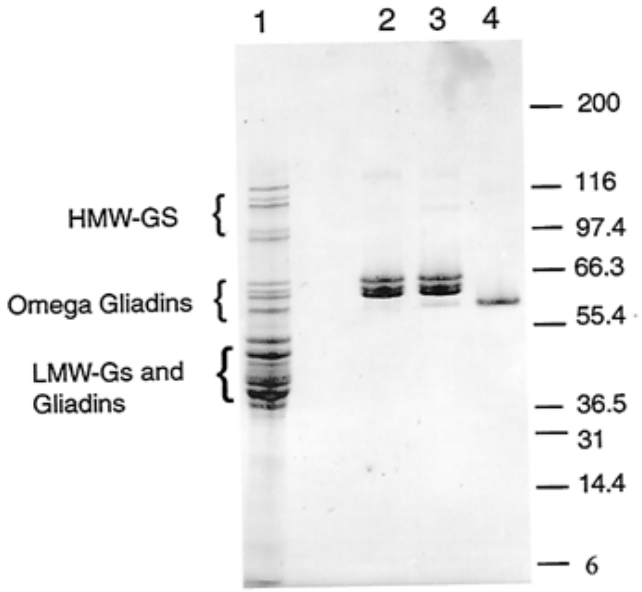


**Fig. 2**. SDS-PAGE analysis of a 50% propanol extract of Butte flour (lane 1) and three fractions obtained by reverse-phase HPLC of the propanol extract: 1B1, 1B2, and 1D ω-gliadin fractions (lanes 2–4, respectively). Positions of molecular mass markers (× $10^{-3}$) indicated on the right. All samples reduced with dithiothreitol.

**Edman Sequencing**

Proteins and peptides were sequenced using an automatic protein sequencer (model 477A, Applied Biosystems, Foster City, CA) equipped with an online 120A HPLC system for identification of phenylthiohydantoin (PTH)-amino acids. The sample of protein or peptide to be sequenced was dissolved in 20 µL of 50% (v/v) acetonitrile containing 0.1% TFA, immediately applied to a preconditioned coated glass fiber filter (Biobrene, Applied Biosystems) and dried. A standard cleavage time of 5 min at 48°C was used for the ω-gliadins. The peptides from the thermolysin digest were sequenced using a cleavage time of 10 min at 53°C (Vensel and Kasarda 1991). Sequence determination was made by visual inspection of the chromatograms and by examination of the data as analyzed by the data analysis software (vers. 1.61, Applied Biosystems).

**CD**

CD spectroscopy was conducted using a spectropolarimeter (model J-715, Jasco, Easton, MD). Measurements were made at 20°C in a 0.01-cm pathlength cell with a scan speed of 50 nm/min and resolution of 0.1 nm. Sensitivity was 50 millidegrees. Spectra were recorded as an average of 10 scans. Bandwidth was 1.0 nm and response was 4 sec. The solvent was 1 m*M* phosphoric acid (pH 3), and a solvent blank run under the same conditions was subtracted from the protein scan. The HPLC-purified protein samples were repurified by HPLC, freeze-dried, redissolved in water, and again freeze-dried to diminish the amount of TFA in the sample. Initially, protein concentrations of the two fractions were determined relative to one another by weight. The dried protein samples (≈2–3 mg) were placed in a freeze-drying flask and equilibrated overnight (≈15 hr) on the freeze-dryer at a pressure of 12 Pa. The exact weight of the protein sample was determined immediately after the sample was removed from the freeze-dryer and it was dissolved in a weighed amount of solvent at a concentration of ≈1 mg/mL. The samples dissolved readily and there was no obvious turbidity. Before CD measurements were made, the sample was clarified by centrifugation at 10,000 × *g*, which did not change the concentration significantly as indicated by the ultraviolet (UV) absorption at 275 nm. Although the solution was clear, there was slight aggregation noted as light scattering in the 350–300 nm range of the UV spectrum for the 1B fraction.

The actual concentration of the 1D ω-gliadin sample was determined from the UV spectra of the protein solutions using the extinction coefficient of 0.332 (absorption of a 1 mg/mL solution, 1-cm path, at 275 nm) for a similar protein, ω-gliadin 17, (Popineau et al 1986). The concentrations of the 1B ω-gliadin fractions were adjusted on the basis of weight relative to those of the 1D fractions. Although the UV spectrum of ω-gliadin 30 (Popineau et al 1986) was similar to that of our 1B fractions (data not shown), the ratio of the absorbances at 258–275 nm for ω-gliadin 30 appeared to be different from the ratio for our 1B components. [Note: the designations for the two spectra, component 30 vs. component 17, were accidentally reversed in Fig. 6 of the publication of Popineau et al (1986) according to a personal communication from Y. Popineau.] Spectra were analyzed for secondary structures by the method of Johnson (1999).

