Nucleotide Sequence of a γ-Type Glutenin Gene from a Durum Wheat: Correlation with a γ-Type Glutenin Subunit from the Same Biotype

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ABSTRACT

A gene coding for an apparent γ-type glutenin subunit has been cloned from the biotype Lira 45 of the durum wheat cultivar Lira and sequenced. The nucleotide sequence of the gene from clone pTD9 showed almost perfect homology with a complete γ-type glutenin gene of *Triticum aestivum* L. and with an incomplete, presumably γ-type glutenin gene isolated from a different cultivar of *Triticum durum* Desf. All three genes code for proteins that have an additional cysteine residue in the N-terminal region at residue 26; there are nine cysteines in the molecule rather than the eight that are typical of γ–gliadins. This odd cysteine makes it reasonably certain that the equivalent protein will be incorporated into glutenin polymers, because all other cysteines were homologous to those in classical γ–gliadins, making it likely that they form the usual four intramolecular disulfide bonds characteristic of γ–gliadins. Accordingly, the ninth (odd) cysteine should be available for intermolecular disulfide bond formation. The ability to form only a single intermolecular disulfide bond would cause the molecule to act as a chain terminator during formation of glutenin polymers. In support of this, a γ-type glutenin was purified from a glutenin preparation derived from the same biotype (Lira 45) and characterized by N-terminal amino acid sequencing to show perfect homology at the N-terminus with the protein sequence derived from sequencing of the pTD9 clone, including the cysteine at residue 26. A computer molecular model of the protein equivalent to pTD9, based on the sequence and on the disulfide bond arrangements found for γ-gliadins (Köhler et al 1993), provides a hypothetical three-dimensional structure for the γ-type glutenin subunit.

The Italian durum wheat cultivar Lira possesses two biotypes; they are designated Lira 42 or Lira 45 because they posses either the γ-42 or γ-45 type gliadin components. They also have the expected types of low molecular weight (LMW) glutenin subunits, LMW-1 and LMW-2, that are linked, respectively, with γ–42 and γ-45. The strong correlation of these two types with differences in gluten quality (Lira 42 with poor quality and Lira 45 with good quality) make these biotypes a valuable tool for exploring the basis for differences at the molecular level.

Previous studies have shown that it is possible to obtain polymerase chain reaction (PCR) amplification of γ-gliadin sequences, and that the PCR amplification pattern from genotypes with γ-45 differs from that of genotypes with γ-42 (D'Ovidio et al 1990, 1992). The primers used in the earlier work did not, however, amplify the whole coding sequence. In particular, one of the triplets coding for cysteines and located at the 3′ end of the gene was excluded. The difficulties with the primers were overcome. We now report the nucleotide sequence of a complete γ-type glutenin gene from the biotype Lira 45 of *T. durum* cv. Lira and its correlation with the N-terminal sequence of a γ–type glutenin subunit purified from the same biotype.

To avoid misunderstanding, we will refer to γ-type glutenin when the amino acid or nucleotide sequence is similar to that of classical monomeric γ-gliadins, but is found in, or presumably belongs to, the polymeric fraction.

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MATERIALS AND METHODS

DNA Extraction

Genomic DNA was extracted from 5 g of leaves and extracted according to the procedure of D'Ovidio et al (1992).

PCR Amplification

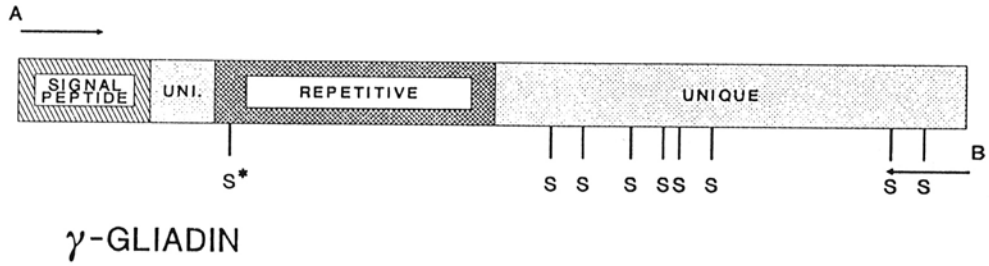
PCR reactions from genomic DNA (50–100 ng) were performed in a reaction volume of 100 µl with 2.5 units of Taq DNA polymerase, 1× Taq PCR buffer (Boehringer), 250 ng of each of the two primers, and 300 µM of each deoxyribonucleotide. Amplification conditions were 30 cycles at 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min. The oligonucleotides used as primers were synthesized on the basis of a γ-type glutenin gene isolated from *T. aestivum* (Scheets and Hedgcoth 1988) and have the following sequences: a) 5′ ATG AAG ACC TTA CTC ATC CT 3′, b) 5′ TTG GCC ACC AAT GCC AGC GT 3′. Amplified products were analyzed on 1.5% agarose gel. The amplified product was cloned into the *Sma*I site of the pUC18 vector before phosphorylation by T4 polynucleotide kinase.

