Theor Appl Genet (2002) 104:422–428

S. Masci · L. Rovelli · D.D. Kasarda · W.H. Vensel
D. Lafiandra

Characterisation and chromosomal localisation of C-type low-molecular-weight glutenin subunits in the bread wheat cultivar Chinese Spring

Received: 5 April 2001 / Accepted: 4 May 2001

Abstract Low-molecular-weight glutenin subunits are classically divided into the B, C and D groups. Most attention has been paid to the characterisation of the B and D groups, whereas C subunits, although represented by a large number of protein components, have not been thoroughly characterised, mainly because they tend to separate with the gliadins in many fractionation procedures. Here we describe a procedure for obtaining a fraction strongly enriched in C subunits that has allowed us to determine the chromosomal location of these subunits in the bread wheat cultivar Chinese Spring. This analysis has shown that these subunits are coded on chromosome groups 1 and 6. Comparison between N-terminal amino acid sequencing of B and C subunits has shown that, whereas the former group includes mainly subunits with typical LMW-GS type sequences (76%), the C subunit group is made up almost completely of subunits with gliadin-like sequences (95%), including the α-type. These results indicate that the LMW-GSs are likely to be coded not only by the typical *Glu-3* loci, but also by loci tightly linked to, and possibly included within, the *Gli-1* and *Gli-2* loci.

Keywords C subunits · Low-molecular-weight glutenins · Chromosomal localisation · Wheat storage proteins · Glutenin · Protein sequencing

Communicated by H.F. Linskens

S. Masci · L. Rovelli · D. Lafiandra (✉)
Dipartimento di Agrobiologia e Agrochimica,
Università degli Studi della Tuscia, Via S. Camillo de Lellis,
01100 Viterbo, Italy
e-mail: lafiandr@unitus.it
Tel.: +39-761 357243, Fax +39-761 357238

D.D. Kasarda · W.H. Vensel
U.S. Department of Agriculture, Agriculture Research Service,
Western Regional Research Center, 800 Buchanan Street,
Albany, CA 94710, USA

Introduction

Gliadins and glutenins are the main components of the wheat storage proteins and make up almost all of the proteins found in gluten. These fractions and their individual proteins are extensively studied because of their importance in determining dough rheological properties (Lafiandra et al. 2000).

Gliadins are monomeric proteins either because they do not possess cysteine residues, as in ω-gliadins, or because any cysteine residues present are involved in intramolecular disulfide bonds. Gliadins are classified as α-, β-, γ- and ω-gliadins in order of decreasing mobility in gel electrophoresis at acid pH (Woychik et al. 1961). The α- and β-gliadins have closely similar amino-acid and DNA sequences (Kasarda et al. 1987), so that they are currently indicated as either α-type or α/β-gliadins, and have molecular weights in the approximate range 30–45 k. The γ-gliadins have a similar amino-acid composition to α-gliadins and to typical low-molecular-weight glutenin subunits, although differing considerably in their sequences from these latter proteins, and all three of these protein types have molecular weights in the range 30–45 k. The ω-gliadins have higher apparent molecular weights by SDS-PAGE (44000–74000) and distinctive amino-acid compositions. Most of the α/β-gliadins are encoded at the *Gli-2* loci, located on the short arms of chromosomes 6A, 6B and 6D, while most γ- and ω-gliadins are encoded at the *Gli-1* loci, located on the short arms of chromosomes 1A, 1B and 1D. The *Gli-1* loci are linked to the *Glu-3* loci, the latter coding for typical LMW-GS having N-terminal sequences beginning with METS or SHIP.

The glutenin subunits, high-molecular-weight (HMW-GS) and low-molecular-weight (LMW-GS), have both intra- and inter-molecular disulfide bonds, with the latter resulting in the formation of the ”glutenin polymer.” The size distribution and composition of the polymers in the glutenin fraction are strongly correlated to flour technological characteristics (Wrigley 1996). HMW-GS are coded by gene pairs on the long arms of group-1 chro-

mosomes (*Glu-1* loci), whereas typical LMW-GS are coded by gene families located on the short arm of the same chromosomes (*Glu-A3*, *Glu-B3* and *Glu-D3* loci).