## RESULTS

**Purification of ω-Gliadins**

The proteins in the 50% propanol extract were reduced and separated by RP-HPLC (Fig. 1). The protein peaks were identified

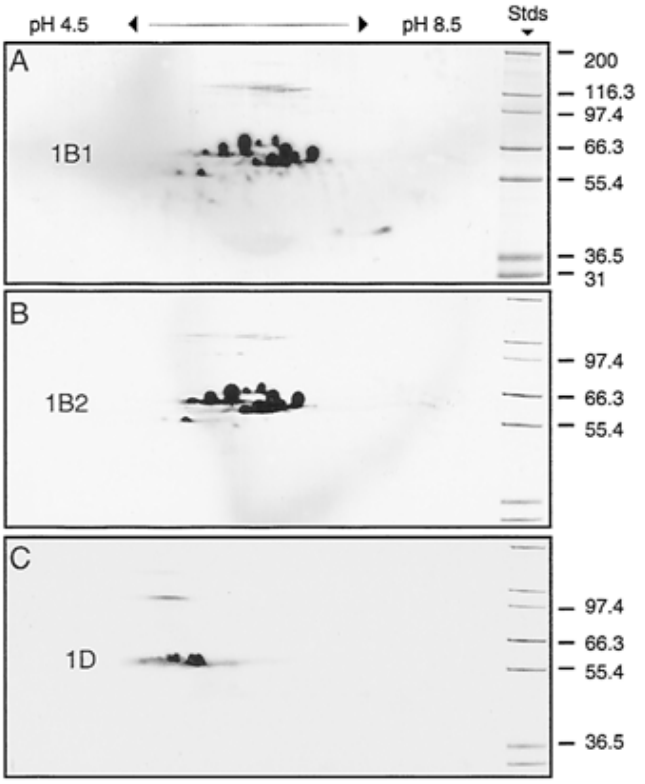


**Fig. 3**. Two-dimensional PAGE analysis of the 1B1 (**A**), 1B2 (**B**), and 1D (**C**) ω-gliadin HPLC fractions.

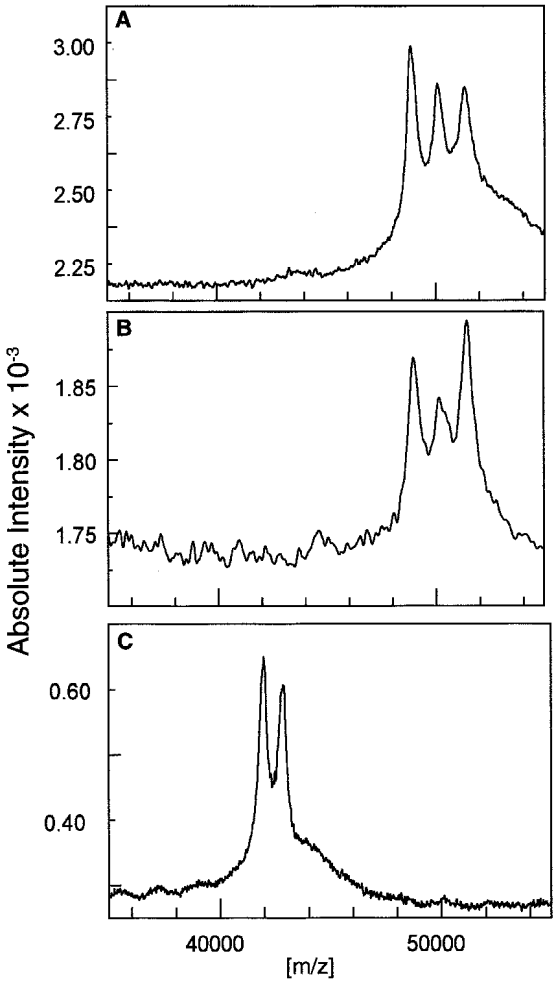


**Fig. 4**. Molecular mass of proteins in the ω-gliadin fractions determined by MALDI-TOF analysis. Spectra, plotted as absolute intensity vs. mass-to-charge ratio (m/z) are shown for the 1B1 (**A),** 1B2 (**B),** and 1D (**C)** ω-gliadin fractions.

based on retention times, SDS gel analysis of the apparent molecular mass of the proteins in each peak, and previous experience with separation of wheat storage proteins (Kasarda et al 1983, 1998). The proteins in the first two peaks that eluted were classified as 1B ω-gliadins (Figs. 1 and 2). They were followed by a peak of 1D ω-gliadins. Two additional peaks of moderate height followed the 1 D ω-gliadins. One contained an ω-gliadin-like protein, possibly a 1A encoded ω-gliadin, and the other an unidentified protein of similar mass to the ω-gliadins, which was not characterized further. Small amounts of HMW-GS eluted in the same range as the later two peaks. For convenience, the 1B ω-gliadin peaks are referred to as 1B1 and 1B2, in order of elution. In the example shown in Fig. 1, the flour was from heat-treated plants with a high percent protein. This flour sample was used because it was enriched in ω-gliadins compared with the other proteins in the 50% propanol fraction.

