Theor Appl Genet (2003) 106:629–635
DOI 10.1007/s00122-002-1091-1

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The characterisation and mapping of a family of LMW-gliadin genes: effects on dough properties and bread volume

Received: 20 February 2002 / Accepted: 5 July 2002 / Published online: 19 October 2002

Abstract Analysis of a cDNA library from wheat cv Wyuna endosperm, indicated a significant size and sequence variation among seed-endosperm protein genes. In this study, a family of low-molecular-weight seed protein genes are analysed that are related to the gliadins and the low-molecular-weight glutenin subunits. Sequence analysis and comparison of these proteins showed that they are closely related to a 17-kDa protein from barley, ε hordein, which plays a role in beer foam stability in the brewing industry. Mapping of these genes in wheat shows that they are located on group 7 and 4 chromosomes, as opposed to a group 1 and 6 location for the glutenins and gliadins. It is possible that this family of proteins forms a new class of seed-endosperm proteins important in defining the quality characteristics of wheat flour. Therefore, a representative gene from this family was expressed in *Escherichia coli* and the purified protein was supplemented into a base wheat flour. Rheological analysis showed that the protein effected dough strength and resistance break down during mixing of the dough, and provided a 20% increase in loaf height after baking.

Keywords Wheat endosperm · Low-molecular-weight gliadin · Seed protein · Dough rheology · Mapping of SNPs

Introduction

Large-scale sequence analysis of cDNA libraries provides expressed sequence tags (ESTs) which specify the transcripts from genes that are expressed in a particular tissue. The analysis of ESTs from wheat endosperm tissue (Clarke et al. 2000, 2001; Anderson et al. 2001) has shown that the complexity of the seed protein gene families is far greater than has so far been indicated by the analysis of the proteins.

Wheat seed storage proteins consist of two classes, gliadins and glutenins. Gliadins are monomeric proteins that, on the basis of their electrophoretic mobility in A-PAGE (pH 3.1), have been classified as α-, β-, γ- and ω-gliadins. These gliadins are polypeptides having intramolecular disulphide bonds with the exception of ω-gliadins, which have no cysteine in their structure (Müller and Wieser 1995, 1997). Glutenins are polymeric structures formed by polypeptides that are held together by disulphide bonds. They have molecular weights up to several million Daltons and determine gluten strength and elasticity. After reduction of disulphide bonds, glutenin subunits are divided into two major groups: high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (GS) determined by their electrophoretic mobility in SDS-PAGE (Payne 1987; Gupta and Shepherd 1990).

The number and distribution of cysteine residues are very important structural features within the gluten proteins because of their involvement in forming intra- and inter-molecular bonds.

Usually, α-gliadins have six cysteine residues in their structure while γ-gliadins and LMW glutenin subunits contain eight cysteines. While the α- and γ-gliadins form only intra-molecular disulphide bonds, the LMW glutenin subunits have two cysteines involved in inter-molecular disulphide bonds (Kohler et al. 1993; Müller et al. 1998). The disulphide residues available for inter-molecular bonding are not randomly distributed in the protein but are located in specific positions in the peptide chain (Grosch and Weiser 1999). Only a small number of cysteine residues are able to form inter-molecular disulphide bonds. These cysteine residues named C^{b*} and C^{x} according to Kohler et al. (1993), observed in LMW glutenin subunits, are responsible for the high aggregation tendency of these type of proteins. These cysteines are not present in monomeric gliadins (Müller et al. 1998).

Communicated by J.W. Snape

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The contribution of the seed storage protein groups to dough properties has been studied extensively. However, due to the difficulty of obtaining sufficient quantities of purified native protein for these studies, the bacterial expression of proteins has been adopted as a model. In this model the proteins are incorporated into flour by first partially reducing the dough, to allow them access into the glutenin matrix, then re-oxidizing it to include them in the polymers. In the case of HMW glutenin subunits, excellent agreement has been obtained between experiments where bacterially expressed proteins and native proteins, isolated from wheat, have been incorporated into flour (Bekes et al. 1994a; Dowd and Bekes 2002).

When HMW and LMW glutenin subunits are supplemented into flour, it is necessary to chemically incorporate them into the protein matrix of the dough, to observe their full effect. However, when gliadins are supplemented into flour (Bekes et al. 1994a), with or without chemical incorporation, they behave in the same way (Murray et al. 1998).