LMW-GS are classically divided into B, C and D groups on the basis of molecular weight and isoelectric point (Jackson et al. 1983). Typical LMW-GS are mostly present in the B group (Lew et al. 1992; Masci et al. 1995), whereas the D group corresponds to mutated ω-gliadins which, by virtue of the presence of only a single cysteine residue, become part of the polymeric fraction (Masci et al. 1993, 1999). The LMW-GS group that has been least characterised is the C group, mainly because of technical problems with their fractionation and purification. The C group of LMW-GS is quantitatively present in a lower amount with respect to the B group, although the number of subunits is comparable to those present in the B group (Payne et al. 1985). In the absence of a specific procedure aimed at obtaining a fraction enriched in C subunits, it has been extremely difficult to analyse and characterise them in detail. The B subunits are more abundant, and because the properties of B- and C-type subunits are fairly similar, separation prodedures, such as those involving electrophoretic gels or chromatographic columns, usually result in overlaps in which the B subunits predominate.

C subunits have a lower-molecular-weight range (30000–40000) compared to B and D subunits, and N-terminal amino-acid sequencing has shown that they are mostly made up of α/β-and γ-gliadin-like components, with some typical LMW-GS exhibiting the SHIP- and METS-types (Tao and Kasarda 1989; Lew et al. 1992). The presence of gliadin-like subunits in glutenin preparations is very likely due to mutations that affect the number and/or the distribution of cysteine residues, as has been demonstrated for D subunits (Masci et al. 1993). In support of such a hypothesis, D'Ovidio et al. (1995) sequenced a γ-type glutenin gene from a c-DNA durum wheat library, which had nine cysteine codons, instead of the eight present in typical γ-gliadins, and which very likely corresponds to a C-LMW glutenin subunit of the corresponding durum wheat cultivar. Moreover, Anderson and Greene (1997) have sequenced different α-gliadin genes including an odd number of cysteine codons rather than the usual six. Although these sequences have been obtained from genomic clones, if such genes are active they might well code for the α–gliadin-like subunits found in the glutenin fraction.

Mutation is a rare event and it is likely that mutated genes will have acquired only one extra or missing cysteine residue, so that the encoded polypeptides frequently have odd numbers of cysteine residues. An odd number of cysteines makes such subunits chain terminators of growing glutenin polymer chains, which would presumably have a negative effect on flour quality (Tao and Kasarda 1989). Additionally, changes in position of the cysteine residues might affect the pattern of disulfide bond formation, resulting in a failure of two cysteine residues in a protein with an even complement to form an intramolecular bond. These two cysteines would then be available for intermolecular disulfide bond formation and such a subunit might then act as a chain extender.

The finding that some C subunits have N-terminal amino-acid sequences corresponding to γ- and α/β-gliadins implies that LMW-GS are possibly coded by genes at the *Gli-1* and *Gli-2* loci, as well as at the *Glu-3 locus.* Accordingly, it may be that some subunits are coded also on group-6 chromosomes (the location of *Gli-2* loci), which has not yet been established. In order to study C-type LMW-GS and the locations of the genes coding for them in more detail, we have combined and extended different procedures (Morel 1994; Verbruggen et al. 1998) for their preparation. This has allowed us to compare N-terminal sequences of the polypeptides present in the B and C fractions of LMW-GS, and to analyse the chromosomal locations of genes coding for C subunits that had been separated by two-dimensional electrophoresis. The extensively studied cultivar 'Chinese Spring' has been used in this study because of the availablity of its aneuploid and substitution lines.

Materials and methods

Wheat lines

The following materials were used in the analysis of the chromosomal locations of genes coding for C subunits of CS: the bread wheat cultivar Chinese Spring (CS); its nullisomic-tetrasomic lines involving chromosomes 1 and 6; CS ditelosomic lines involving chromosomes 1 and 6; the intervarietal 1A, 1B, 6A and 6B substitution lines of the cultivar 'Cheyenne' (CNN) in Chinese Spring; the 1D/1B and 6D/6B substitution lines of CS in the durum wheat cultivar Langdon; the durum wheat genotype Lira, null at the *Gli-A2* locus; and the bread wheat genotype Saratovskaja, null at the *Gli-A2* and/or *Gli-D2* loci.