DNA Sequencing Analysis

The DNA sequence was determined by the dideoxy chain-termination method (Sanger et al 1977). Overlapping deletions of the pTD9 clone were generated by exonuclease III digestion (Henikoff 1984).

High-Performance Liquid Chromatography and N-Terminal Amino Acid Sequence Analyses

The procedures reported in Masci et al (1995) were used to isolate the glutenin fraction and obtain purified γ–type glutenins by reversed-phase high-performance liquid chromatography (RP-HPLC). Late-eluting fractions corresponding to γ-type glutenins were collected and checked for purity on a mini sodium dodecyl sulfate polyacrylamide gel-electrophoresis (SDS-PAGE) system (Bio-Rad, Richmond, CA). N-terminal amino acid sequencing was performed for 29 cycles with an automatic sequencer (477A, Applied Bio-systems, Foster City, CA) on pyridylethylated proteins (Lew et al 1992).



γ-GLIADIN

Fig. 1. Diagram of a γ-type glutenin deduced from nucleotide sequence of pTD9 clone. S indicates the position of sulfur amino acids (cysteine); arrows indicate the position of primers used for polymerase chain reaction amplification; asterisk indicates the cysteine residue peculiar to γ–type glutenin genes as compared to classical γ-gliadin genes.

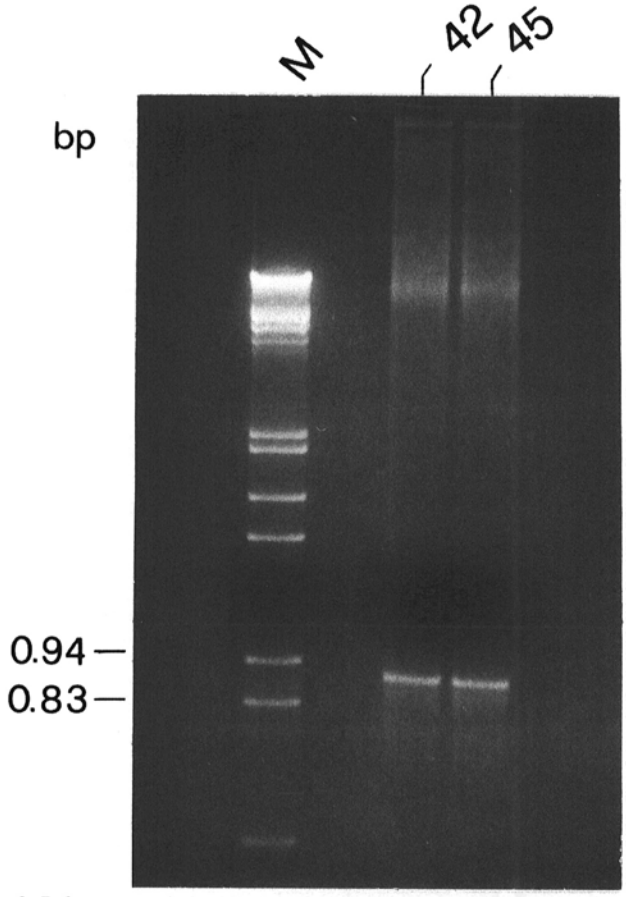


Fig. 2. Polymerase chain reaction amplification on 1.5% agarose gel of γ-gliadin or γ-type glutenin from Lira 42 and Lira 45. M = molecular weight marker.

Molecular Modeling

Molecular modeling was done by computer (Silicon Graphics Personal Iris) using Quanta-CHARMm software (Molecular Simulations, Inc., Burlington, MA). Approaches to modeling were largely as described by Kasarda et al (1994). Secondary structure predictions were made by the PHD method of Rost and Sander (1993). The initial model was constructed from the derived amino acid sequence of the mature protein corresponding to the pTD9 clone. Eight of the nine cysteines (in the C-terminal half) were patched into the sequence as four disulfide bonds according to the assignments for γ–gliadins by Köhler et al (1993). The connected cysteines in the model were Cys 152 to Cys 186; Cys 160 to Cys 179; Cys 187 to Cys 258; and Cys 199 to Cys 266. Only polar hydrogens were included in the calculations. The model was energy-minimized (Adopted Basis Newton Raphson method). Heating to 300°K was simulated. The resulting model was subjected to dynamic equilibrium calculations for 140 psec at that temperature.