The proteins in the 50% propanol extract and in the HPLC fractions were analyzed by SDS-PAGE (Fig. 2). Results are shown only for the extract and the first three HPLC fractions. The five HMW-GS were resolved at apparent molecular mass of 80,000–115,000 (lane 1), the cluster of ω-gliadins gave three bands with apparent molecular mass of 57,000–65,000 (lanes 1–4), and LMW-GS and other gliadins were resolved at apparent molecular mass of 34,000–55,000 (lane 1). The 1B1 and 1B2 peaks from HPLC were each resolved by SDS-PAGE into three bands with apparent molecular mass of 60,000, 61,700, and 64,000 (lanes 2 and 3) and the 1D peak was resolved into a single band with apparent molecular mass of 57,000 (lane 4). Two-dimensional PAGE resolved the 1B1 and 1B2 fraction into 10 proteins each, with nearly identical patterns for the two fractions (Fig. 3A–B). The 1D fraction was resolved into two proteins (Fig. 3C). The apparent molecular mass estimated from the gel of Fig. 3 was slightly higher than those estimated from Fig. 2. This is not unusual when one-dimensional gels are compared with two-dimensional gels, possibly because the initial retardation of the proteins by the first dimension gel is greater than when solution is loaded directly as in one-dimensional gels.

**TABLE I**
**Amino Acid Compositions of 1B and 1D ω-Gliadin Fractions from Butte Flour**

| Amino Acid[a] | nmol | | | % | | |
|---|---|---|---|---|---|---|
| | 1B1-ω | 1B2-ω | 1D-ω | 1B1-ω | 1B2-ω | 1D-ω |
| | nmol | nmol | nmol | % | % | % |
| Asp | 0.2 | 0.2 | 0.3 | 0.4 | 0.4 | 0.5 |
| Thr | 0.3 | 0.3 | 0.9 | 0.6 | 0.7 | 1.6 |
| Ser | 1.3 | 1.4 | 2.9 | 2.8 | 2.9 | 5.2 |
| Glu | 26.0 | 25.8 | 24.5 | 54.4 | 53.8 | 43.7 |
| Pro | 9.4 | 9.4 | 16.5 | 19.8 | 19.5 | 29.5 |
| Gly | 0.6 | 0.9 | 0.7 | 1.2 | 1.8 | 1.2 |
| Ala | 0.2 | 0.2 | 0.4 | 0.4 | 0.5 | 0.7 |
| Val | 0.1 | 0.1 | 0.2 | 0.3 | 0.3 | 0.4 |
| Met | 0.04 | 0.04 | 0.01 | 0.1 | 0.1 | 0.0 |
| Ile | 1.9 | 1.9 | 1.0 | 4.0 | 3.9 | 1.7 |
| Leu | 1.4 | 1.5 | 2.2 | 2.8 | 3.1 | 3.9 |
| Tyr | 0.4 | 0.5 | 0.8 | 0.9 | 1.1 | 1.5 |
| Phe | 4.5 | 4.4 | 4.7 | 9.4 | 9.1 | 8.4 |
| His | 0.8 | 0.7 | 0.4 | 1.6 | 1.5 | 0.7 |
| Lys | 0.4 | 0.4 | 0.2 | 0.7 | 0.7 | 0.4 |
| $NH_3$[b] | 26.2 | 26.2 | 23.3 | | | |
| Arg | 0.4 | 0.4 | 0.3 | 0.8 | 0.8 | 0.6 |
| Total | 47.8 | 48.0 | 57.0 | 100.0 | 100.0 | 100.0 |

[a] Tryp and Cys were not determined.
[b] Not included in total.

**TABLE II**
**N-Terminal Amino Acid Sequences for Proteins in 1B and 1D ω-Gliadin Fractions from Butte Flour Compared with Published N-Terminal Amino Acid Sequences**

| Protein | N-Terminal Amino Acid Sequence |
|---|---|
| *T. longissima* ω-gliadin -1[a] | SRQLSPI |
| *T. longissima* ω-gliadin -2[a] | SRQISPIGKELXXP |
| *T. longissima* ω-gliadin -2[a] | SRQLSPIGKELQTP |
| *T. aestivum* 1B ω-gliadin[b] | SRLLSPRGKELHTPQQQFPQQ |
| ***T. aestivum* 'Butte' 1B1 ω-gliadin** | **SRLLSPRGKELHTPQEFQFPQQQ** |
| | **G M** |
| | **T** |
| ***T. aestivum* 'Butte' 1B2 ω-gliadin** | **SRLLSPRGKELHTPQEQFPQQQ** |
| | **G M** |
| | **T** |
| *T. aestivum* 1D ω-gliadin[c] | ARELNPSNKELQSPQQSFS |
| ***T. aestivum* 'Butte' 1D ω-gliadin** | **ARELNPQNKEL** |
| *T. aestivum* 1D ω-gliadin[d] | KELQSPQQSFSHQQQPFPQQ |
| ***T. aestivum* 'Butte'1D ω-gliadin** | **KELQSPQQSF** |

[a] Sequence for ω-gliadins from *Triticum longissima* (Odintsova and Egorov 1990).
[b] ω-5 (Kasarda et al 1983).
[c] ω-5 (Kasarda et al 1983).
[d] ω-5 (Kasarda et al 1983).