Isolation of the B and C groups of low-molecular-weight glutenins

In order to obtain fractions enriched in B or C subunits, we used different precipitation steps involving increasing concentrations of propan-1-ol, based mainly on the methods described by Verbruggen et al. (1998). The procedure used was as follows: wheat seeds (1–3 kernels) were crushed with a mortar and pestle, and gliadins were removed by triple extraction with 1 ml of 50% propan-1-ol at room temperature for 30 min. Each step was followed by 5 min of centrifugation at 14000 g. Total glutenin subunits were extracted (1 mg:5 µl) with a buffer containing 50% propan-1-ol, 80 mM Tris-HCl, pH 8.5, 20 mM dithiothreitol (DTT) at 60°C for 30 min. After centrifugation at 14000 g for 10 min, a freshly prepared solution of propan-1-ol (at room temperature) and 1% DTT was added to the supernatant containing the glutenin subunits to reach the final propan-1-ol concentration of 60%. The precipitation was performed at 4°C for 1 h. After 20 min of centrifugation at 12,000 g, sequential precipitation was performed on the supernatants by adjusting the propan-1-ol concentration first to 75%, then to 80% (this latter step resulted in pellets strongly enriched in B subunits) and finally to 85% in the same conditions as above. In this last step, the pellet was discarded, and the supernatant was stored at 4°C overnight, in order to allow specific precipitation of C subunits, which were obtained as a pellet after centrifugation for 20 min at 12000 g. Pellets containing either B or C subunits were dried-down in a Savant Speed-Vac (Savant, Farmingdale, N.Y.) concentrator and stored at –20°C until used.

Reversed-phase high performance liquid chromatography (RP-HPLC) of B and C subunits

The dried pellets obtained from the above isolation procedures were dissolved in 100 µl of 50% propan-1-ol, 40 mM of Tris-HCl (pH 8.5), and 10 mM of dithiothreitol. Before injection, an additional 100 µl of 50% acetonitrile (ACN) containing 0.05% trifluoroacetic acid (TFA) was added. The protein solution was filtered through a 0.45-µm filter and injected into the sample loop of the RP-HPLC system. A System Gold HPLC apparatus (Beckman, Palo Alto, Calf.), composed of the solvent delivery module 126 and UV-detector module 166, was used for the fractionations. Protein separations were carried out with a Supelco (Bellafonte, Pa.) C_8 analytical column (25 cm×4.7 mm). Solvent A was 0.05% (v/v) TFA, and solvent B was ACN containing 0.05% (v/v) TFA. The gradient used was 29%–43% solvent B over 50 min. The flow rate was 1 ml/min. The column was thermostated at 50°C and proteins were detected by UV absorbance at 210 nm. All peaks obtained from the B-subunit preparation or all peaks obtained for the C-subunit preparation were collected in a bulk, dried-down, dissolved in 29% ACN containing 0.05% TFA, and re-injected under the same conditions in order to improve the purification. After the final purification step, fractions were dried down for N-terminal amino-acid sequencing.

N-terminal amino-acid sequence analyses

The bulks of B and C subunits were subjected to N-terminal amino-acid sequencing as mixtures in a Procise Model 492 protein sequencer (PE-Applied Biosystems, Foster City, Calif.). In order to quantitate the different sequence types present as a mixture in one or the other of the fractions, the following approach was used. Sequencing was carried out for 5–10 cycles. The possible protein sequences in each mixture were assumed to correspond to the following four main types of glutenin subunits: (1) SH**I**PGLERPS; (2) **M**ETSHIPGLE; (3) **V**R**V**PVPQLQP; and (4) **N**MQVDPSGQV. The amino acids indicated in bold type for each sequence were chosen for quantitative analysis because they are unique markers of a single sequence in the indicated cycle and the sequences of interest did not show variations in the amino acid found at that cycle. Results were examined for evidence of other types of sequences, such as those corresponding to the HMW-GS and D subunits, but we saw no indication that these sequences were represented in our mixtures at significant levels. Amino acids were, of course, analyzed as the phenylthiohyndantoin derivatives, which is the end result of the Edman degradation cycles used for sequencing; but for purposes of simplification, we shall just refer to the parent amino acid here.

The amino acids chosen as markers for quantitative analysis of the proportions of subunits in the mixtures give relatively stable and reproducible yields in sequencing. For example, the isoleucine at cycle 3 in sequence 1 is not found in any other type of gluten protein sequence and is frequently used for calculating sequencer repetitive yields because of its reproducible recovery during the Edman degradation cycles. When the amino-acid marker did not appear in the first cycle, its yield in the cycle preceding the cycle of interest was subtracted as a background correction. A repetitive yield of 93%, which is fairly typical for the Procise sequencer, was assumed and when the chosen amino acid did not appear in the first cycle, the yield for that sequence was corrected to a first-cycle equivalent by the appropriate factor. Finally, the percentage of each of the four sequences in the mixture was calculated.