RESULTS

PCR amplifications from genomic DNA of Lira 42 and Lira 45 were performed with primers specific for the 5′ and 3′ ends of γ–gliadin genes (Fig. 1). A single amplification band with the same mobility for both the genotypes was obtained (Fig. 2). The amplification products from biotype 45 were cloned and the nucleotide sequences determined. The nucleotide sequence of one of the PCR clones obtained, pTD9, was 906 bp long. The open reading frame encoded a γ-type glutenin of 302 amino acid residues with a predicted molecular weight of 34,302 (Fig. 3). Like other γ–gliadins and γ-type glutenins, the polypeptide encoded by the pTD9 clone contained three domains: N-terminal domain that included a 19 amino acid residue signal sequence; repetitive sequence domain, and C-terminal domain. The predicted molecular weight of the mature protein was 32,230; the calculated pI was 6.37. The short N-terminal domain of the mature protein (residues 21–35) was followed by the repeating sequence domain (residues 36-156) made up of glutamine- and proline-rich repeats that also included a phenylalanine residue. The repeats were not perfect and were somewhat irregular in size, varying in length from 7 to 11 residues. However, a consensus repeat can be derived from the sequence: Q-P-Q-Q-P-F-P (Fig. 4). The upstream part of the repeat domain contains a cysteine residue that is absent from classical γ-gliadins. The C-terminal domain, residues 157–302, is nonrepeating and contains eight cysteine residues. Figure 1 shows the overall features of the pTD9 clone and encoded protein. The hydropathy index of the mature protein encoded by the pTD9 clone shows the hydrophilic character of the repetitive region and the more hydrophobic character of the C-terminal domain (Fig. 5).

The deduced amino acid sequence of the pTD9 clone (Fig. 3) was compared to the previously reported γ–gliadin and γ-type glutenin sequences from durum and bread wheat (Table I). These comparisons revealed an almost perfect homology between the pTD9 and the pTDA16 and pW1020 clones. All three clones possess a cysteine residue at position 26 (unusual for γ-gliadin components). Moreover, differences observed with other γ-gliadin genes are mainly due to deletions and insertions preferentially located in the repetitive region. The polypeptide encoded by the pTD9 clone was also compared to γ–secalins, B_1-hordeins, and γ-hordeins (Cameron-Mills and Brandt 1988). Similarity ranged between 60 and 80% (data not shown). Table II gives the amino acid composition of the mature protein encoded by the pTD9 clone as compared with those of purified γ–gliadins (Aibara and Morita 1988, Tatham et al 1990, Du Cros 1991). The amino acid composition (%) of the N-terminal, repetitive, and C-terminal domains is also reported (Table II). The remarkable difference in glutamine and proline content between the repetitive domain (43.9%) and the C-terminal domain (17.7%) is noteworthy

The pTD9 clone coded for a protein with eight cysteines in the C-terminal half, which probably form the normal complement of four intramolecular disulfide bonds characteristic of γ-gliadins, and a ninth cysteine near the N-terminus, which probably forms