## Determination of Molecular Weight by Mass Spectrometry

The actual mass of the ω-gliadins was determined by MALDI-TOF analysis (Fig. 4). The 1B1 fraction was resolved into peaks with molecular mass of 48,900, 50,100, and 51,300 (Fig. 4A), the 1B2 fraction was resolved into three peaks with molecular mass of 49,085, 50,300, and 51,500 (Fig. 4B), and the 1D fraction was resolved by MALDI-TOF into two peaks with molecular mass of 41,923 and 42,770 (Fig. 4C). The mass of the 1B ω-gliadins was 18–20% lower, and the mass of the 1D ω-gliadins was 27% lower than the apparent molecular mass determined by SDS-PAGE.

## Amino Acid Composition

The total amino acid compositions of the three ω-gliadin fractions were determined (Table I). The amino acid compositions of the 1B1and 1B2 ω-gliadin fractions were nearly identical. As for most gluten proteins, the principal amino acid components were glutamine and proline. The 1B ω-gliadin fractions had glutamine-to-proline ratios of 2.75, and the 1D ω-gliadin fraction had a glutamine-to-proline ratio of 1.48. All glutamic acid was assumed to be in the form of glutamine (Wieser et al 1982), which is approximately correct, as indicated by the amount of ammonia released.

## Determination of N-Terminal Amino Acid Sequences

N-terminal amino acid sequences were determined for the proteins in the 1B1, 1B2, and 1D ω-gliadin fractions (Table II). The sequence data for the 1B1 and 1B2 ω-gliadins are compared with the N-terminal sequences of three ω-gliadins from *T. longissima* and to the previously published N-terminal sequence of a 1B-encoded ω-gliadin from *T. aestivum*. The 1B1 and 1B2 fractions

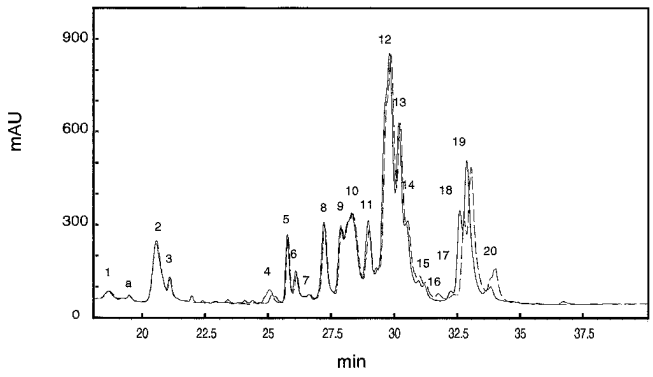


**Fig. 5.** Reverse-phase HPLC analysis of peptides obtained by digesting proteins in 1B1 (——) and 1B2 (- - - -) ω-gliadin fractions with thermolysin. Numbers indicate fractions collected for further analysis.

each were composed of at least three different proteins, on the basis of the N terminal amino acid sequences, differing in at least two positions. Although leucine was the predominant amino acid at cycle 3, a definite methionine residue was also present in cycle 3 at ≈67% of the leucine amount. The N-terminal sequence data for the 1D ω-gliadin fraction gave two strong amino acid signals at each amino acid position. The sequence pattern was almost identical to that from a combination of the previously published sequences for 1Dω-1 and 1Dω-2, in agreement with the two-dimensional PAGE and the mass spectrometry data that indicated that the 1D ω-gliadin fraction contained at least two major peptides.

**Analysis of Thermolysin Digests**

To obtain internal sequence data for the 1B ω-gliadins, the 1B1 and 1B2 fractions were separately digested using thermolysin, which cleaves proteins N-terminal to hydrophobic resides, such as L, I, T and F, unless a proline is on the C-terminal side of the residue. The extent of the digestion was limited by performing the reaction at room temperature. When the peptides were separated by RP-HPLC, the profiles for the 1B1 and 1B2 thermolysin digests were almost identical, giving ≈20 peaks each (Fig. 5). RP-HPLC fractions containing the principal peptide peaks were collected, and the molecular mass of the peptides in each fraction were determined by MALDI-TOF analysis. The results for the corresponding fractions from the 1B1 and the 1B2 digests were compared. The MALDI-TOF patterns of nine representative pairs of peptide fractions are shown in Fig. 6. Some fractions contained a single peptide as illustrated for fractions 1, 3, and 5, but others contained several peptides. The MALDI-TOF patterns for most of the 1B1 and 1B2 peptide fractions were nearly identical, although some peptides differed a small amount in mass, and a few peaks were faint and harder to visualize in one or the other of the pairs.