Studies of the impact of gliadin on dough mixing properties (MacRitchie 1987) have shown that they result in weaker and less-stable dough preparations, as indicated by decreases in mixing time and peak resistance, and an increase in resistance breakdown (Hussein and Lukow 1997; Uthayakumaran et al. 1999). These changes result in increased extensibility and poorer baking performance of the dough. Dough strength and stability are directly related to the molecular size of the glutenin polymer. Thus, in general, the HMW-GS make a larger contribution to dough strength and stability than the same molar amount of LMW-GS.

In describing dough or bread-making properties, only a percentage of the variation in quality traits can be related to the composition of the glutenin and gliadin proteins. Studies by Pomeranz (1988) indicate that some non-gluten proteins, and other flour components such as lipids, pentosans and starch, also influence quality traits. An example is the negative effects of certain globulin proteins on dough properties (MacRitchie 1987).

In our analysis of EST sequences (Clarke et al. 2000) we identified several ‘novel’ LMW endosperm proteins (designated as LMW-gliadin in this manuscript) that were similar to the LMW-gliadin classes of prolamins (Salcedo et al. 1979; Rocher et al. 1996) and ε-hordein storage proteins in barley (*Hordeum vulgare* L.). The 17-kDa ε-hordein protein has been studied (Vaag et al. 2000) in relation to beer foam stability. Since many applications of wheat flour in the production of food products involve the formation of foams, further characterisation of the LMW-gliadin genes was undertaken.

Materials and methods

cDNA library construction, DNA sequencing and sequence alignments

RNA was isolated from 8 to 12 days post-anthesis (DPA) endosperm tissue from wheat cv ‘Wyuna’ and used to generate a cDNA library. The EST clones were sequenced in a single pass from the 5′ end and interesting candidate genes were sequenced in both orientations (Clarke et al. 2000).

Wheat mapping lines

DNA from the nulli-tetra and deletion line wheat cytogenetic stocks (Endo and Gill 1996) were kindly supplied by Qi and Gill, Kansas State University. M. Pallotta (University of Adelaide, Waite campus) kindly provided the DNA from the deletion lines that were originally supplied by A. Lukaszewski (UC, Riverside USA).

Data storage and sequence analyses were carried out through the Australian National Genomic Information Service (ANGIS), Bay 16/104, Australian Technology Park, Eveleigh NSW 1430, Australia (http://current.angis.org.au/).

Amplification of LMW-gliadin seed protein genes from genomic DNA

PCR (polymerase chain reaction) primers used for the amplification of LMW-gliadin seed protein genes from genomic DNA were as follows: (5′TT(A/G)TAGCCA(G/A)CCACCATGAAGA3′) which spans the start codon and part of the signal peptide region for these genes, and (5′CAGATCTTAGCAGG(T/C)(C/A)CCACCA 3′) which covers the 3′ end of the gene and spans the stop codon. The reactions were done in PCR SuperMix (GibcoBRL Life Technologies Inc. N.Y., USA) containing 3.2 pmol of the 5′ primer, 5.6 ρmol of the 3′ primer and 50 ng of template DNA in a 25-µl reaction. The amplification conditions were as follows: 1 cycle of 94 °C/1 min; 25 cycles of 94 °C/30 s, 77 °C/1.5 min.

Detecting single nucleotide polymorphisms (SNPs) and mapping of the LMW-gliadin genes

Gene family members of the LMW-gliadin seed proteins were aligned using ECLUSTALW (a multiple sequence alignment program from the Wisconsin Computer Group). SNPs characterizing the different gene family members were used to generate gene-specific primers (Table 1). These primers were used for PCR analysis of DNA from the nulli-tetra and deletion-line wheat cytogenetic stocks (Clarke et al. 2001) to map the chromosomal location of these genes.

Synthesis and cloning of the LMW-gliadin seed protein gene

The genic region coding for the mature peptide (minus the signal peptide) was amplified from clone LMGli1111 using the following two primers.

(1) *Nco*I primer 5′ TCTAAACCATGGTTGCGCAGCTGGACAC,
(2) *Bam*HI primer 5′ TCTAAAGGATCCTTAGCAGGTACCACCAAC.