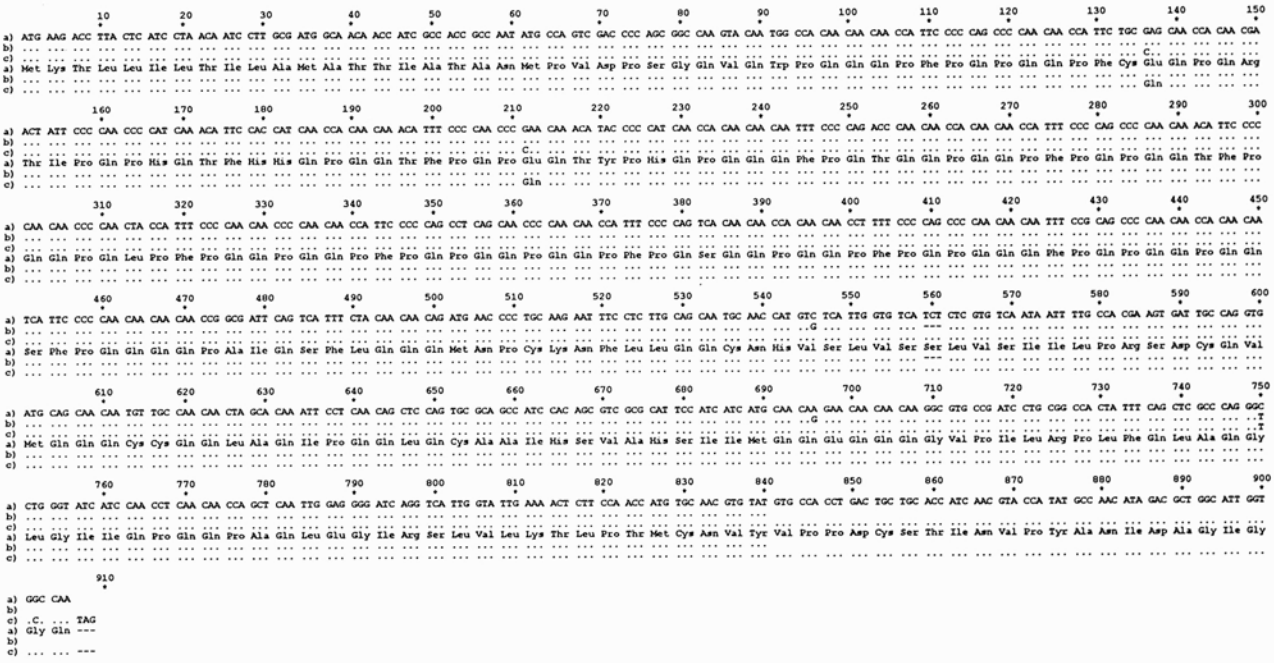


Fig. 3. Alignment of γ-type glutenin nucleotide and deduced amino acid sequences: a) clone pTD9, *T. durum* cv. Lira, biotype 45; b) clone pTDA16, *T. durum*, cv. Langdon (D'Ovidio et al 1991); c) clone pW1020, *T. aestivum* cv. Yamhill (Scheets and Hedgcoth 1988).

A) Nucleotide sequence

1)	CCA	CAA	CAA	CAA	CCA	TTC	CCC				
2)	CAG	CCC	CAA	CAA	CCA	TTC	TGC	GAG			
3)	CAA	CCA	CAA	CGA	ACT	ATT	CCC				
4)	CAA	CCC	CAT	CAA	ACA	TTC	CAC	CAT			
5)	CAA	CCA	CAA	CAA	ACA	TTT	CCC				
6)	CAA	CCC	GAA	CAA	ACA	TAC	CCC	CAT			
7)	CAA	CCA	CAA	CAA	CAA	TTT	CCC	CAG	ACC	CAA	
8)	CAA	CCA	CAA	CAA	CCA	TTT	CCC				
9)	CAG	CCC	CAA	CAA	ACA	TTC	CCC	CAA			
10)	CAA	CCC	CAA	CTA	CCA	TTT	CCC	CAA			
11)	CAA	CCC	CAA	CAA	CCA	TTC	CCC	CAG	CCT	CAG	
12)	CAA	CCC	CAA	CAA	CCA	TTT	CCC	CAG	TCA	CAA	
13)	CAA	CCA	CAA	CAA	CCT	TTT	CCC				
14)	CAG	CCC	CAA	CAA	CAA	TTT	CCG	CAG	CCC	CAA	
15)	CAA	CCA	CAA	CAA	TCA	TTC	CCC	CAA	CAA	CAA	CAA
c)	caa	ccc	caa	caa	cca	ttt	ccc				

B) Deduced aminoacid sequence

1)	P	Q	Q	Q	P	F	P				
2)	Q	P	Q	Q	P	F	C	E			
3)	Q	P	Q	R	T	I	P				
4)	Q	P	H	Q	T	F	H	H			
5)	Q	P	Q	Q	T	F	P				
6)	Q	P	E	Q	T	Y	P	H			
7)	Q	P	Q	Q	Q	F	P	Q	T	Q	
8)	Q	P	Q	Q	P	F	P				
9)	Q	P	Q	Q	T	F	P	Q			
10)	Q	P	Q	L	P	F	P	Q			
11)	Q	P	Q	Q	P	F	P	Q	P	Q	
12)	Q	P	Q	Q	P	F	P	Q	S	Q	
13)	Q	P	Q	Q	P	F	P				
14)	Q	P	Q	Q	Q	F	P	Q	P	Q	
15)	Q	P	Q	Q	S	F	P	Q	Q	Q	Q
c)	Q	P	Q	Q	P	F	P				

Fig. 4. Nucleotide and amino acid composition of the repetitive region of the pTD9 clone. Triplets and corresponding heptapeptides are aligned. The first nucleotide corresponds to base 94. Lane c = consensus sequence.