Two-dimensional electrophoresis (APAGE vs SDS-PAGE)

In order to analyse the pattern of C subunits in more detail, two-dimensional electrophoresis (APAGE vs SDS-PAGE) was performed on fractions obtained after the propan-1-ol precipitation steps reported above. The procedure used, partly based on that proposed by Morel (1994), was as follows:

First dimension (APAGE)

The first-dimension electrophoresis was carried out in acidic solution with a Hoefer SE600 Apparatus (Amersham Pharmacia, Uppsala, Sweden). Polyacrylamide gels (14 cm×16 cm×1 mm, T=6 and C=2.67) contained 4 M urea and 0.75% (v/v) acetic acid, and were polymerised by using 7.5 mM ammonium persulfate and 0.6% (v/v) TEMED. A pre-run with normal polarity at constant current (27 mA per gel) at 20°C was performed with 0.75% (v/v) acetic acid until the initial voltage reached 180–190 V (about 40–50 min.).

The dried samples were dissolved in 25 µ l of a solution containing 0.14% acetic acid, 6 M urea, and methyl violet as a tracking dye. Solubilisation was improved by sonication (Branson 3200, Bransonic, Shelton, Conn.) for 10 min. After centrifugation at 14000 g for 5 min at room temperature, 20 µl were loaded on the first-dimension gel.

For the first-dimension electrophoresis, the upper chamber contained 0.14% (v/v) acetic acid and the lower contained 0.25% acetic acid. The analysis was carried out at constant current (27 mA per gel) at 20°C with reversed polarity (upper electrode positive), and was stopped 5 min after the dye reached the bottom of the gel (about 3 hours). The gels were stained according to Neuhoff et al. (1988) and de-stained in distilled water.

Second dimension (SDS-PAGE)

The second-dimension electrophoresis was performed in the same apparatus used for the first dimension, and gels were the same size. The main gel was T=12 and C=2, and contained 0.38 M Tris-HCl, pH 8.8 and 0.1% SDS. The stacking gel (T=3.75, C=2.67, 0.125 M Tris-HCl, pH 6.8, 0.1% SDS) was poured up to about 1 cm from the top of the glass plates, without a comb. Once the first-dimension gel had been stained, lanes containing samples to be submitted to the second-dimension separation were cut and equilibrated for 30 min at room temperature in a solution containing 10% glycerol, 2% SDS, 0.125 M Tris-HCl at pH 6.8, and 0.5% DTT. The equilibrated gels were placed on top of the second-dimension gel without using agarose to seal them to the second-dimension gel. The run was carried out at 12°–14°C, at a constant current (30 mA per gel), and was stopped 15 min after the Coomassie Brilliant Blue used to stain the first dimension reached the bottom of the gel (about 4 h). The running buffer contained 0.2 M glycine, 25 mM Tris at pH 8.8 and 0.1% SDS. Gels were stained overnight with 12% trichloroacetic acid solution containing 0.05% Coomassie Brillant Blue R-250 in absolute ethanol (1%, w/v) and de-stained in tap water.

Results and discussion

The purification of the B and C groups of LMW-GS obtained by the procedure described above is illustrated in Fig. 1. Although there is a partial overlapping of the electrophoretic mobilities between the two groups, both in one- and two-dimensional electrophoresis, there is clearly a strong enrichment of B subunits in the fraction corresponding to the pellet obtained after 80% propan-1-ol precipitation and of C subunits in the pellet corresponding to the final 85% propan-1-ol precipitation step. The final yield is strongly influenced by temperature, the amount of flour used and the precipitation time, and the procedure is qualitative rather than quantitative.

The bulks of B and C subunits were also submitted to RP-HPLC in order to obtain fractions for N-terminal amino-acid sequencing. The effectiveness of the procedure used in separating B and C subunits was further