Table 1 Sequence primers used for mapping the LMW-gliadin genes in wheat

Primer name	Primer sequence 5′ to 3′
LMGli1058F	CAGCCACCATGAAGAACC
LMGli1199F	CAGCCACCATGAAGACCA
LMGli1111F	CTCGCGGCGACTAGCGTC
LMGli2482F	CCATGAAGACCATGTTCC
LMGli1058R	GGACATATGCTGTCTGGC
LMGli1199R	GGACATATGGTGTCTGGT
LMGli1111R	ATGACCTGGGCCACACCG
LMGli2482R	ACCATCCTCATTATCTCG

The amplified DNA and the pET11d expression vector were cut with *Nco*I and *Bam*HI restriction endonucleases, ligated overnight at 16 °C and transformed into competent cells of strain GJ1158 by electroporation following standard protocols. Transformed colonies were screened for inserts of the correct size (486 bp) and these were sequenced in both directions to ensure the integrity of the cloned fragment relative to the original clone LMGli1111.

Bacterial strains and plasmids

Escherichia coli strain GJ1158 (Bhandari and Gowrishanker 1997) was used as the expression host strain in this work, with the expression vector plasmid pET11d (Novagen Inc., Madison Wis. 53711, USA) as described below. Bacterial cell cultures were grown using Luria-Bertani medium supplemented where appropriate with ampicillin (200 mg/l). Large-scale cultures for protein expression were also supplemented with glycerol (30 mM) as a carbon source.

Expression and purification of LMGli1111

The procedure was that used by Solomon and Appels (1999). The induced and control cultures were analysed by SDS-PAGE using the tris-tricine discontinuous electrophoresis system (Schagger and von Jagow 1987). Large-scale (1 litre) cultures for purification of LMGli1111 were inoculated with a 5-ml overnight culture and induced to express LMGli1111 by the addition of NaCl to 280 mM and IPTG to 1 mM at a cell density of 0.4–0.6. The culture was incubated with shaking overnight at 37 °C and cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 °C. The cell pellet was resuspended in 5 mM DTT at approximately 5 ml/g (wet weight) and absolute ethanol was added to a final concentration of 70% (*v*/*v*). The LMGli1111 protein was purified from the cell pellet by aqueous ethanol extraction as described in Solomon and Appels (1999). The purified acetone precipitate of the protein was dried at room temperature before resuspension in 70% ethanol and dialysed for 72 h against 0.1% acetic acid at 4 °C using a 12,000 Da cut-off dialysis membrane and then lyophilised. Protein yield was determined by dry weight and was about 22 mg from 4 l of culture.

N-terminal amino-acid sequencing

N-terminal amino acid sequencing of the expressed protein, by automated Edman degradation, was carried out at the Australian Proteome Analysis Facility, Macquarie University, Sydney, Australia, 2109.

Dough testing and baking studies

The impact of the LMGli1111 protein on mixing and baking properties were investigated by supplementing the purified protein into bread-making flour, milled from the cultivar Janz (protein content: 10.2%). Two experimental designs were used. A reversible reduction/oxidation procedure (Békés et al. 1994b), in which the chemical bonds holding together the glutenin polymer are broken by reduction and, on re-oxidation, the added polypeptides can incorporate into the polymeric glutenin structure, the other straightforward addition (without oxidation and reduction). The same procedure was applied to both, but the reducing and oxidising agents were replaced with the same volume of water for the 'simple addition' method.

Mixing studies were carried out, in triplicate, with a prototype Mixograph (Gras et al. 1990) using 2 g of flour into which 5 mg of the test protein (LMGli1111) was added. Mixing parameters were determined using a modification of a previously reported computer program (Gras et al. 1990). The parameters determined were: mixing time (MT); peak resistance (PR); bandwidth at peak resistance (BWPR); resistance breakdown (RBD); bandwidth breakdown (BWBD); time to maximum bandwidth (TMBW) and maximum bandwidth (MBW).

Test baking was carried out, in duplicate, using a procedure employing 2.4 g of dough and optimal conditions of reversible reduction/oxidation, prepared in the 2-g Mixograph (Uthayakumaran et al. 2000). Loaf height (LH) was used to determine the baking performance. The reproducibility is indicated by the least significant difference (LSD).