Seven peptides from the 1B1 and 1B2 digests were sequenced, along with an additional peptide from the 1B2 digest (Table III). The peptides that were sequenced were from RP-HPLC fractions that had one major component in the MALDI-TOF analysis. The sequences for the 1B1 and 1B2 peptides were nearly identical. The sequence of the peptide in the 1B1 fraction 1 was identical to that of one of the 1B1 N-terminal amino acid sequences, except that it appeared to be truncated, because it was missing the first two amino acids as a consequence of the thermolysin cleavage N-terminal to leucine. The sequence of the 1B2 fraction 1 was identical to the 1B2 N-terminal sequence that began with T. The cleavage between residues 2 and 3 did not occur, presumably because of incomplete digestion by the thermolysin at room temperature. The other peptides that were sequenced were rich in proline and glutamine. Some had unique patterns, but most contained repeated patterns of two, three, or four glutamines (Q), followed predominantly by FP, but sometimes with the F substituted by I or L. The repeated sequences are underlined in Table III.

**Circular Dichroism**

Because the amino acid composition and amino acid repeat sequences of the 1B ω-gliadins had more glutamine and less proline than those of the 1D ω-gliadins, we compared their secondary structures using circular dichroism spectroscopy (Fig. 7). There was no significant difference between the spectra for the 1B1 and 1B2 fractions (not shown). The CD spectra of the 1B and 1D components were qualitatively quite similar, but the negative band near 200 nm was more intense for the 1D fraction (Fig. 7).

## DISCUSSION

Despite extensive efforts to clone genes for gliadins and glutenins, some gene sequences appear to be rare or absent from the available cDNA and genomic libraries (Masci et al 1998, Altenbach 1998). It is possible that some highly repetitive gene sequences are not replicated properly by the usual bacteria and phage combinations used to make the DNA libraries. In the case of the 1B ω-gliadins, no cDNA or genomic clone has been reported, and the mass and peptide sequences reported in this article are the only information available on the exact size and internal protein sequence. Also, most physical studies of the ω-gliadins and C-hordeins were conducted using protein preparations that were probably similar to our 1D ω-gliadin fraction. (Tatham and Shewry 1985, Field et al 1986, Tatham et al 1989, I'Anson et al 1992,

**TABLE III**
**N-Terminal Amino Acid Sequences for Selected Peptides from Thermolysin Digests of 1B ω-Gliadins Fractions**

| Fraction[a] | Cycle Number 1 5 10 15 20 25 30 35 |
|---|---|
| **1B1-ω** | |
| 1 | L L S P R G K E – |
| 2 | I S Q Q P Q Q L P Q Q Q Q I P[b] – |
| | L |
| 3 | I S Q Q P Q Q L P Q Q Q I P Q Q P – |
| | L |
| 5 | I S Q Q P Q Q L P Q Q Q Q I P – |
| 9 | F H Q Q Q L P Q Q Q F P Q Q Q F P Q Q Q F P Q – |
| | I A |
| 13 | L H Q P Q E Q F P Q Q Q Q F P Q P Q Q F P Q L P I – |
| | F L Q F P P H F P |
| 19 | L T Q Q Q F P R P Q Q S P E Q Q Q F P Q Q Q F P Q Q P P Q – F P Q Q – F P I – |
| | R |
| **1B2-ω** | |
| 1 | T R L L S P R G K E L – |
| a | I S Q Q P Q Q L P Q Q Q Q I P – |
| | L G G |
| 3 | I S Q Q P Q R L P Q Q Q Q I P – |
| | L Q |
| 4 | L Q Q Q F P Q L K L P Q Q I F P Q Q Q Q F – |
| | I P Q P Q E P P |
| 5 | I S Q Q P Q Q L P Q Q – Q I P Q Q P Q – |
| 9 | F H Q Q Q L P Q Q Q F P Q Q Q F P Q Q Q F P Q – |
| | I D P L P I P |
| 13 | L L Q P Q E Q F P Q Q Q Q F P H P F P F P Q L I P Q – |
| | F H T Q F F P P Q Q Q |
| 19 | L T Q – – – – – – Q Q S P E Q Q Q F P Q Q Q F P Q Q P P Q – F P Q Q – F P I – |
| | R |

[a] Fraction numbers as in Fig. 5.
[b] Repeat regions with sequences consisting of QQ, QQQ, or QQQQ followed by FP, IP, or LP are underlined.

Thomson et al 1999). The electrophoretic patterns in Tatham and Shewry (1985) indicate that their ω-gliadin fraction 1 was likely to be similar to our 1D fraction, possibly also including 1A ω-gliadins, and their fraction 2 was probably similar to our 1B fraction, though the results for fraction 2 were not shown. Because 1D ω-gliadins and C-hordeins are similar to one another in sequence and composition, physical studies of one are likely to apply to the other. However, we are not aware of any physical studies that have clearly focused on proteins similar to our 1B ω-gliadins.