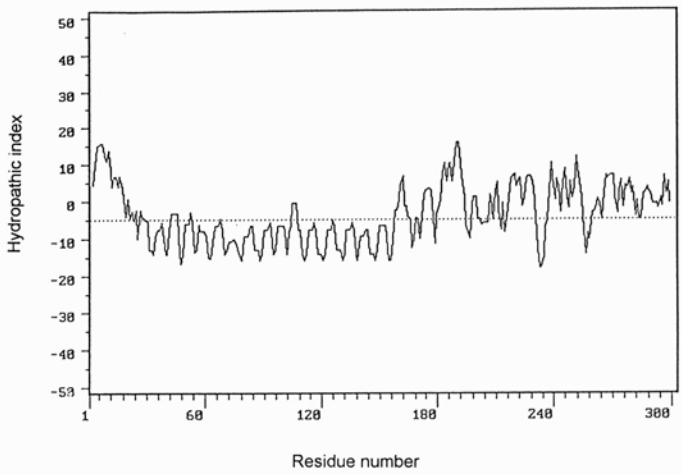


Fig. 5. Hydropathic profile of the pTD9 polypeptide calculated according to Kyte and Doolittle (1982) using an interval of five amino acid residues. Positive and negative values indicate hydrophobic and hydrophilic regions, respectively.

TABLE I
References to Nucleotide Sequences of γ-Gliadin Genes

Clone	Species	Reference
L311A	*Triticum aestivum* cv. Yamhill	Rafalski (1986)
L311B	*T. aestivum* cv. Yamhill	Rafalski (1986)
pW1621	*T. aestivum* cv. Yamhill	Sugiyama et al (1986)
pTAG1436	*T. aestivum* cv. Chinese Spring	Bartels et al (1986)
pW1020	*T. aestivum* cv. Yamhill	Scheets and Hedgcoth (1988)
pTDA16	*T. durum* cv. Langdon	D'Ovidio et al (1991)

an intermolecular bond and consequently the protein should be found in the glutenin fraction. On the basis of this assumption, the residue (corresponding to the glutenin fraction) from Lira 45 was extracted, and the reduced glutenin subunits were analyzed by RP-HPLC. The last four eluting peaks (data not shown), which have γ-type glutenin N-terminal sequences (Masci et al 1995), were collected and analyzed by SDS-PAGE. This analysis showed multiple bands with mobility in both B and C LMW glutenin subunit regions (Fig. 6). Because only fraction 2 contained a single band, it was analyzed by N-terminal sequencing. On the

TABLE II
Amino Acid Compositions (%) of a Purified γ-Gliadin (A) and the Polypeptide Encoded by the pTD9 Clone[a]

Amino Acid	A[b]	pTD9M (21–302)[c]	N-Terminal (21–35)	Repetitive (36–156)	C-Terminal (157–302)
Ala	3.7	3.1	0.0	0.7	5.9
Arg	1.2	1.4	0.0	0.7	2.2
Asn	2.5[d]	2.4	6.2	0.0	4.4
Asp		1.4	6.2	0.0	2.2
Cys	2.3	3.1	0.0	0.7	5.9
Gln	37.2[d]	31.0	37.5	43.9	17.7
Glu		1.4	0.0	1.5	1.4
Gly	5.6	2.8	6.2	0.0	5.1
His	2.3	2.4	0.0	3.0	2.2
Ile	5.3	5.3	0.0	1.5	9.6
Leu	7.2	6.0	0.0	1.5	11.1
Lys	1.0	0.7	0.0	0.0	1.4
Met	2.3	1.7	6.2	0.0	2.9
Phe	3.9	5.6	0.0	10.6	1.4
Pro	10.5	17.6	12.5	28.0	8.1
Ser	6.5	4.5	6.2	2.2	6.6
Thr	3.4	3.1	0.0	4.5	2.2
Trp	nd[e]	0.3	6.2	0.0	0.0
Tyr	0.7	1.0	0.0	0.7	1.4
Val	4.6	4.2	12.5	0.0	7.4

[a] Excluding the signal peptide.
[b] From Du Cros (1991).
[c] Mature polypeptide encoded by the pTD9 clone.
[d] Value referred to is Asx (Asn + Asp) or Glx (Gln + Glu).
[e] Not determined.

