

Biochemical, Genetic, and Molecular Characterization of Wheat Glutenin and Its Component Subunits

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The emphasis of this review is on the subunits of glutenin. See the AACC web site for a fuller version of this review, in which glutenin subunits are placed in the context of the full spectrum of gluten proteins.

Of all the cereal grains, wheat is unique because wheat flour alone has the ability to form a dough that exhibits the rheological properties required for the production of leavened bread and for the wider diversity of foods that have been developed to take advantage of these attributes. The unique properties of the wheat grain reside primarily in the gluten-forming storage proteins of its endosperm. It is these dough-forming properties that are responsible for wheat being the most important source of protein in the human diet.

The bread and durum wheats are polyploid species containing three (AABBDD) and two (AABB) related genomes, respectively. The genetic constitution of wheat is important because all quality traits result from the expression of genes and their interaction with the environment. The full spectrum of wheat-endosperm proteins has been exhibited in proteome studies involving the two-dimensional fractionation of the polypeptides (after disulfide-bond rupture), followed by dissection of the individual components for identification. This display (Fig. 1) shows that there are at least 1,300 polypeptides, over 300 of which have been identified by N-terminal amino acid sequencing and matching established protein database information (Skylas et al 2000). This approach offers the opportunity to examine gene expression in the specific tissue under prevailing growth conditions, thereby complementing research at the purely genetic level.

Genetic studies indicate that storage-protein genes exhibit simple co-dominant Mendelian inheritance (Sozinov et al 1974, 1975; Meehan et al 1978; Metakovsky et al 1986; Payne et al 1981a; Payne 1987; Gupta and Shepherd 1990a). For most traditional uses, wheat quality derives mainly from two interrelated characteristics: grain hardness and protein content, with each end-use requiring a particular "protein quality". Quality is determined by the molecular structure of the storage proteins of wheat which, in turn, controls the interactions of the proteins during the breadmaking process (Bushuk 1998; Shewry et al 1999).

Within the context of improving protein quality (e.g., high extensibility, appropriate dough strength) by wheat breeding, research has been conclusive about the importance of glutenin, with emphasis on subunits of high molecular weight (HMW), particularly those controlled by the D genome (Payne et al 1981b, 1987; Branlard and Dardevet 1985; Gupta and MacRitchie 1994; Popineau et al 1994). The emphasis on the HMW glutenin subunits (GS) initially arose from their accessibility for analysis, appearing as they do at the top of an electrophoresis gel pattern (using the routine method

with SDS), well separated from all the other polypeptide bands. The research focus on the HMW-GS has proved to be justified, showing them to be particularly important components of the gluten complex (Shewry et al 1992, 1997). By contrast, the low molecular weight (LMW) GS are present in gluten at about three times the amount of the HMW-GS, but their size distribution means that they are difficult to study, being mixed with many other polypeptides in the SDS gel electrophoresis pattern of flour. Yet LMW-GS play a significant role in gluten structure. This role has attracted relatively little attention in the literature, due largely to the difficulty of studying this quantitatively important family of gluten proteins. Thus, it is important that they should receive similar emphasis in a review of the gluten family of polypeptides.

NATIVE GLUTENIN

With the molecular weights of glutenin polymers reaching over twenty million daltons, based on gel filtration (Huebner and Wall 1976; Bietz and Simpson 1992) and flow field-flow fractionation (FFF) studies (Stevenson and Preston 1996; Wahlund et al 1996), the glutenin proteins are among the largest protein molecules in nature (Wrigley 1996). Nevertheless, it should be stated that all measurements were based on the use of globular proteins as standards. These standards are relatively compactly folded, whereas glutenin polymers are largely not compactly folded. Thus, this type of calibration may tend to give anomalously high results. These proteins are heterogeneous mixtures of polymers formed by disulfide-bonded linkages of polypeptides that can be classified in four groups according to their electrophoretic mobility in SDS-PAGE after reduction of the S-S bonds (the A-, B-, C- and D-regions of electrophoretic mobility). The A-group (with an apparent molecular weight range of 80,000 to 120,000 Da) corresponds to the HMW-GS (Payne and Corfield 1979). The B- (42,000 to 51,000 Da) and C- (30,000 to 40,000 Da) groups are LMW-GS, distantly related to γ - and α -gliadins (Payne and Corfield 1979; Payne et al 1985; Thompson et al 1994). Finally, the D-group, also belonging to the LMW-GS group, is highly acidic and related to ω -gliadins (Jackson et al 1983; Masci et al 1993) (Fig. 2). The GS may also be characterized by capillary electrophoresis (Lookhart and Bean 1996; Bean et al 1998; Bean and Lookhart 2000) and by reversed-phase HPLC (Bietz 1983; Burnouf and Bietz 1984, 1985). These are valuable techniques that offer excellent resolution, automation, quantitation, and computerization. Based on separation by differences in charge and hydrophobicity, they can be used alone, or complementary to other separation methods (mainly SDS-PAGE). For RP-HPLC, the component proteins show a wide range of hydrophobicities (Marchylo et al 1989, 1992a; Margiotta et al 1993).

The HMW-GS are very difficult to solubilize. To do so, native glutenin must be treated with a reducing agent (to break disulfide bonds). However, recent studies have established that the central repetitive domain of the HMW-GS is soluble in water (van Dijk et al 1997; Bekkers et al 1999). Complementary studies of solubility of

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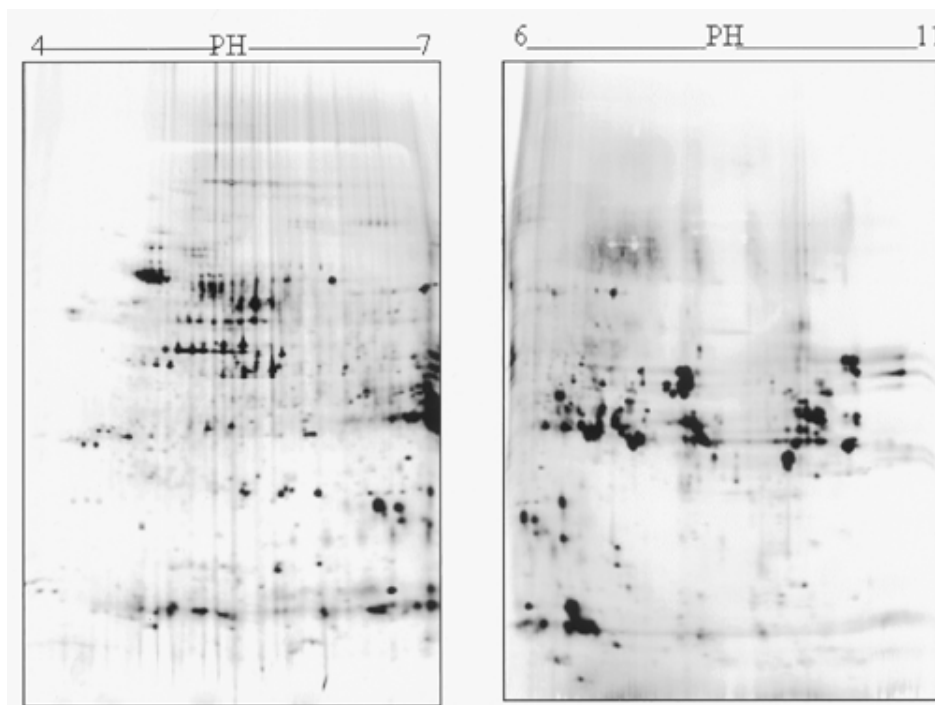