Results and discussion

Identification of the LMW-gliadin seed-protein gene family by EST analysis and mapping of genes

From our EST sequence analysis, several clones encoding the LMW-gliadin seed-protein family were identified. Sequence analysis of these clones showed that they are similar to LMW-GS and gliadins, as determined by a BLASTX search of the GenBank database, but the expected scores were lower than true orthologs, being in the range of 1E–13 to 1E–17 (Clarke et al. 2000). Characterisation of these sequences, at the nucleotide and amino-acid level, identified them as being closely related to the 17-kDa foam-stabilising ε hordein (88% identity) protein (Vaag et al. 2000) and an LMW gliadin, 11dc7 (93% identity), reported by Anderson et al. (2001) (Fig. 1). EST clones of these LMW-gliadin genes were relatively abundant with 69 clones identified from the 4,374 clones sequenced. Sequence alignments suggested a gene family comprising four different family members represented by the clones LMGli1111, LMGli2482, LMGli1058 and LMGli1199 (Fig. 1). The LMGli designation indicates their similarity to the low-molecular-weight gliadin proteins mentioned above and the number, their clone ID, in our database. The transcript abundance for each family member was 27, 9, 11 and 22 ESTs, respectively.

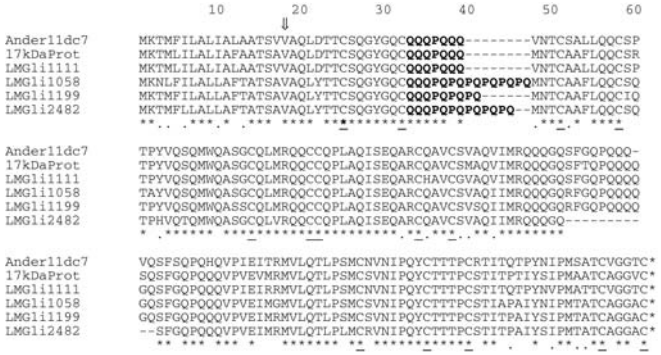

Fig. 1 Predicted amino-acid sequence for the gene 11dc7 isolated by Anderson et al. (2001), the 17-kDa ε-hordein protein of Vaag et al. (2000) and the four representatives, LMGli1111, –1058, –2482 and –1199, of the LMW-gliadin seed-protein family members (novel seed storage protein) from Clarke et al. (2000). Sequence data is from the start of translation to the stop codon (*). The ⇓ indicates the putative start of the mature peptide. The repetitive PQ amino-acid domain is indicated by *bold type* and cysteine residues are *underlined*

Fig. 2 Nucleotide sequences of the four members of the LMW-gliadin seed-protein family discussed in the manuscript. The primers used for the amplification of all family members from genomic DNA are *underlined*. The primers designed to specifically amplify the genes LMGli1111, LMGli1199, LMGli2482 and LMGli1158 are in *bold type* and those used to sub-clone the LMGli1111 gene into the expression vector are *shaded and underlined*

Primers were selected close to the start and termination sites of translation for these genes (Fig. 2) and used for PCR amplification of total DNA from wheat cv Wyuna. An approximately 480-bp band of predicted size was amplified and sequenced, which confirmed that these cDNAs were present in the genome and were not post-transcriptional modifications of previously identified seed-storage protein genes. These proteins do not contain introns, as is also the case with the seed-storage protein genes.

Sequence alignment of the four family members using ECLUSTALW revealed single nucleotide polymorphisms (SNPs) amongst the different genes, and oligonucleotide primers of 18 bp were generated which terminated 3′ at these sequence differences (Fig. 2). The primers are listed in Table 1. The chromosomal locations of these genes were determined by PCR amplification of genomic DNA from nulli-tetra and deletion line wheat cytogenetic stocks, as discussed in Clarke et al. (2001). Primers specific for the clone LMGli1111 amplified a product from chromosome 7DS, and the primers for clone LMGli2482 a band from chromosome 7D. Primers specific for clone LMGli1058 amplified a band from chromosome 4A, and primers for clone LMGli1199 from chromosome 7AS. It was not possible to determine a chromosomal arm location for genes LMGli2482 and LMGli1058, probably because graded deletions covering the whole arm are not available within the deletion-line stocks.

The LMW-gliadin genes and their relationship to the seed-storage protein genes

The major sequence variation that discriminates the four family members is the diversity that occurs in a repetitive proline glutamine $(PQ)_n$-rich domain (Fig. 1). Family members have deletions of 2, 6 and 8 amino acids within this region when compared to the largest gene member LMGli1058. In addition, clone LMGli2482 has another 11 amino acid-deletion, 64 amino acids downstream from the first, in a glutamine-rich domain (Fig. 1).

The amino-acid sequence of the LMW-gliadin proteins consists of a signal peptide of 20 amino acids followed by a short N-terminal sequence of 12 amino acids, containing the first two cysteines (positions 5 and 12). The usual repetitive sequence observed in gliadins (α- and γ-type) and LMW glutenin subunits is truncated in this new type of protein, and only a few amino acids (i.e. 7 in LMGli1111 and 15 in LMGli1058) remain. Following the repetitive domain there is a C-terminal region with high homology to the γ-gliadins.