We found that the molecular weights of the 1B and 1D ω-gliadins were greatly overestimated by SDS-PAGE. This is often true for gluten subunits, probably because the gluten peptide-SDS complexes are more elongated than those of the protein molecular weight standards (Hamauzu et al 1974). It is also possible that they do not bind SDS as well as most other proteins because of the hydrophilic nature of these proteins and a scarcity of charged side chains. Published estimates of the molecular weight of ω-gliadins obtained by SDS-PAGE, gel filtration, and sedimentation equilibrium ultracentrifugation (Popineau et al 1986, Tatham and Shewry 1995) vary widely. They are generally much higher than the molecular mass obtained by mass spectrometry in this study, except for one estimate, which is much lower (Hamauzu et al 1974). The surface hydrophobicity of the ω-gliadins must be lower than for other gluten subunits because they were the first peptides to elute from the RP-HPLC column and they are the most hydrophilic of the gluten proteins in terms of total amino acid composition.

The Butte 1B ω-gliadin fractions were complex. They were separated into two peaks by RP-HPLC, which were each separated into three polypeptides by SDS-PAGE and mass spectrometry, and into 10 polypeptides by two-dimensional PAGE. N-terminal sequence data indicated that there were at least six different proteins. The primary structures of the 1B ω-gliadins must be very similar, however, because RP-HPLC of the thermolysin digests of both HPLC fractions gave almost identical elution profiles, peptide mass determined by MALDI-TOF was nearly identical, and the N-terminal sequences were essentially the same. The 1B ω-gliadin N-terminal sequences were similar to those of the ω-gliadins of *T. longissima* (Odintsova and Egorov 1990), which is related to *T. speltoides*, the ancestor of the B genome. It is likely that all of the 1B ω-gliadins originated by gene duplication from a single gene and that subsequent changes in protein sequence have been relatively minor.

There was only one 1D ω-gliadin HPLC peak, and only one band was observed in SDS-PAGE. However, two peaks were observed by mass spectrometry and there were at least two different proteins, with N-terminal sequences beginning with ARE and KEL. Previously, these were separated by ion-exchange chromatography, char-

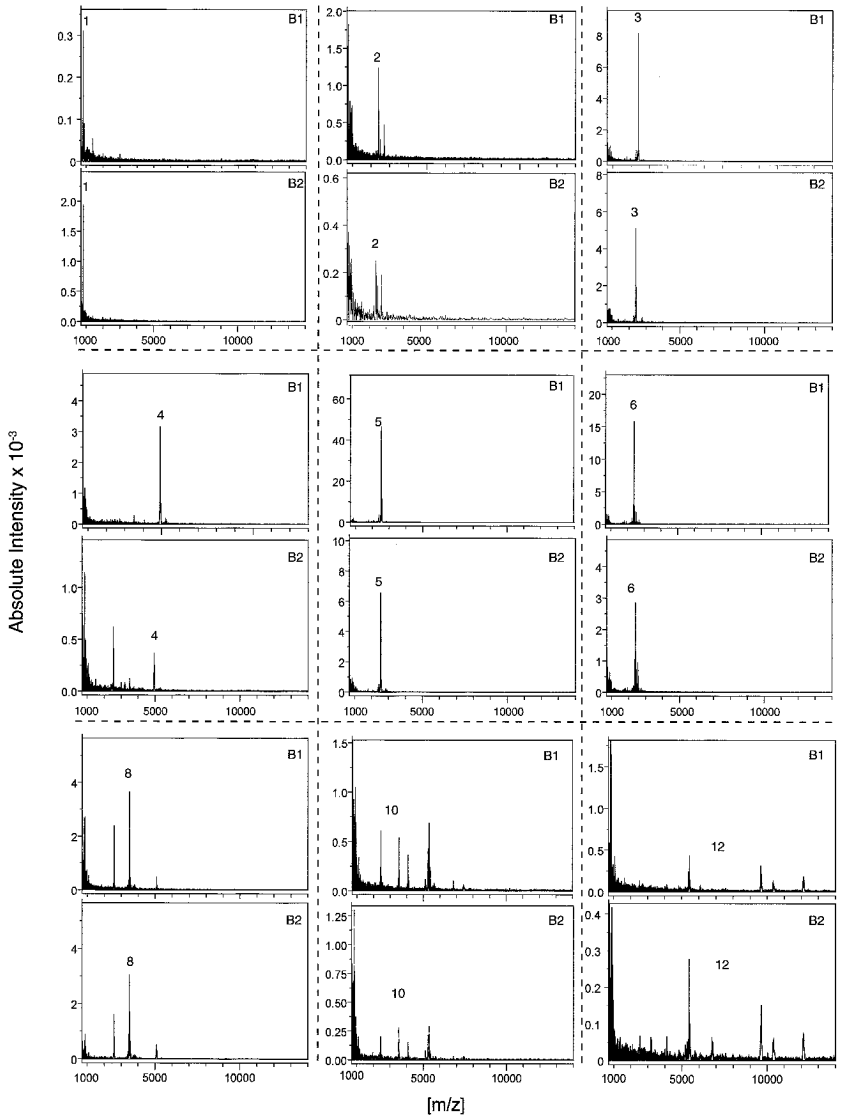


**Fig. 6.** Molecular mass of peptides obtained by MALDI-TOF for nine of 20 fractions collected by reverse-phase HPLC after digesting 1B1 and 1B2 ω-gliadin fractions with thermolysin. *x*-Axis scale for mass-to-charge ratio (m/z) of the peptides begins at 1,000; lower values were mostly noise. Numbers above the principal peak or peaks in the spectra refer to RP-HPLC fraction numbers in Fig. 4; B1 or B2 indicate fractions from 1B1 or 1B2 digests.