Fig. 1. Proteome maps of proteins from immature endosperm of the wheat cultivar Wyuna at 17 days after flowering (Skylas et al 2000), obtained in two stages of isoelectric focusing to fractionate the acidic (left) and basic polypeptides. (Reproduced with permission from Skylas and Wrigley 2000).

peptides with sequences corresponding to the N- and C-terminal regions of these proteins have shown that the former is responsible for the insoluble behavior of the HMW-GS (Bekkers et al 1998; van Dijk et al 1998).

HMW-GS

HMW-GS are minor components in terms of quantity, but they are key factors in the process of breadmaking, being major determinants of gluten elasticity (Tatham et al 1985) to the extent that they appear to promote the formation of larger glutenin polymers. SDS-PAGE was used to study the glutenin composition of bread wheat. The pioneer studies of Bietz and Wall (1972) showed that two types of subunits were present, LMW-GS (10,000 to 70,000 Da) and HMW-GS (\approx 80,000 to 130,000 Da). Using nullisomic-tetrasomic, nullisomic-trisomic, and ditelocentric lines of Chinese Spring, Bietz et al (1975) showed that HMW-GS were controlled by genes at the long arms of the chromosomes 1D and 1B. More detailed studies about the genetics of the HMW-GS and their relation to bread-making quality were conducted by Payne et al (1979, 1980, 1981a,b) and Lawrence and Shepherd (1980, 1981). As indicated above, the “apparent” molecular weights estimated by SDS-PAGE range from \approx 80,000 to 130,000 Da; however true estimates, calculated from derived amino acid sequences, indicate lower molecular weights (60,000 to 90,000 Da) (Anderson et al 1988, 1989; Anderson and Green 1989). RP-HPLC analysis indicates the HMW-GS to be less hydrophobic than the LMW-GS.

Nomenclature

The numbering system in current use (Fig. 3) was developed by Payne and Lawrence (1983) to identify HMW-GS. It also provides a chromosomal location of the genes. Originally, the assignment of ascending numbers was related to the mobility in SDS-PAGE; lower numbers equating to lower mobility. As new subunits were identified, there was difficulty in following this logical order. Thus, there are some subunits, such as 21, with lower mobility and higher number than the original subunits. When identifying subunits numerically, it is customary to include both the genome from which the subunit is derived and the indication of whether it is an x-type or y-type subunit (e.g., Dx5, By9).

Genetics and Polymorphism

The HMW-GS are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes (1A, 1B, and 1D) (Bietz et al 1975; Payne et al 1980, 1984a, 1987). These loci are *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively (Fig. 4). Each locus includes two genes linked together encoding two different types of HMW-GS: x- and y-type subunits (Payne et al 1981b; Payne 1987; Shewry et al 1992) (Fig. 2). The x-type subunits generally have a slower electrophoretic mobility in SDS-PAGE and higher molecular weight than the y-type subunits. Electrophoretic studies have revealed appreciable polymorphism in the number and mobility of HMW-GS in both bread wheats (Lawrence and Shepherd 1980; Payne et al 1980) and pasta wheats (Waines and Payne 1987; Branlard et al 1989). Consequently, the *Glu-1* loci present multiple allelism. Payne and Lawrence (1983) summarized the range of the alleles at the *Glu-1* loci as three allelic forms at the *Glu-1A*, 11 alleles at the *Glu-1B*, and six alleles at the *Glu-1D*. Since the publication of this catalog of alleles at *Glu-1* loci, more have been identified, as reported by McIntosh et al (1994). Wild species, such as *Aegilops* (Fernandez-Calvin and Orellana 1990), *Triticum tauschii* (Lagudah and Halloran 1988; Williams et al 1993; Gianibelli et al 2000), einkorn species (Waines and Payne 1987; Ciaffi et al 1993, 1998) and emmer species (Nevo and Payne 1987) have also shown extensive polymorphism of HMW-GS.

Gene Expression

Bread wheats could, in theory, contain six different HMW-GS, but due to the “silencing” of some of these genes, most common wheat cultivars possess three to five HMW-GS (one to three subunits in durum wheats). Thus, all hexaploid wheats contain at least the 1Bx, 1Dx, and 1Dy subunits, while some cultivars also contain a 1By subunit and a 1Ax subunit as well. It appears that the gene encoding the 1Ay subunit is always silent. Nevertheless, 1Ay subunits have been reported in the A-genome diploid species *T. monococcum* and *T. urartu* (Waines and Payne 1987) and, more recently, some bread wheats with six HMW-GS have been reported (Johansson et al 1993; Margiotta et al 1996). Extensive electrophoretic analyses show genotypes in both hexaploid and tetraploid wheats that lack certain subunits (Lafiandra et al 1988). Mutants lacking the subunits controlled by chromosome 1D in bread wheat were

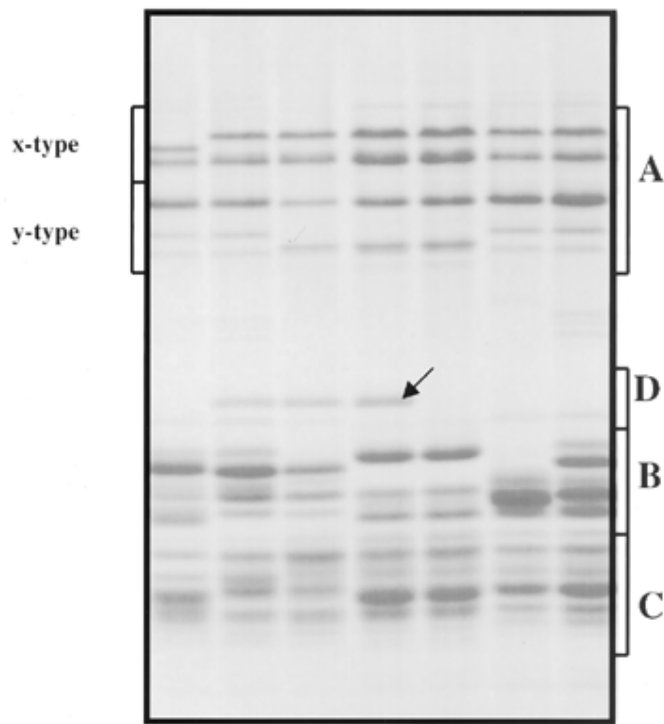


Fig. 2. SDS-PAGE of polymeric protein (after reduction to subunits), performed according to the one-step one-dimensional procedure of Gupta and MacRitchie (1991) (Gianibelli unpublished results). Group A: high molecular weight glutenin subunits showing x- and y-type glutenin subunits. Groups B-, C-, D-: low molecular weight glutenin subunits. Arrow indicates subunit D.