In addition to the similarity of the two genes compared in Fig. 1, two small secalins named rye-15 and rye-18 (Rocher et al. 1996) and the LMW gliadins of Salcedo et al. (1979) are also very similar. The endosperm proteins described by Salcedo et al. (1979) are hydrophobic with a molecular weight in the range of 17,000–19,000 Da and an electrophoretic mobility at pH 3.2, the same as in classical gliadins. These proteins also have a high level of cysteine and a lower content of glutamine and proline, compared with the typical gliadins, and they have been named LMW-gliadins. Salcedo et al. (1980) localized these LMW-gliadin genes to chromosomes 7A, 7D and 4B, the same chromosomal locations as the proteins described here. The fact that we assigned the clone LMGli1058 to chromosome 4A is explained by the misclassification of this chromosome as 4B at the time when Salcedo's group were working with these proteins.

Characteristics and functionality

The number and spacing of the eight cysteine residues present in the γ-gliadins are also conserved in the LMW-gliadin proteins. The C-terminal region of the γ-gliadin protein is known to assume a compact structure with a marked surface hydrophobicity (Popineau and Pineau 1987). In addition, the LMW-gliadin proteins have six

extra cysteines, four in the C-terminal region and two in the N-terminal region. The even number of these cysteine residues might result in the formation of more intra-molecular disulphide bonds and therefore a more compact structure than that observed in γ-gliadins. The even number of cysteines in this protein suggests that none are available for inter-molecular disulphide bonding. This assumption is strengthened by the fact that no protein of a similar molecular weight has been described for the glutenin class of proteins (Gupta and Shepherd 1990; Nieto Taladriz et al. 1997; Gianibelli et al. 2002). Recently, it has been reported that about 5% of the cysteines present in wheat flour are as free SH groups (Grosh and Weiser 1999) and, thus, do not form disulphide bonds. Of the total cysteine composition in the seed-storage proteins, only a small number of residues are able to form inter-molecular disulphide bonds (Hüttner and Weiser 2001) and these cysteine residues are not present in monomeric gliadins. The residue located at position 5 of the N-terminal sequence in the LMW-gliadin proteins, however, is located in a similar position to that of the LMW-mc5 type of glutenin protein (LMW glutenin subunits with a methionine at the beginning of the N-terminal sequence and a cysteine at position 5) that is associated with inter-molecular bonding.

The repetitive domain of the prolamin proteins has been considered to be an important feature in the determination of protein elasticity, particularly in the HMW glutenin subunits. The fact that high levels of glutamine are present in the repetitive domain means high levels of OH^- groups are available to form hydrogen bonds that contribute to the elasticity of the proteins (Shewry et al. 2001). This attribute would be negligible in the LMW-gliadin proteins.

The glutenins and gliadins represent 10% of the EST sequences in our library; 17% of these are LMW-gliadins as discussed here. Therefore, the abundance of the LMW-gliadin gene transcripts in endosperm tissue and their similarity to the seed-storage protein group of genes suggests that they may play an important role in the quality attributes of wheat flour. The novel characteristics of this protein are its high cysteine content and the lack of a large repetitive domain. These properties, and the additional characteristic of gas bubble stabilisation observed by Vaag et al. (2000) for the ε-hordein protein, suggest that the LMW-gliadin proteins may produce novel characteristics in the dough.

Bacterial expression of the LMW-gliadin protein

Clone LMGli1111 was selected as representative of this gene family. The predicted ORF of this cDNA clone was expressed in bacteria, and the purified protein (Fig. 3) was subsequently incorporated into bread dough. Sequence analysis of the N-terminal end of the purified protein showed that the major signal for the first nine amino acids are (VAQLDTTCS) (data not shown) as predicted from the gene sequence used for expression.