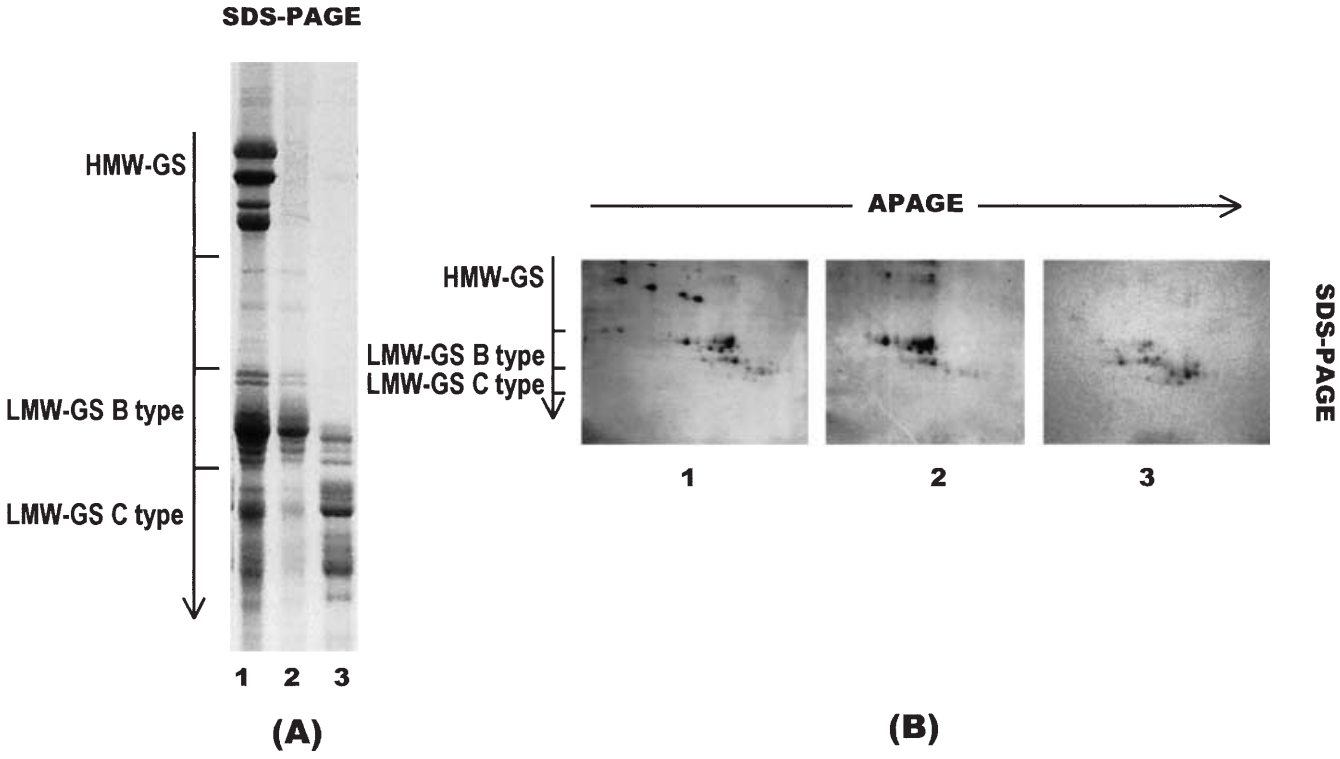

Fig. 1 (**A**) SDS-PAGE and (**B**) two-dimensional (A-PAGE vs SDS-PAGE) electrophoretic separation of (*1*) total glutenin subunits from the bread wheat cultivar Chinese Spring, (*2*) the B subunit fraction, (*3*) the C subunit fraction

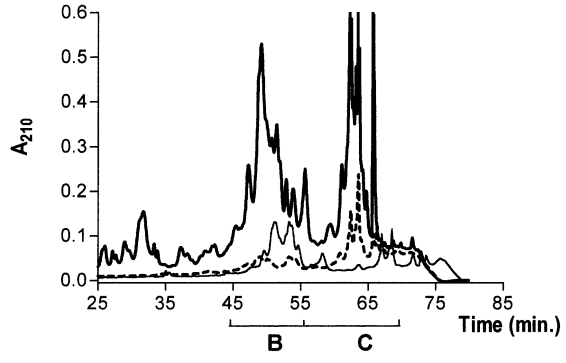

Fig. 2 RP-HPLC of total glutenin subunits (*solid bold line*), *B* (*solid thin line*) and *C* (*dotted line*) LMW-GS from cv Chinese Spring

confirmed by RP-HPLC. Figure 2 shows the RP-HPLC separations of the B and C groups in comparison with the separation of the total glutenin subunits. The peaks of the two groups elute for the most part at different retention times (Fig. 2). In fact, the B-type LMW-GS elutes preferentially between 45 and 55 min, whereas most of C subunits elute in the range of 55–70 min. Fractions eluting between 45 and 70 min were collected together and dried down in a single test tube for both the B and C subunit-fractions, and then submitted to a second RP-HPLC separation as described, again collected, and finally submitted to N-terminal amino-acid sequencing.