first reported by Bietz et al (1975) in landraces from Nepal. However, the production of durum and bread wheat lines with four and six HMW-GS, respectively, has been achieved by replacing silent genes at the *Glu-A1* locus with allelic forms that expressed x-type and y-type subunits. An increment in the amount of polymeric glutenin and better flour performance was indicated (Ciaffi et al 1995; Rogers et al 1997; Lafiandra et al 1998).

In general, there is little variation in the amount of individual subunits synthesized when alleles of the same locus are compared. However, genotypes from Israel (line TAA36), Canada (wheat cultivars such as Glenlea, Roblin and Bluesky), and the United States (cultivar Red River 68) were overexpressing 1Bx subunit 7 when compared with other HMW-GS (Lukow et al 1992; Marchylo et al 1992b; D'Ovidio et al 1997a). In Red River 68, the relatively large proportion of subunit 7 was due to gene duplication (D'Ovidio et al 1997a). The Israeli landrace TAA36 showed similar results. On the other hand, the subunit 7 from Glenlea was encoded by a single gene dose (Lukow et al 1992). In this case, either a more effective transcription or more efficient translation are the probable causes for the overexpression.

Amino Acid Composition and N-Terminal Sequences

With unusually high content of glutamic acid (mostly as the amidated form glutamine), HMW-GS also have high contents of proline and glycine and low contents of lysine. Structural features include a central repetitive domain (composed of short amino acid motifs that constitute $\leq 85\%$ of the protein sequence), and two nonrepetitive terminal domains that contain the majority of the cysteine residues present in the HMW-GS. These domains presumably form the molecular basis of the role of the HMW-GS in gluten functionality (Fig. 5). The amino acid composition of HMW-GS indicates the hydrophilic nature of the central repetitive domain and the hydrophobic characteristics of the N- and C-terminal domains (Shewry et al 1989). The proportion of the different amino acids is mainly

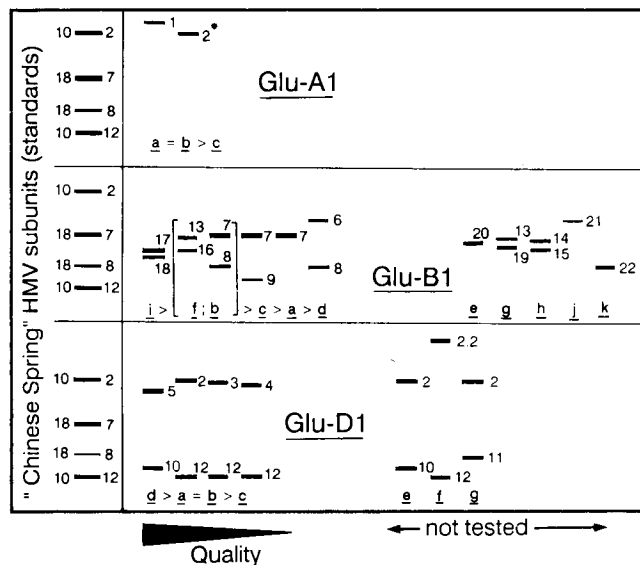


Fig. 3. Allelic variation in high molecular weight (HMW) glutenin subunits at three gene loci and relationship to breadmaking quality (Payne et al 1984a) based on SDS-PAGE fractionation. Chinese Spring patterns on left are included for comparison of relative mobilities. Lowercase letters refer to allele designations of Payne and Lawrence (1983) and ranking is according to assessed quality. (Reproduced with permission from Payne et al 1984a, Fig. 4)

defined by sequences of repeated polypeptide motifs. The sequences PGQGQQ, GYYPTSPQQ form $>90\%$ of the repetitive domain (Anderson and Greene 1989; Shewry et al 1992, 1997). The repetitive domains of x-type also have a tripeptide motif present (GQQ). The central domains of y-type HMW-GS often have the second proline in the GYYPTSPQQ repeat motif replaced by a leucine. Both x-type and y-type are predicted to adopt a β -turn conformation (Tatham et al 1990). Scanning tunneling microscopy of HMW-GS Dx5 (Miles et al 1991) indicates that the β -turns may be organized in a β -spiral structure. However, care needs to be taken when interpreting scanning tunneling microscopy images (Clemmer and Beebe 1991). This region holds repeated sequences of amino acids in numbers ranging from 490 to 700. These regions are rich in glutamine, proline, and glycine, and poor in sulfur (0 or 1 cysteine) (Table I). The N-terminal region has a nonrepetitive sequence ranging from 81 to 140 residues with three to five cysteine residues. Cysteine residues provide intermolecular disulfide bonds between HMW-GS and LMW-GS to form protein polymers with a range of different sizes that could reach up to tens of millions of daltons (Shewry et al 1992; MacRitchie 1992; Wrigley 1996). This is a quite conservative region. Considering the first 16 amino acids residues, it is possible to distinguish only small differences such as the sixth residue that could be E (glutamic acid) in Dx-type glutenin subunits or R (arginine) in Dy-type subunits (EGEAS-QLQCERELQE). At position 10 in all HMW-GS, there is a C residue (cysteine). Some differences have been observed for the By7 subunit at positions 12 and 14 (Shewry et al 1984; Anderson et al 1991). Finally, the C-terminus is a non-repetitive domain consisting of 42 residues that include one residue of cysteine.