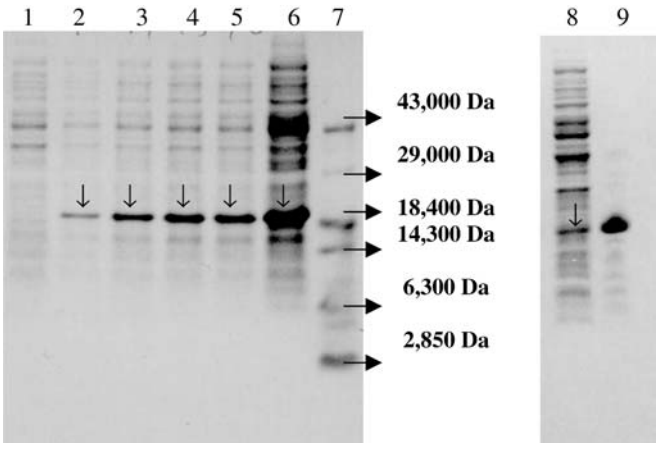

Fig. 3 SDS-PAGE gel using Tris-Tricine discontinuous electrophoresis. *Lanes 1 to 6*, a time-course showing increasing levels of expression of the LMW-gliadin protein in whole-cell extract: *lane 1* is at the time of induction and *lane 6* after overnight expression. *Lane 7* low-range protein molecular-weight standard (GibcoBRL Life Technologies Inc. N.Y., USA). *Lane 8* whole-cell extract after overnight induction of protein. *Lane 9* expressed protein after the purification step

The influence of LMGli1111 protein on dough properties

The addition of 5 mg of the LMGli1111 protein (without chemical incorporation) into the base flour showed that the dough had a decreased mixing time (MT) and time to maximum bandwidth (TMBW) with an increase in resistance breakdown (RBD) (Table 2). This is similar to the effect achieved by the addition of monomeric proteins, which also cause a decrease in dough-mixing parameters. Similar results have been obtained for the addition of unincorporated gliadins (Fido et al. 1997), HMW-GS (Bekes et al. 1994c) and LMW-GS (Lee et al. 1999). Therefore, in these experiments, the LMGli1111 polypeptide when simply added to the base flour acted as a monomeric protein.

Incorporation of the LMGli1111 polypeptide into the base flour resulted in statistically significant increases in MT, TMBW, maximum bandwidth (MBW) and RBD. In addition, slight positive changes were observed in peak resistance (PR), bandwidth at peak resistance (BWPR) and a slight decrease in bandwidth breakdown (BWBD) (Table 2). These changes in mixing properties are analogous to those observed when incorporating glutenin subunits into the polymeric gluten network. The extent of these changes is significantly smaller than that obtained with HMW-GS (Bekes et al. 1994a,1994c) and smaller, but comparable, to those with LMW-GS (Lee et al. 1999).

Changes in RBD caused by the incorporation of LMGli1111 protein are unusual. RBD is a measure of dough stability which shows how sensitive the dough is to over-mixing; a higher RBD value indicates a less-stable dough. Usually, dough strength and dough stability change in parallel with an increase in dough strength leading to increased dough stability (decrease in RBD).

Table 2 Mixing properties and baking performance of flours supplemented with the LMGli1111 polypeptide. All data are expressed as a value relative to the control flour, which is set at 100. (MT) Mixing time, (PR) peak resistance, (BWPR) bandwidth at peak resistance, (RBD) resistance breakdown, (BWBD) bandwidth breakdown, (TMBW) time to maximum bandwidth, (MBW) maximum bandwidth and (LH) Loaf height. LSD, least significant difference at $P = 0.05$

Item	MT	PR	BWPR	RBD	BWBD	TMBW	MBW	LH
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100
Addition	94.8	98.8	96.1	114.7	102.9	91.5	99.1	104
Incorp.	111.4	104.0	104.0	146.9	95.2	117.8	113.0	119
LSD	4.2	5.7	4.7	7.4	4.8	4.6	5.2	9.6

The incorporation of LMGli1111 protein resulted in a significant increase in dough strength (represented by the increased mixing requirement) and, at the same time, a very significant increase in RBD, i.e. decreased dough stability (Table 2). This dual impact clearly indicates that the changes in the dough structure caused by the presence of LMGli1111 protein are not the same as those that occur when the dough is enriched by glutenin subunits.

Effect of LMGli1111 protein on baking

Incorporating the LMGli1111 polypeptide into the dough led to an almost 20% increase in baking performance as characterized by an increase in loaf height (Table 2), which is a significantly larger influence than that observed by adding/incorporating the same amount (5 mg to 2 g of flour) of any gliadin or glutenin polypeptides (Bekes and Gras 1999). This indicates that the effect is not only based on changes in dough properties, such as dough strength or extension, but may be related to other parameters such as gas-holding capacity.