acterized, and termed the ARE- and the KEL-type ω-gliadins (Kasarda et al 1983, Masci et al 1991). The ARE-type ω-gliadin is most similar to the barley C-hordeins and the rye ω-secalins. The KEL-type ω-gliadin is similar to the ARE-type except for the loss of the first eight residues and probably arose from a gene duplication.

The amino acid compositions of the 1B and 1D ω-gliadin fractions from Butte were in good agreement with previously published results (Charbonnier 1974, Kasarda et al 1983). The 1B ω-gliadins with a glutamine-to-proline ratio of ≈2.8:1 have a greater proportion of glutamine than the 1D ω-gliadins or any other known gluten proteins (Kasarda et al 1983, Tatham and Shewry 1995). The glutamine-to-proline ratio for the 1D ω-gliadins, in contrast, is ≈1.5:1. For both the 1B- and 1D-types, the combined glutamine and proline contents amounted to 74 mol% of the total. When the third most predominant amino acid, phenylalanine, was included, these three amino acids made up 83% of the total. The 1B1 and 1B2 internal peptides characterized in this study had glutamine-rich repeats of QQXP, QQQXP, or QQQQXP, where X is F, I, or L in order of predominance. This repeat sequence is distinct from the PQQPFPQQ repeating sequence found both in hordeins (Tatham et al 1989) and in the gene sequence for a 1D-type ω-gliadin (O. D. Anderson, *personal communication*). When the genes for the 1B ω-gliadins are cloned and sequenced, a full analysis of the repetitive and nonrepetitive sequence regions will be possible. Based on the amino acid composition, we expect that the high ratio of glutamine to proline will be present throughout most of the entire sequence.

Because the 1B ω-gliadins appear to have a repeating sequence different from that for the 1D ω-gliadins and C hordeins (Tatham et al 1989), we asked whether the 1B and 1D types also differed in conformation. Our studies were made in dilute solution at room temperature. Although the conformation under these conditions probably does not closely mimic the situation in a dough where the proteins are highly concentrated and have been subjected to considerable shear, determination of the conformations in dilute solution is of scientific interest and to a large extent necessary for the interpretation of studies made under other conditions.

In the 1D w-gliadins, the residues PFP, and possibly QPFP would be expected to assume the left-handed polyproline II helical structure (PPII) (Stapley and Creamer 1999), so that if the 1D ω-gliadins consisted of perfect repeats, the proteins would include 38–50% PPII structure. In contrast, because glutamine residues in series of two, three, or four may tend to assume a random coil conformation (Masci et al 1998), and a single proline in a sequence may not promote significant amounts of the PPII (Creamer 1998), the 1B ω-gliadins might be expected to have almost no PPII structure.

The CD spectra were generally similar for the 1B and 1D ω-gliadins, and Tatham and Shewry (1985a) reported that the CD spectra of their fraction 1 and 2 ω-gliadins were almost identical to one another. Our spectra were also highly similar to the spectrum expected for polypeptides in the flexible, unordered conformation (Toumadje and Johnson 1995), both in shape and band intensity. Our spectra were similar in shape but not intensity, to that expected for polypeptides in the PPII conformation (Jenness et al 1976, Woody 1992, Bienkiewicz et al 2000). Although the spectra for the random coil and PPII conformations resemble each other qualitatively, they differ in the intensity of the negative band near 200 nm, which is ≈ 60,000 deg cm$^2$/decimole in the spectrum of polyproline itself when in the PPII conformation, but only 16,000 deg cm$^2$/decimole in the spectrum of flexible, unordered peptides (Jenness et al 1976, Rabanal et al 1993, Park et al 1997). If we assume that the intensity of the negative band at 200 nm is a measure of the degree of PPII structure in the ω-gliadins, then the 1D ω-gliadins in Fig. 7 would have ≈18% PPII structure and the 1B fraction would have approximately none. This result may be compared to the prediction above that the 1D fraction would have 38–50% PPII structure if the entire molecule were made up of perfect consensus repeats and the 1B fraction would have almost none.