Structure

Little was known about the structure of these proteins until 20 years ago. Then, boosted by new technology developments such as molecular cloning, it became possible to isolate cloned cDNA and genes for all the major groups of gluten proteins (Forde et al 1985; Okita et al 1985; Halford et al 1987; Anderson and Green 1989; Cassidy et al 1998; Masci et al 1998; D'Ovidio et al 1999; Hsia and Anderson 2001). This has allowed the complete amino acid sequences of the proteins encoded by these DNA to be deduced, provi-

ding a basis for modeling and biophysical studies. Such molecular and biophysical studies have been combined to give a detailed picture of HMW-GS structure (Shewry et al 1989, 1992, 1997). Contrary to the central repetitive domain, where a regular spiral structure has been proposed, the structure for both terminal regions is of α -helical type (Miles et al 1991; Shewry et al 1992, 1997) (Fig. 6). Tatham et al (1985) proposed a model based on an analogy to the mammalian connective tissue protein elastin, in which the β -reverse turns give HMW-GS their distinctive elastic properties. Although this model was widely accepted, recent studies (Belton et al 1994) have shown that the HMW-GS may not be elastin-like in their interaction with water. The high level of glutamine residues has a very high capacity to form both intra- and intermolecular hydrogen bonds. Belton (1999) has postulated that this feature may be involved in elasticity through formation of intermolecular hydrogen bonds. In the dough, some of these bonds break on stretching, giving rise to unbonded mobile regions (loops) and bonded regions (trains). Thus, the loops can be stretched and then reform when the stress is removed, accounting for the elastic restoring force, as in rubber elasticity. The theory of trains and loops has been applied to adsorption of polymers at interfaces (Fleer and Scheutjens 1982). This is the first time it has been applied to bulk systems.

Molecular Characterization of HMW-GS Genes

The recent use of molecular biology to clone and sequence several glutenin subunits has provided advances in our understanding of basic aspects of these proteins. Analyses of isolated genes provided the complete nucleotide and derived amino acid sequences for six HMW-GS genes from cultivar Cheyenne: Ax2*, Bx7, By9, Dx5, Dy10, and the silent Ay subunit gene (Forde et al 1985; Halford et al 1987; Anderson and Greene 1989; Anderson et al 1989). In addition, a similar procedure was used to obtain the complete sequence of an allelic pair 1Dx2+1Dy12 from cultivars Yamhill (Sugiyama et al 1985) and Chinese Spring (Thompson et al 1985). A third subunit from the 1A chromosome, Ax1, was characterized from cultivar Hope by Halford et al (1992). Other sequences (e.g., Bx17) were

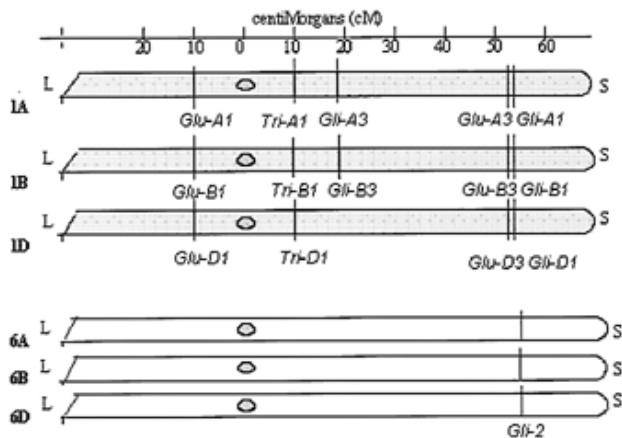


Fig. 4. Chromosomal location of genes for major wheat protein groups of hexaploid wheats (adapted from Payne 1987, Singh and Shepherd 1988). (L) long arm and (S) short arm of homoeologous groups 1 and 6 chromosomes. *Glu-1* loci located on the long arm of homoeologous group 1 chromosomes, controlling high molecular weight glutenin subunits (*Glu-A1*, *Glu-B1*, and *Glu-D1*). *Gli-1* loci controlling the synthesis of ω -, γ - and a few β -gliadins (*Gli-A1*, *Gli-B1*, and *Gli-D1*) (Payne et al 1982). Some ω -gliadins are controlled by minor loci such as *Gli-A3* (Sobko 1984, Metakovsky et al 1986) and *Gli-B3* (Jackson et al 1985, Metakovsky et al 1986). *Gli-2* loci are located on the short arm of the group 6 chromosomes, controlling mostly α -, β - and a few γ -gliadins (Metakovsky 1991). *Glu-3* loci controlling low molecular weight glutenin subunits are tightly linked to *Gli-1* loci (Singh and Shepherd 1988, Gupta and Shepherd 1990a). *Tri-A1* and *Tri-D1* are two loci controlling tritamins (Singh and Shepherd 1985), while *Tri-B1* does not express protein (Dubcovsky et al 1997).

also deduced (Reddy and Appels 1993). Polymerase chain reaction (PCR) is a fast and reliable alternative to more conventional methods for the study of wheat protein genes. This technique, which in the few years since its introduction has become a widespread research tool, allows the specific amplification of a target DNA segment using a pair of flanking oligonucleotides as primers (D'Ovidio et al 1990, 1994, 1995a). D'Ovidio et al (1995b) reported specific amplifications of the complete coding region of all six HMW-GS genes present in hexaploid wheat by means of PCR. This permitted ready analysis of the genetic polymorphism of HMW-GS genes, as well as the isolation of new allelic variants, the estimation of molecular size, and verification of the numbers of cysteine residues. It has been proposed that the length variation observed for the HMW-GS encoded at the *Glu-1* locus is mainly due to variations in the length of the central repetitive domain (Table II) (Halford et al 1987; Anderson et al 1988, 1989; Shewry et al 1989, 1992; D'Ovidio et al 1994, 1995b). This hypothesis has been confirmed by PCR analyses using primers specific for the N-terminal, C-terminal, and repetitive regions of HMW-GS genes at the *Glu-D1* locus in hexaploid wheats (D'Ovidio et al 1995b). As pointed out by other authors (Payne et al 1983; Shewry et al 1989; D'Ovidio et al 1996), the most likely mechanism giving rise to variation in size of the glutenin subunit is an unequal crossing-over event. This is a relatively common process among genes belonging to multigene families in eukaryotes (Baltimore 1981). Unequal crossing-over could also produce very long genes (from insertion of several blocks of repetitive motifs) according to D'Ovidio et al (1996) or very short ones (from deletion of several blocks) such as subunit 12.4¹ in *T. tauschii* (Gianibelli et al 1996a; Gianibelli 1998).

Relationship to Wheat Quality

Several HMW-GS have been closely associated with bread-making quality. Payne et al (1981a) analyzed progenies of crosses between common wheat cultivars for both SDS sedimentation volume and subunit composition. They showed that certain allelic subunits impart different effects on gluten quality. One example is the allelic variation at the *Glu-D1* locus of bread wheats, where the alternative pairs of subunits 5+10 (associated with good quality) and subunits 2+12 (associated with weaker dough quality) were identified. Such results have been confirmed in laboratories elsewhere. For example, Branlard and Dardevet (1985) reported that alveograph parameters *W* (gluten strength) and *P* (tenacity) and the Zeleny sedimentation value are correlated positively with subunits 7+9 and 5+10, and negatively with bands 2+12, whereas subunit 1

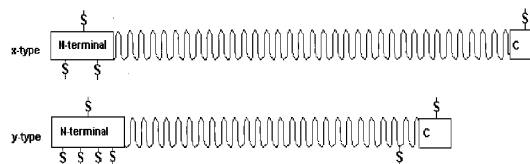


Fig. 5. Schematic of x- and y-type high molecular weight glutenin subunits based on Kasarda et al (1994).