Conclusion

Analysis of ESTs from a particular tissue can indicate not only the range of genes being expressed but also the relative levels of expression of these genes. Using our knowledge of the biological process under investigation, in this case the types and interaction of the seed-storage proteins, new genes were identified. From sequence information, the structure, chromosomal locations and expression levels of the individual gene members were determined. In turn, bacterial expression, purification and subsequent incorporation into bread dough, of a representative protein from the LMW-gliadin gene family, has revealed the characteristics these proteins exhibit in dough rheology and baking studies.

This is the first experimental finding on the effect of incorporation of a seed protein lacking the central repetitive domain on mixing properties of dough. Another feature that make this protein unique is its high cysteine content. The reduction and oxidation of flours containing the LMW-gliadin proteins is likely to result in their incorporation into the glutenin structure of the dough. Incorporation of 5 mg of the proteins showed excellent reproducibility and a significant impact on dough properties. This amount of LMW-gliadin protein increased the protein content of the flour by 2.5%.

An unexpected outcome of incorporating this protein into dough is the breaking of the nexus between dough strength and dough stability. In all proteins tested to-date, increases in dough strength (increased mixing time) have led to more stable dough (reduction in resistance break down). A novel characteristic of these genes, observed in sequence comparisons to gliadin and LMW glutenin genes, is the lack of a central repetitive domain. The dough rheology results suggest the role of this region in the expressed protein, once it is introduced into the glutenin polymer, to provide elasticity to the dough. Consequently, these proteins add to dough strength by incorporating into the glutenin polymer during dough formation. Once formed, however, the RBD is reduced because these proteins lack the elasticity to resist overmixing.

In addition, an almost 20% increase in baking performance was obtained. This increase is beyond that which can be accounted for in the changes to the rheology of the dough. Dough rheology gives a physical measure of the characteristics of the dough, in terms of strength (MT) and elasticity (RBD) to resist CO_2 gas expansion. However, the chemical composition provides a barrier to trap the gas in pockets preventing its diffusion throughout the dough. An explanation for this also relates to the lack of the repetitive domain. This domain is hydrophilic and, therefore, polymers formed with proteins that lack this region would become more hydrophobic thus enabling better CO_2 retention in the dough during baking. This would be consistent with the action of the 17-kDa hordein protein in foam stabilisation.

The addition of this protein into bread flours would provide a significant increase in loaf volume without the accompanying increase in mixing energy that would normally be required for stronger dough.

References

Anderson OD, Hsia CC, Adalsteins AE, Lew EJ-L, Kasarda DD (2001) Identification of several new classes of low-molecular-weight wheat gliadin-related proteins and genes. Theor Appl Genet 103:307–315

Bekes F, Gras PW (1999) In vitro studies on gluten protein functionality. Cereal Foods World 44:580–586

Bekes F, Anderson OD, Gras PW, Gupta RB, Tam A, Wrigley CW, Appels R (1994a) The contributions to mixing properties of 1D HMW glutenin subunits expressed in a bacterial system. In: Henry RJ, Ronalds JA (eds) Improvement of cereal quality by genetic engineering. Sydney, Australia, pp 97–103

Bekes F, Gras PW, Gupta RB (1994b) Mixing properties as a measure of reversible reduction/oxidation of doughs. Cereal Chem 71:44–50

Bekes F, Gras PW, Gupta RB, Hickman DR, Tatham AS (1994c) Effects of 1B × 20 HMW glutenin on mixing properties. J Cereal Sci 19:3–7

Bhandari P, Gowrishankar J (1997) An *Escherichia coli* host strain useful for efficient overproduction of cloned gene products with NaCl as an inducer. J Bacteriol 179:4403–4406

Clarke BC, Hobbs M, Skylas D, Appels R (2000) Genes active in developing wheat endosperm. Funct Integrative Genom 1:44–55

Clarke BC, Larroque O, Bekes F, Somers D, Appels R (2001) The frequent classes of genes expressed in wheat endosperm tissue as possible sources of genetic markers Aust J Agric Res 52:1181–1193

Dowd C, Bekes F (2002) Large-scale expression and purification of high-molecular-weight glutenin subunits. Protein Express Purif 25:97–104

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Fido R, Bekes F, Gras PW, Tatham A (1997) The effects of added gliadin classes on the mixing properties and extension of dough. J Cereal Sci 26:271–277

Gianibelli MC, Wrigley CW, MacRitchie F (2002) Polymorphism of low M_r glutenin subunits in *Triticum tauschii*. J Cereal Sci 35:277–286