Table 1 Percentages of N-terminal sequences present in B and C subunits. Each sequence type corresponds to the following N-terminal amino-acid sequences: α: VRVPV; γ: N(M/I)QVD; LMW-GS Met-type: METS(H/C); LMW-GS Ser-type: SHIPG

	Sequence type	Percentage
B subunits	α	9
	γ	15
	LMW-GS Met-type	24
	LMW-GS Ser-type	52
C subunits	α	40
	γ	55
	LMW-GS Met-type	5
	LMW-GS Ser-type	0

Mixture sequencing showed that, as expected, B subunits consist mostly of typical Ser-type (52%) or Met-type (24%) LMW-GS sequences, for a total of 76%; whereas C subunits have gliadin-like sequences almost exclusively (95%), with a slight prevalence of γ-type (55%) over α-type (40%) gliadin sequences. The percentages attributed to each sequence type are reported in Table 1. It is interesting to note that no LMW-GS Ser-type proteins are present in the C subunit fraction. LMW-GS Ser-type sequences seem to correspond only to the highest molecular weight polypeptides among LMW-GS, namely B subunits (Tao and Kasarda 1989; Lew et al. 1992; Masci et al. 1995), and apparently this type does not have molecular variants in the molecular-weight range of C subunits, whereas LMW-GS corresponding to the Met-type are known to have variant

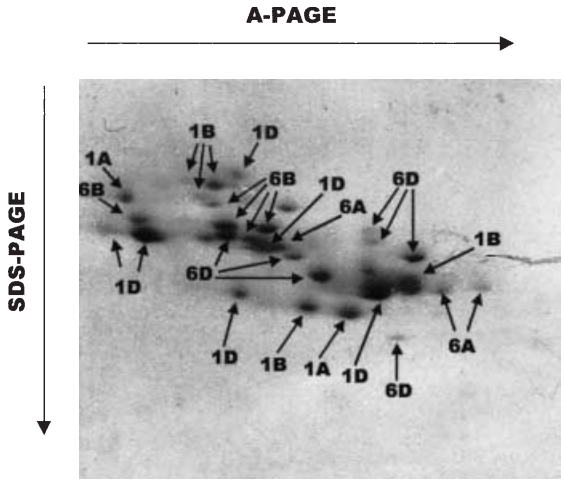

Fig. 3 Chromosome assignments for the components of the C LMW-GS fraction from cv Chinese Spring

Table 2 Number of components belonging to the C fraction of LMW-GS assigned for each chromosome

Chromosome	Number of components
1A	2
1B	5
1D	5
6A	3
6B	4
6D	6

forms that fall in the molecular-weight ranges of both the B and C groups (Lew et al. 1992). Solubility fractionation, as applied here, might be expected to separate components somewhat on the basis of protein type, which, for example, might explain the presence of a few components having α-type and γ-type sequences in the molecular-weight range of B subunits for the C subunit fraction (compare Figs. 1 and 3).

The possibility to obtain fractions strongly enriched in C subunits, which we have shown to be composed mainly of modified α- or γ-gliadin type subunits, along with the development of a high-resolution two-dimensional procedure, has enabled us to analyze the chromosomal locations of the genes coding for the different spots belonging to the C-type LMW-GS (Fig. 3). The use of appropriate genetic stocks allowed us to assign the resolved subunits to the short arms of the homoeologous group-1 and -6 chromosomes. Analysis of null-type lines (either at the *Gli-A2* or *Gli-D2* loci, or both) showed consistency between the absence of particular gliadin components and C components, suggesting a tight linkage between loci coding for gliadins and those coding for C-type LMW-GS (data not shown). The distribution of chromosome assignments is shown in Table 2. Although we resolved about 30 components in the C fraction of low-molecular-weight glutenin subunits, some minor components were not assigned. An extensive analysis of two-dimensional patterns of progenies of specific crosses will give information about the genetic linkage between *Gli-1* and *Gli-2* loci, and the loci coding for gliadin type glutenin subunits. It is, of course, possible that γ-type and α-type glutenin subunit genes are found within the complex loci coding for the gliadin genes and intermixed with them. The *Gli-1* and *Gli-2* loci are complex and include multiple gene copies, many of which show indications of slight divergence. Because a mutation of a single codon to a cysteine codon is likely to be sufficient to turn a monomeric gliadin into a chain-terminating glutenin subunit, it would not be surprising to find that many C subunit genes are part of the *Gli-1* or *Gli-2* loci.