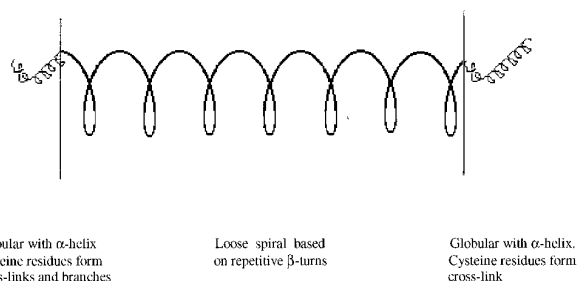


Fig. 6. Structural model for high molecular weight glutenin subunits (Shewry et al 1989).

is correlated to *W*, and subunits 2* and 17+18 with *G* (extensibility). A scoring system for HMW-GS was developed based on analyses of large numbers of cultivars (Payne et al 1987) (Fig. 3) in which individual subunits are graded with numbers based on quality evaluations. A given cultivar can then be assigned a *Glu-1* score, which is the sum of the contributions of each of the three HMW-GS loci. The HMW-GS score has more influence in some sets of wheats than in others (MacRitchie et al 1990). This is likely to be due to the complex interaction of factors that define wheat quality. These factors, in which HMW-GS have a major role, also include LMW-GS, gliadins, and abiotic stresses. One aspect that is sometimes overlooked when using this scoring system is that subunits with the same electrophoretic mobility in SDS-PAGE differ in some other features, like small differences in their protein sequences and surface hydrophobicity. For example, after the *Glu-1* score was established, Sutton (1991) found differences in retention time for subunit 8 in some cultivars when subjected to RP-HPLC. The author concluded that two different subunit 8 were involved (8 and 8*). Also, different electrophoretic mobilities were reported for subunit 7 (7 and 7*). Thus, instead of just one, four different alleles are expected for this pair: 7+8; 7*+8; 7+8*; 7*+8* (Marchylo et al 1992b). Interestingly, there are contrasting effects on quality within these pairs and the score originally given to the pair 7+8 is sometimes misleading. Nevertheless, reference to HMW-GS composition has proved valuable in the segregation of lines in breeding for specific quality targets (Cornish 1995; Cornish et al 1999).

It has been well documented that the *Glu-D1*-encoded HMW-GS pair 5+10 is associated with greater dough strength while the allelic variant pair 2+12 is associated with lesser strength. Similar results have been found for other allelic variant pairs (*Glu-B1* subunits 17+18 [strong] vs. subunits 20x+20y [weak]). These differences in dough strength are due to differences in molecular size of the glutenin polymers deduced from solubility measurements (Gupta and MacRitchie 1994). The origins of the allelic differences have not been established. However, in the 5+10 vs. 2+12 comparison, an extra cysteine residue in Dx5 may be a possible explanation (Anderson and Green 1989; Kasarda 1999).

The effect of individual proteins (HMW-GS and LMW-GS, hordeins, gliadins) on dough properties can be evaluated by studying the mixing behavior of a base flour, modified either by incorporation or addition of specific proteins (Bekes et al 1994a,b). Some recent advances in microscale mixing and protein-engineering systems have proved to be valuable in elucidating the structure and functional relationship in gluten proteins (Bekes et al 1998). Chain-extender proteins such as HMW-GS and LMW-GS increased dough strength and stability as estimated by mixograph parameters (Sissons et al 1998; Lee et al 1999a). Likewise, chain-extender proteins with longer repetitive domains increased the stability and strength more than proteins with shorter domains (Bekes et al 1998). Polypeptides containing a single cysteine can act as chain terminators during formation of the glutenin polymers, decreasing dough strength and stability (Buonocore et al 1998; Greenfield et al 1998; Tamas et al 1998).

LMW-GS

The LMW-GS (B-, C-, and D-subunits) represent about one-third of the total seed protein and ≈60% of total glutenins (Bietz and Wall 1973). Despite their abundance, they have received much less research attention than the HMW-GS. This has been mainly due to the difficulty in identifying them in one-dimensional SDS-PAGE gels. The resolution of the problem, which was principally due to overlapping between LMW-GS and gliadins, was largely resolved when Singh and Shepherd (1988) developed a simplified two-step SDS-PAGE method. Earlier, Jackson et al (1983), using a more complicated two-dimensional electrophoresis procedure, improved resolution and began systematic work on this group of subunits. More recently, Singh et al (1991a) and Gupta and MacRitchie (1991) reported similar methods to analyze polymeric proteins

after prior extraction of monomeric proteins with either 50% propan-1-ol or dimethyl sulfoxide (DMSO), respectively. Advances in the characterization of LMW-GS have also been enhanced by the production of wheat-rye translocation lines (single, double, and triple) (Gupta and Shepherd 1993), permitting the simplification of the electrophoretic pattern for closer study of the alleles of LMW-GS. RP-HPLC has also proved useful for the study of LMW-GS, showing that these proteins have higher hydrophobic surfaces than those from HMW-GS and comparable with the hydrophobic surfaces of gliadins. Recent improvements in capillary electrophoresis reported by Bean and Lookhart (2000) allowed clear characterization of all glutenin subunits.

Recently, two new LMW-GS, with molecular weights of ≈30,000 to 31,000 Da (*Glu-D4* locus) and 32,000 Da (*Glu-D5* locus) were reported (Sreeramulu and Singh 1997). These glutenin subunits could be seen only in alkylated glutenin, and the one encoded at *Glu-D5* locus has an α-type N-terminal sequence. The genes encoding them (*Glu-D4* and *Glu-D5*) are located on chromosomes 1D and 7D, respectively, although their exact localization within the chromosome has not been established. By comparison of *T. aestivum* and *T. tauschii*, it was possible to identify glutenin subunits with similar electrophoretic mobilities in the latter species, the donor of the D genome to wheat (Gianibelli 1998).

Nomenclature

The nomenclature for the major LMW-GS is based on genetic analysis and on the chromosomal location of the encoding genes. It is extensively described for bread wheat (*T. aestivum* L.) by Gupta and Shepherd (1990a) and more recently by Jackson et al (1996). Recently, Nieto-Taladriz et al (1997) described the allelic variation of the B-type LMW-GS in durum wheat (*T. durum*). Both systems are based on the relative electrophoretic mobility of subunits in SDS-PAGE. Nevertheless, a system based on the similarity of sequences instead of SDS-PAGE mobility would be a better way to designate them (Lew et al 1992). The sequences corresponding to LMW-GS have been divided into two groups on the basis of the N-terminal sequences. The first group corresponds to a LMW-m and LMW-s type and the second group corresponds to sequences similar to those of α- and γ-gliadins. The suffix refers to the first

TABLE I
Consensus Repeat Motifs of x-type and y-type High Molecular Weight Glutenin Subunits^a

Motif	Subunits	
	x-type	y-type
Tripeptide	GQQ	
Hexapeptide	PGQGQQ	PGQGQQ
Nonapeptide	GYPTSPQQ	GYPTSLQQ

^a From Shewry et al (1989).