We used the algorithm of Johnson (1999) to analyze the CD spectra for structure content of the 1B and 1D ω-gliadins as an exercise, even though the algorithm is not intended for use with proteins other than globular proteins that have essentially static structure. The resulting predictions were, for the 1B fractions: 1% α-helix, 4% $3_{10}$-helix, 21% β-strands, 15% turns, 13% PPII, and 46% other (unordered); for the 1D fraction: 1% α-helix, 8% $3_{10}$-helix, 16% β-strands, 21% turns, 9% PPII, and 46% other (unordered).

The ω-gliadins seem likely to have dynamic structures to a considerable extent but may include sequences that assume a stable conformation in solution. Although PPII regions might be considered static structures, because PPII does not involve any internal hydrogen bonding, it may have some aspects of a dynamic structure in essentially linear proteins and peptides in solution as well as well-defined PPII structures that are fixed by tertiary interactions in globular proteins.

Other structures beyond flexible coil and PPII may be present to some degree and contribute to the observed spectra. Extended linear structure (β-strand) and, possibly, β- or γ-turns would contribute positive ellipticity at 200 nm (Madison and Kopple 1980, Woody 1996), thereby diminishing the negative ellipticity at this wavelength. Nevertheless, we suggest that the structure of the 1D ω-gliadins consists mainly of a mixture of flexible coil and PPII structure by way of an interpretation similar to that applied by Johnson and Toumadje (1995) to the peptide systemin, while not ruling out smaller amounts of β-strand and β- or γ-turn structure. We suggest that the structure of the 1B ω-gliadin fraction is similar but with significantly less PPII structure. Our model should result in a stiffened, extended coil, in accord with the worm-like chain of I'Anson et al (1992). The tendency for glutamine side chains to form multiple hydrogen bonds might result in interactions among the glutamine side chains and with main chain amides of the same polypeptide chain in a way that stabilizes an extended backbone chain as proposed for the repeat region of HMW-GS (Kasarda 1994). Although such interactions would be in competition in aqueous solutions with hydrogen bonding to the surrounding water, a dynamic equilibrium might result so that significant numbers of such hydrogen-bonded side chain loops exist at any given time. Such a hydrogen bond-stiffened chain would contribute to the apparent asymmetry of ω-gliadins and would apply to both our 1D and 1B ω-gliadin fractions. It has been proposed that C-hordeins, and thus the similar 1D ω-gliadins, have frequent, regular β-turns organized in a loose spiral form, similar to the β-spiral proposed for elastin, interspersed with PPII structure (Tatham et al 1989). Our model may be considered an alternative to the model of Tatham et al (1989).

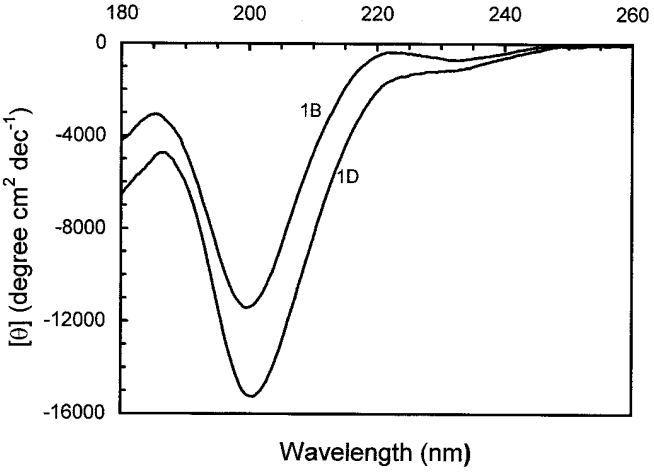


**Fig. 7.** Circular dichroism spectra of 1B1 and 1D ω-gliadin fractions in 0.001*M* phosphoric acid. Results presented as molar (mean residue) ellipticity (θ) plotted as a function of wavelength in nm. Residue weight assumed to be 110.

Wheat flour is one of the most complex food ingredients in use, and the processes by which the gluten proteins interact to form a high quality dough are still not well understood. In this article, we present information about the structure of one group of dough constituents, the sulfur-poor ω-gliadins. The repetitive structure of gluten subunits may play an important role in the unique viscoelastic properties of the gluten protein complexes, although evidence for this is lacking, as well as having a role in the immunogenic activity of these proteins (Ensari et al 1999). Our data support the idea that the somewhat flexible but extended ω-gliadin molecules interact cohesively with other dough proteins. The extremely high proportion of glutamine in the ω-gliadins, especially the 1B ω-gliadins, is likely to enhance this cohesiveness through strong hydrogen bonding interactions. On this basis, the contributions of ω-gliadins to dough properties might be larger than would be expected on the basis of relatively low proportions in gluten.

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# Erratum

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Characterization of the 1B-Type ω-Gliadins from *Triticum aestivum* Cultivar Butte

*Frances M. DuPont, William H. Vensel, Ronald Chan, and Donald D. Kasarda*

Cereal Chem. 77(5):607–614

'Butte' should read 'Butte 86' throughout.

The sequence 1B1 in Table II should read: SRLLSPRGKELHTPQEQFPQQQ