Conclusions

C subunits of low-molecular-weight glutenins are represented by a large number of components, although their relative amount is smaller than that of the B subunits. Because of this latter aspect, they have been overlooked, although they are significant components of gluten. Lew et al. (1992) estimate that α- and γ-type glutenin subunits account for 20–40 mol % of total LMW-GS, although some subunits of this type actually fall within the B subunit molecular-weight range.

N-terminal amino-acid sequencing of two-dimensional spots corresponding to C subunits in 2-D electrophoretic patterns of glutenin (Tao and Kasarda 1989) previously had indicated that gliadin-like polypeptides were present in the fraction. Contrasting hypotheses have been formulated in order to explain their presence. In one hypothesis, the gliadin subunits were considered to be physically ”entrapped” in the glutenin polymer, due to its entangled structure, even though electrophoresis of purified, unreduced glutenin polymers denatured by SDS shows no components migrating in the range of LMW-GS when analyzed by SDS-PAGE (Tao et al. 1989). An alternative explanation was that such proteins were present in the glutenin polymer because of a different organisation of their cysteine residues that enabled them to form intermolecular disulfide bonds. This latter hypothesis has been supported by various experimental studies (Masci et al. 1993, 1999; D’Ovidio et al. 1995; Anderson et al. 1997) and in particular by Köhler et al. (1993) and Keck et al. (1995), who identified an intermolecular disulfide bond between peptides belonging to typical LMW-GS and γ-gliadin type subunits.

If the different cysteine organisation is such that the mutated subunit is able to form only one intermolecular disulfide bond, such a subunit will act as a terminator of the growing glutenin polymer chains, resulting in shorter lengths of the polymer chain. The consequent downward shift in average molecular-weight of the glutenin polymer should result in a negative contribution to gluten quality in consideration of evidence for a positive correlation existing between polymer size and viscoelastic

properties (Huebner and Wall 1976; Dachkevitch and Autran 1989; Gupta et al. 1993). If, instead, C subunits form more than one intermolecular disulfide bond, they might behave as chain extenders or even chain branchers, and contribute positively to dough quality. D subunits of LMW glutenins (Masci et al. 1993, 1999) and the γ-type glutenin subunit isolated in the durum wheat cultivar Lira (D'Ovidio et al. 1995) are likely to be chain terminators because of the presence of a single extra cysteine residue relative to the absence of cysteine residues in the related normal ω-gliadins and the normal complement of eight cysteine residues found in normal γ-gliadins. The characterisation of a clone corresponding to an ω-secalin gene from rye, which is highly homologous to genes for ω-gliadins (completely lacking cysteines), showed that a single mutation event, like a frameshift mutation, can produce two cysteine codons (Clarke and Appels 1999), a finding that opens the possibility of at least some C subunits being chain extenders.

On the basis of the above exposition, we suggest that the study of C subunits is important for an unravelling of glutenin polymer structure. Here we have reported a combination of methods that allow a detailed characterisation of C subunits. N-terminal amino-acid sequencing has demonstrated that C subunits are mainly composed of gliadin-like sequences (95%), approximatively equally distributed between α– and γ-types, with a slight favoring of γ-types (55% vs 40%), whereas these α- and γ-types are present in a minor amount in B subunits (24%).

For the first time, the chromosome localisation of C subunits has been reported. The two-dimensional analysis of the C group of LMW glutenins has shown that they are coded on chromosome groups 1 and 6, as was thought likely based on the finding of α- and γ-type subunits in the C group. In fact, because most C subunits are almost identical to α- and γ-gliadins, it is very likely that they are coded by novel genes located on loci tightly linked to *Gli-1* and *Gli-2* loci or by genes located actually within the *Gli* loci, whereas the typical LMW-GS present in the C fraction are likely to be coded at *Glu-3*. This hypothesis is supported by the observation of a parallelism between the presence/absence of particular gliadin components and the presence/absence of some C subunits.

The study of C subunits, in addition to offering the possibility to understand the glutenin polymer structure and its relation to dough quality in more detail, also stimulates a better understanding of the evolutionary path that has resulted in the diversification of wheat storage protein genes, giving rise to the multiple polypeptides that make up the complex structure of gluten.