TABLE II
Numbers of Repeat Motifs in Central Repetitive Domain of 10 High Molecular Weight Glutenin Subunits (HMW-GS)^a

HMW-GS	Number of Repeats		
	Tripeptide	Hexapeptide	Nonapeptide
Dx5	23	73	21
Ax1	15	65	23
Dx2	20	73	21
Ax2*	16	67	23
Bx7	4	66	25
Bx17	4	63	23
By9	0	56	22
Dy12	0	49	21
Dy10	0	47	21
Ay null	0	47	17

^a From Shewry et al 1992.

amino acid in the sequence, serine (-s) or methionine (-m), respectively. LMW-s sequences are more common than LMW-m (Lew et al 1992; Masci et al 1995). Almost all B-type subunits have LMW-m or LMW-s N-terminal sequences, whereas those with sequences similar to the α - and γ -gliadin have C-type electrophoretic mobilities.

Genetics and Polymorphism

The LMW-GS are controlled by genes at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on the short arms of chromosome 1AS, 1BS, and 1DS, respectively (Fig. 4). On the basis of screening a collection of 222 hexaploid wheats from 32 countries, Gupta and Shepherd (1990a) detected 20 different band patterns (LMW-GS blocks), six for the *Glu-A3* locus, nine for the *Glu-B3* locus, and five for the *Glu-D3* locus (Fig. 7). Analysis of substitution and translocation lines of the group 1 chromosome showed that the different patterns in the groups were controlled by genes on the short arms of chromosomes 1A, 1B, and 1D. Chromosome 1A encodes relatively few LMW-GS. Some cultivars do not exhibit any LMW-GS encoded by *Glu-A3*. On the other hand, there is extensive polymorphism for LMW-GS encoded by chromosome 1B. There is also evidence that some LMW-GS are controlled by genes on group-6 chromosomes (Lew et al 1992, Gupta and Shepherd 1993). A wide variability in number and electrophoretic mobility of LMW-GS was also observed in *Triticum* species like *T. monococcum* and *T. urartu* (A genome) (Rodriguez-Quijano et al 1997; Lee et al 1999b), *T. dicoccoides* (AB genomes) (Ciaffi et al 1993), and *T. tauschii* (D genome) (Gianibelli 1998). Because the actual number of electrophoretic band combinations in LMW-GS is much lower than the total of randomly possible combinations, it is assumed that the genes controlling LMW-GS are closely linked. They form clusters that are inherited together, similar to those controlling gliadin blocks (Gupta and Shepherd 1990a,b; Lagudah et al 1991). Close linkage between the *Glu-3* loci encoding LMW-GS and the *Gli-1* loci has been reported (Singh and Shepherd 1984, 1988; Payne et al 1984b; Pogna et al 1990). The *Gli-1* multigene loci encode γ - and ω -gliadins and some β -gliadins at the distal ends of the short arms of chromosomes 1A, 1B, and 1D. This close linkage (estimated as 2cM between *Glu-B3* and *Gli-B1* on the short arm of chromosome 1B in both bread and durum wheat) is useful for identifying *Glu-B3* alleles and some *Glu-D3* alleles in breeding programs. Because the gliadin composition can be screened more readily than specific LMW-GS, gliadins are potentially useful as indicators of LMW-GS alleles (Singh et al 1991b; Jackson et al 1996). Earlier studies identified γ -gliadins 45 and 42 as reliable markers for good and poor pasta quality, respectively (Damidaux et al 1978; Kosmolak et al 1980). It is now known that the effect of γ -gliadins on pasta quality is related to genetic linkages with the LMW-GS (Payne et al 1984b).

The D-subunits of the SDS-PAGE pattern appear in the glutenin fraction separated by gel-filtration chromatography (Jackson et al 1983; Payne et al 1988). Genes encoding D-subunits are completely linked to the *Gli-D1* locus on chromosome 1D (Payne et al 1986; Pogna et al 1995). On chromosome 1B they are located at a separate locus originally designated as *Glu-B2*. This locus was mapped between *Glu-B1* and *Gli-B1* (Jackson et al 1985). These genes are completely linked with those mapped at the locus originally designated as *Gld-B6* (Galili and Feldman 1984). Later, Payne et al (1988) redesignated the locus as *Gli-B3* due to the close relationship between ω -gliadins and D-subunits encoded at the *Gld-B6* and *Glu-B2* loci, which were presumed to be one locus. Furthermore, the *Gld-2* locus on chromosome 1A (Sobko 1984) was assumed to be homologous with the *Gli-B3* locus and was redesignated by Payne et al (1988) as *Gli-A3* (Dubcovsky et al 1997). Recently, Masci et al (1993) characterized D-subunits encoded by chromosome 1D with linkage to the *Gli-D1* locus of hexaploid wheat. These polypeptides, considered LMW-GS because of their electrophoretic mobilities, have proved to be modified ω -gliadins with at least one cysteine codon in their sequence. Clearly, the D-subunits are part of the aggregated glutenin structure, but in terms of functional properties, they are quite different from subunits encoded at the *Gli-B3* and *Gli-A3* loci described above. However, it is still not clear whether the D-subunits described by Masci et al (1993) are encoded at the *Gli-D1* locus or if they are encoded by some of the remote gliadin genes located halfway between *Gli-1* and the centromere, as reported for the other group-1 chromosomes (1A and 1B) by Payne et al (1988), Ruiz and Carrillo (1993), and Pogna et al (1995).