Gras PW, Hibberd GE, Walker CE (1990) Electronic sensing and interpretation of dough properties using a 35 gram Mixograph. Cereal Foods World 35:568–571

Grosh W, Weiser H (1999) Redox reactions in wheat dough as affected by ascorbic acid. J Cereal Sci 29:1–16

Gupta RB, Shepherd KW (1990) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. I. Variation and genetic control of the subunits in hexaploid wheats. Theor Appl Genet 80:65–74

Hussain A, Lukow OM (1997) Influence of gliadin-rich subfractions of glenlea wheat on the mixing characteristics of wheat flour. Cereal Chem 74:791–799

Hüttner S, Wieser H (2001) Studies on the distribution and binding on the endogenous glutathione in wheat dough and gluten. II. Binding sites of endogenous glutathione in glutenins. Eur Food Res Technol 213:460–464

Köhler P, Belitz H-D, Weiser H (1993) Disulphide bonds in wheat gluten: further cysteine peptides from high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits of glutenin and from γ-gliadins. Z Lebensm Unters Forsch 196:239–247

Lee YK, Bekes F, Gras PW, Appels R, Morell M (1999) The low-molecular-weight glutenin subunit proteins of primitive wheats. IV. Functional properties of products from individual genes. Theor Appl Genet 98:149–155

MacRitchie F (1987) Evaluation of contributions from wheat protein fractions to dough mixing and bread making. J Cereal Sci 6:259–268

Müller S, Wieser H (1995) Disulphide bonds of α-type gliadins. J Cereal Sci 22:21–27

Müller S, Wieser H (1997) The location of disulphide bonds in monomeric γ-gliadins. J Cereal Sci 26:169–176

Müller S, Vensel WH, Kasarda DD, Köhler P, Wieser H (1998) Disulphide bonds of adjacent cysteine residues in low-molecular-weight subunits of wheat glutenin. J Cereal Sci 27:109–116

Murray DJ, Bekes F, Gras PW, Copeland L, Savage AWJ, Tatham AS (1998) Hydrogen bonding and the structure/function relationship of wheat flour gliadins. In: Ross AS, Wrigley CW (eds) Proc 48th Australian Cereal Chem Conf, Cairns, Queensland, Australia, pp 12–16

Nieto-Taladriz MT, Ruiz M, Martinez MC, Vazquez JF, Carrillo JM (1997) Variation and classification of B low-molecular-weight glutenin subunits alleles in durum wheat. Theor Appl Genet 95:1155–1160

Payne PI (1987) Genetics of wheat storage protein and the effect of allelic variation on breadmaking quality. Annu Rev Plant Physiol 38:141–153

Pomeranz Y (1988) Composition and functionality of wheat flour components. In: Pomeranz Y (ed) Wheat chemistry and technology, vol 2. AACC, St Paul, USA pp 219–370

Popineau Y, Pineau F (1987) Investigation of surface hydrophobicities of purified gliadins by hydrophobic interaction chromatography, reversed-phase high performance chromatography and apolar ligand binding. J Cereal Sci 5:215–231

Rocher A, Calero M, Soriano F, Méndez E (1996) Identification of major rye secalins as coeliac immunoreactive proteins. Biochim Biophys Acta 1295:13–22

Salcedo G, Prada J, Aragoncillo C (1979) Low MW gliadin-like proteins from wheat endosperm. Phytochemistry 18:725–727

Salcedo G, Prada J, Sanchez-Monge R, Aragoncillo C (1980) Aneuploid analysis of low-molecular-weight gliadins from wheat. Theor Appl Genet 56:65–69

Schagger H, von Jagow G (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166:368–379

Shewry PR, Popineau Y, Lafiandra D, Belton P (2001) Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project. Trend Food Sci Technol 11:433–441

Solomon RG, Appels R (1999) Stable high-level expression of a type I antifreeze protein in *Escherichia coli*. Protein Express Purif 16:53–62

Uthayakumaran S, Gras PW, Stoddard F, Bekes F (1999) Effects of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. Cereal Chem 77:737–743

Uthayakumaran S, Stoddard FL, Gras PW, Bekes F (2000) Optimized methods for incorporating glutenin subunits into wheat dough for extension and baking studies. Cereal Chem 77:731–736

Vaag P, Cameron-Mills V, Sorensen MB (2000) 17 kDa foam protein. International patent application number WO 00/14237