Acknowledgements S.M., L.R. and D.L. acknowledge the contribution of the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T.), National Research Project "Studio delle Proteine dei Cereali e loro relazioni con aspetti tecnologici e nutrizionali", and the contribution of the Italian Consorzio per la Ricerca sulla Filiera Cerealicola "Gian Pietro Ballatore." The technical help of Emanuele Cannarella in preparing photographic material is gratefully acknowledged. The experiments here reported comply with the current Italian laws.

References

Anderson OD, Greene FC (1997) The α-gliadin gene family. II. DNA and protein sequence variation, subfamily structure, and origins of pseudogenes. Theor Appl Genet 95:59–65

Clarke BC, Appels R (1999) Sequence variation at the *Sec-1* locus of rye, *Secale cereale* (*Poaceae*). Plant Syst Evol 214:1

Dachkevitch T, Autran JC (1989) Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. Cereal Chem 66:448–456

D'Ovidio R, Simeone M, Masci S, Porceddu E, Kasarda DD (1995) Nucleotide sequence of a γ-gliadin type gene from a durum wheat: correlation with a γ-type glutenin subunit from the same biotype. Cereal Chem 72:443–449

Gupta RB, Khan K, MacRitchie F (1993) Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J Cereal Sci 18:23–41

Huebner FR, Wall JS (1976) Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem 53:258–269

Jackson EA, Holt LM, Payne PI (1983) Characterisation of high-molecular-weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. Theor Appl Genet 66:29–37

Kasarda DD, Adalsteins AE, Laird NF (1987) Gamma-gliadins with alpha-type structure coded on chromosome 6B of the wheat (*Triticum aestivum* L.) cultivar ''Chinese Spring." In: Lasztity R, Bekes F (eds) Proc 3rd Int Workshop on Gluten Proteins, Budapest, Hungary, pp 20–29

Keck B, Köhler P, Wieser H (1995) Disulfide bonds in wheat gluten: cystine peptides derived from gluten proteins following peptic and thermolytic digestion. Z Lebensm Unters Forsch 200:432–439

Köhler P, Belitz H-D, Wieser H (1993) Disulphide bonds in wheat gluten: further cystine 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

Lafiandra D, Masci S, D'Ovidio R, Margiotta B (2000) The genetics of wheat gluten proteins: an overview. In: Shewcy PR, Tatham AS (eds) Wheat Gluten, pp 3–10

Lew EJ-L, Kuzmicky DD, Kasarda DD (1992) Characterization of low-molecular-weight glutenin subunits by reversed-phase high-performance liquid chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal amino-acid sequencing. Cereal Chem 69:508–515

Masci S, Lafiandra D, Porceddu E, Lew EJ-L, Tao HP, Kasarda DD (1993) D Glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal Chem 70:581–585

Masci S, Lew EJL, Lafiandra D, Porceddu E, Kasarda DD (1995) Characterization of low-molecular-weight glutenins Type 1 and Type 2 by RP-HPLC and N-terminal sequencing. Cereal Chem 72:100–104

Masci S, Egorov TA, Ronchi C, Kuzmicky DD, Kasarda DD, Lafiandra D (1999) Evidence for the presence of only one cysteine residue in the D-type low-molecular-weight subunits of wheat glutenin. J Cereal Sci 29:17–25

Morel MH (1994) Acid-polyacrylamide gel electrophoresis of wheat glutenins: a new tool for the separation of high- and low-molecular-weight subunits. Cereal Chem 71:238–242

Neuhoff V, Arold N, Taube D, Ehrhardt W (1988) Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with a clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. Electrophoresis 9:255–262

Payne PI, Holt LM, Jarvis MG, Jackson EA (1985). Two-dimensional fractionation of the endosperm proteins of bread wheat (*Triticum aestivum*): biochemical and genetic studies. Cereal Chem 62:319–326

Tao HP, Kasarda DD (1989) Two-Dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. J Exp Bot 40:1015–1020

Tao HP, Cornell DG, Kasarda DD (1989) Surface and optical properties of wheat glutenin monolayers. J Cereal Sci 10:5–18

Verbruggen IM, Veraverbeke WS, Vandamme A, Delcour JA (1998) Simultaneous isolation of wheat high-molecular-weight and low-molecular-weight glutenin subunits. J Cereal Sci 28:25–32

Woychik JH, Boundy JA, Dimler RJ (1961) Starch gel-electrophoresis of wheat gluten proteins with concentrated urea. Arch Biochem Biophys 94:477–482

Wrigley CW (1996) Giant proteins with flour power. Nature 381:738–739