Amino Acid Composition and N-Terminal Sequences

Seven main types of LMW-GS were identified by N-terminal sequences of the proteins according to the first amino acid present in this region. LMW-s are the most abundant sequences observed, starting with SHIPGL-. LMW-m have N-terminal sequences of METSHIPGL-, METSRIPGL-, or METSCIPGL- (Kasarda 1989; Tao and Kasarda 1989; Lew et al 1992). A further three types were identified with N-terminal sequences that resemble those of α -, γ -, and ω -type gliadins. The last three have odd numbers of cysteine residues, which allow their incorporation into glutenin by means of an intermolecular disulfide bond (Kasarda 1989). They are rich in proline and glutamine residues, with most of them showing electrophoretic mobilities similar to those of C-subunits (Lew et al 1992). The three variants from the LMW-m sequence often have a basic residue (histidine or arginine) at position 5 (LMW-mh5, LMW-mr5), but cysteine was sometimes present instead (LMW-mc5). Complete sequences for C-type LMW-GS have LMW-mc5 terminal sequence, while other sequences, incomplete at the protein N-terminus, also appear to correspond to LMW-mc5 subunits. All LMW-m sequences

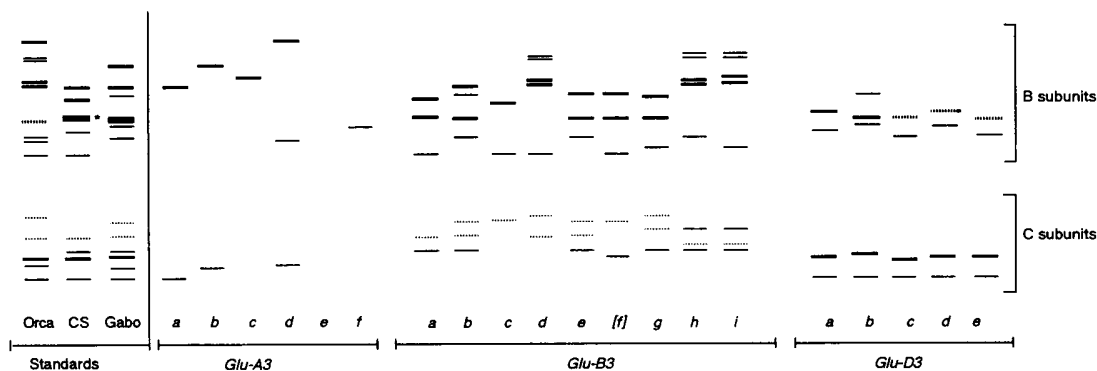


Fig. 7. Analysis of over 200 bread wheat cultivars showing three groups (A, B, and D genome, left to right) of B and C low molecular weight glutenin subunit combinations identified by 2-step SDS-PAGE. Three reference patterns include Orca, Chinese Spring (CS), and Gabo. Dotted lines represent faint bands. Patterns *a* and *b* in each group are from Chinese Spring and Gabo, respectively. (Reproduced with permission from Gupta and Shepherd 1990a, Fig. 3.)

contain six conserved cysteines, corresponding to SC1-5 and SC8 of γ -gliadin (Thompson et al 1994; D'Ovidio et al 1995a).

Structure

Several DNA sequences have been reported for LMW-GS genes (Anderson et al 1991). The secondary structures of LMW-GS, except for D-subunits, have an overall similarity to the structure of S-rich gliadins (Tatham et al 1987; Thompson et al 1994; D'Ovidio et al 1995a) (Fig. 8). Most polypeptides consist of 250 to 300 residues. They have a clear two-domain structure, although further divisions within these have been reported (Kasarda et al 1984; Wieser 1995). N-terminal repetitive domains are rich in β -turns, possibly forming a regular spiral structure, while the short nonrepetitive domains are rich in α -helix and appear to be more compact (Thomson et al 1992). Repeated sequences account for ≈ 30 to 50 mol% of these proteins, in contrast to the more extensive repeats in the S-poor gliadins and HMW-GS. These proteins have a cysteine residue within the N-terminal domain (Okita et al 1985; Colot et al 1989; Tao and Kasarda 1989; Lew et al 1992) that is unlikely to form intramolecular disulfide bonds with cysteine residues in the C-terminal domain because of the rigidity imposed by the repetitive sequence. In addition, LMW-GS have seven cysteine residues in the C-terminal domain, at least one of which is unpaired, making it available for intermolecular bonding.

In terms of electrophoretic mobility and N-terminal sequences, the D-subunits are very similar to the S-poor ω -gliadins (Masci et al 1991a,b, 1993), supposedly being mutant forms of ω -gliadins in which a single cysteine residue allows cross-linking into the glutenin polymer. According to Masci et al (1999), only one cysteine was involved in the structure of D-subunits, allowing them to act as chain terminators. Similarly, Gianibelli et al (1996b, *in press*) and Nieto-Taladriz et al (1998) reported LMW-GS with a M_r of $\approx 70,000$ Da and a N-terminal sequences similar to that of the ω -gliadins encoded at the *Gli-B1* locus. This subunit participated in the glutenin polymeric structure (Gianibelli et al *in press*).

Molecular Characterization of LMW-GS genes

The LMW-GS genes, like all other prolamin genes, do not have introns in their sequences (Kreis et al 1985; Anderson et al 1991; Cassidy et al 1998; Ciaffi et al 1999; Lee et al 1999c; D'Ovidio et al 1999). Several total and partial sequences of LMW-GS genes have been reported (Bartels and Thompson 1983; Okita et al 1985; Pitts et al 1988; Colot et al 1989; Cassidy and Dvorak 1991; D'Ovidio et al 1995a; Van Campenhout et al 1995; Benmoussa et al 2000). DNA-sequencing of LMW-GS predominantly shows an N-terminal sequence of METSCIPGL- with a cysteine in position 5 that is not representative of the major groups of LMW-GS as indicated by N-terminal amino acid sequences. The most abundant subunit corresponds to the LMW-s type of sequence. Sequences of LMW-GS genes corresponding to this type were obtained in durum wheat (D'Ovidio et al 1997b) and bread wheat (Masci et al 1998). These type of subunits present molecular weights higher than those of LMW-m, ranging from 30,000 to 45,000 Da. The LMW-m is represented by three different N-terminal sequences: METSHIPGL-, METSRIPGL- and METSCIPGL-, the first of which is the most abundant. The third group is typical of the cloned sequences and is less common. The LMW-GS gene sequences show a clear structural organization of the polypeptide. A general model LMW-GS structure was generated from gene sequences (Cassidy et al 1998) (Fig. 9). The sequences encoded a protein with a typical signal peptide of 20 amino acids close to the N-termini, followed by a short N-terminal sequence of 13 amino acids (Fig. 9, area I). This N-terminal sequence was present in most of the LMW-GS genes analyzed, with the exception of two genes reported by Pitts et al (1988) (clone LP1211) and Cassidy et al (1998) (clone L4). A cysteine residue in the N-terminal region located in position five is a common feature for most of the LMW-GS genes. Nevertheless, this cysteine does not appear in clones such as LP1211 and L4 where a deletion of

13 amino acids was observed. Further evidence of this cysteine not being present comes from studies by D'Ovidio et al (1997b, 1999) and Masci et al (1998, 2000) on *T. durum* and *T. aestivum* clones, respectively. In these cases, a cysteine at position 45 was found in the repetitive region. The next region corresponds to the repetitive domain (area II), which is formed by a variable number of amino acids from 70 (Cassidy and Dvorak 1991) to 186 (D'Ovidio et al 1999). Intermediate values (94–99) were reported by Cassidy et al (1998) and Colot et al (1989). As for HMW-GS, the length of the repetitive domain defines the size variation for LMW-GS genes. A very conservative C-terminal domain follows. Three areas have been defined in the C-terminal domain (Cassidy et al 1998). The first area has five cysteine residues present (area III); the second area corresponds to a more variable area that has one cysteine residue in either of two positions (area IV); the third is separated from the other by only 15 amino acids. The extreme of the C-terminal region is very conservative, with eight cysteine residues (area V). It has been suggested that cysteines in areas I or II (either in position 5 or 45) and the one in area IV are likely to participate in intermolecular disulfide bond formation, while the others participate in intramolecular disulfide bond formation (Fig. 9) (Lew et al 1992; Shewry and Tatham 1997; Müller et al 1998; D'Ovidio et al 1999; Masci et al 1998, 2000).

Most of the LMW-GS genes sequenced, in terms of molecular weight, belong to the C-subunit region. Only recently have sequences been reported for genes with molecular weights corresponding to B-subunits (D'Ovidio et al 1997b, 1999; Masci et al 1998, 2000). These genes are encoded at the *Glu-B3* locus in both durum and bread wheat. Most of the genes belong to the LMW-m sequences and only two have been reported with sequences that match the LMW-s sequences (D'Ovidio et al 1999; Masci et al 1998, 2000). The polypeptides of both genes belong to the B-type of subunit in terms of electrophoretic mobility with $M_r \approx 42,000$ Da. According to Masci et al (1998), the amino acid methionine was also observed when sequencing the LMW-s GS gene. It was located in a position comparable to that of LMW-m sequence METSH, but a threonine was replaced by an arginine and therefore the MET sequence was converted to MEN. This sequence is part of the signal peptide rather than corresponding to the N-terminal sequence, but close to the signal cleavage site. Masci et al (1998) speculated that this difference in sequence near the putative signal cleavage point might be responsible for the different N-terminal sequences between the LMW-m and LMW-s. All the complete LMW-m or LMW-s sequences that have been reported have eight cysteines and have been considered as chain-extender subunits. In contrast, the α -type and γ -type LMW-GS are most likely to be polypeptides with an odd number of cysteines, very similar to α -type and γ -type gliadins but with an extra cysteine that has allowed the polypeptide to be incorporated

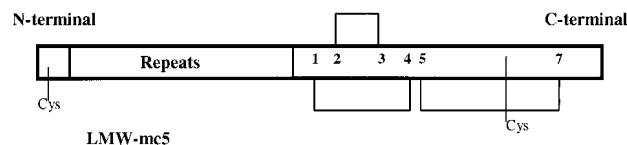


Fig. 8. Structure of a low molecular weight mc5-type glutenin subunit showing disulfide bonds. (Adapted from Thompson et al 1994.)

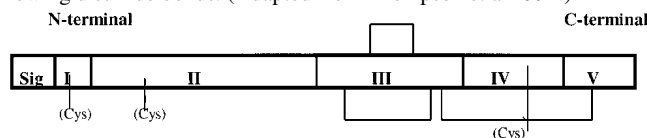


Fig. 9. Polypeptide structure of a low molecular weight glutenin subunit (based on Cassidy et al 1998) and intramolecular disulfide bonds (according to Müller et al 1998). (Cys) nonconservative cysteine residues in different types of low molecular weight glutenin subunits (Cassidy et al 1998; Masci et al 1998). Sig: signal peptide.

into glutenin polymers. Such is the case reported by D'Ovidio et al (1995a) in durum wheat, where an extra cysteine in position 26, located at the beginning of the repetitive domain, was observed. Thus, a total of nine cysteines were detected in this γ -type LMW-GS. Within this type of LMW-GS sequence, the D-subunits should be included insofar as they are modified ω -gliadins that have acquired at least one cysteine in their primary structures (Masci et al 2000; Gianibelli et al *in press*).

Relationship to Wheat Quality

A number of studies have revealed that the allelic variation at the LMW-GS loci is associated with significant differences in dough quality in bread (Gupta et al 1989, 1994) and durum wheat (Pogna et al 1990; Ruiz and Carrillo 1993). LMW-GS have the ability to form large aggregates that are related to dough strength. Payne et al (1984b) were the first to associate LMW-GS with quality characteristics of tetraploid wheat. A preliminary study ranking LMW-GS alleles in order of quality also has been reported (Gupta et al 1989; Cornish 1995; Cornish et al 1999). However, it has been suggested that the effect of these alleles on quality would be more accurately assessed if they are considered in conjunction with the HMW-GS (Gupta et al 1994).

The cysteine residues in the primary structure of the LMW-GS allow us to identify two different polymer-building subunits: chain extenders (subunits with two or more cysteine residues that form intermolecular disulfide bonds) and chain terminators (with only one residue of cysteine available for intermolecular disulfide bonds). While chain extenders allow the formation of stronger doughs, chain terminators have the opposite effect (Greenfield et al 1998; Masci et al 1998; Tamas et al 1998). Functional studies of individual glutenin subunits involving the partial reduction of flour proteins and the subsequent incorporation of the proteins into the polymeric glutenin structure by reoxidation have been very useful in assessing the effect of particular subunits on functional properties of dough (Bekes et al 1994a,b). This approach, combined with the expression of individual glutenin subunits in *E. coli* and the availability of microscale testing to evaluate dough properties, has facilitated the understanding of the effect of particular glutenin subunits in wheat quality (Bekes and Gras 1999). Thus, Sissons et al (1998) showed that the incorporation of LMW-GS with N-terminal sequence METSH produced an improvement of the mixing properties along with an increase in the proportion of the polymeric proteins estimated by SE-HPLC. Likewise, Lee et al (1999a) observed different degrees of increase in dough mixing time when three LMW-GS (LMW-mc5) were incorporated into the dough. LMW-GS from the A genome (*T. boeoticum*) compared with that of D genome from *T. tauschii* were more effective in increasing mixing time. Nevertheless, the effect of incorporating LMW-GS was less important than that observed when HMW-GS 7 was incorporated.

CONCLUSIONS

A great deal of research attention has been focused on the study of wheat gluten proteins in the past three decades. A clear indication of that is the long list of literature citations included in this review. While some areas of study have been thoroughly elucidated, others still need research attention. In particular, the failure to obtain complete solubilization of unaltered polymeric structures hinders a more robust evaluation of the relationship between glutenin and the quality of wheat-based products. Because gluten quality is obviously a complex issue, we would not expect that a solution of the solubility question would result in a complete understanding of the problem. The birth of the proteome approach offers a fresh new look to the problems of gluten functionality, opening the opportunity of identifying the polypeptides that might not have been previously taken into account as wheat-quality modifiers during protein synthesis and processing in the immature endosperm, providing also for the combined study of gene expression and growth conditions.

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