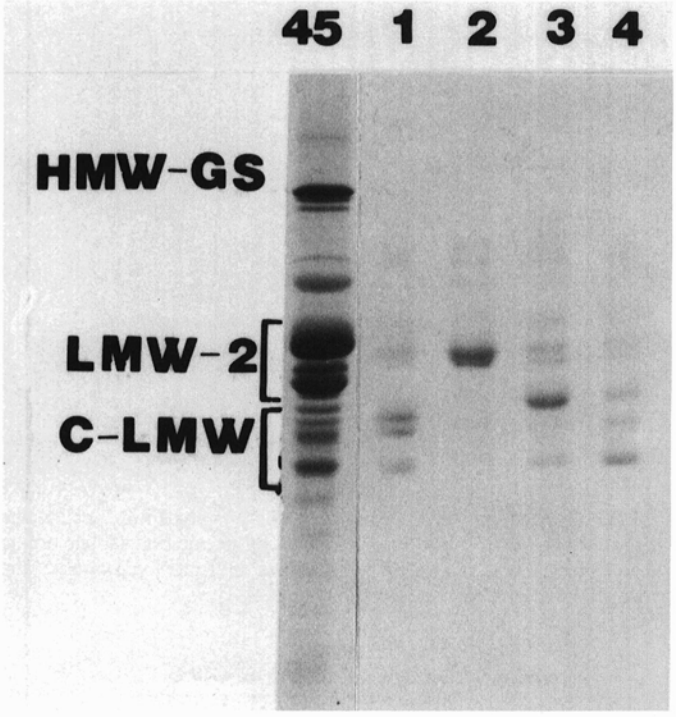


Fig. 6. Sodium dodecyl sulfate polyacrylamide gel-electrophoresis pattern of glutenin subunits in the residue of Lira 45 and of purified fractions corresponding to γ-type glutenins (1–4).

Fig. 7. N-terminal amino acid sequence of γ–type glutenin fraction 2 purified from Lira 45. Minor sequence heretogeneities are reported on the line below.

basis of mobility on SDS-PAGE, the apparent molecular weight of this protein is about 38,000. The N-terminal sequence of this fraction showed a perfect homology with the γ–type glutenin encoded by the pTD9 clone, including a cysteine residue at position 26 (Fig. 7).

Secondary structure prediction (Rost and Sander 1993) for the γ-type glutenin subunit based on the amino acid sequence indicated mainly loop structures for which no torsional angles are specified in the predictive method and which cannot be modeled without obtaining other structural information from physical studies. Only 5% of the total number of residues (near the C-terminal end of the molecule) were predicted to occur in α-helical conformation. To simplify construction of an energy-minimized, disulfide-bonded structure, we decided not to apply any secondary structure to the sequence. The structure obtained after 140 psec of dynamic equilibration calculations is shown in Figure 8. The total energy of the resulting structure was satisfactorily negative. Figure 8A depicts the molecule in licorice-bond style, which makes the interior structure somewhat easier to see. Figure 8B depicts the molecule in space-filling form with atoms having their appropriate Van der Waals radii. Most atoms are shown in blue, but sulfur atoms are shown in yellow, and side chains of nonpolar residues (phenylalanine, tyrosine, tryptophan, leucine, isoleucine, and alanine) are shown in magenta. Only polar hydrogens are shown in the models.

DISCUSSION

Biotypes Lira 42 and Lira 45 constitute a valuable system for analysis of the molecular basis underlying the quality differences of durum wheat cultivars (D'Ovidio et al 1992, Masci et al 1995). Each of the biotypes carries major genes for either poor (γ–42, LMW Type 1) or good (γ–45, LMW Type 2) quality in an otherwise uniform genetic background. As a contribution to the molecular characterization of these biotypes, we have cloned, by PCR methods, a gene from Lira 45 and characterized the gene. We have described characteristics that would be expected for the expressed protein, a γ–type glutenin subunit, and made some comparison with a similar (possibly identical) protein purified from Lira 45. Although γ–type glutenin subunits are found in both Lira biotypes, there is a greater proportion of these subunits in the poor quality Lira 42 biotype (Masci et al 1995).

The almost perfect homology between the sequence of the Lira γ-type glutenin and the genes previously cloned from *T. durum* cv.

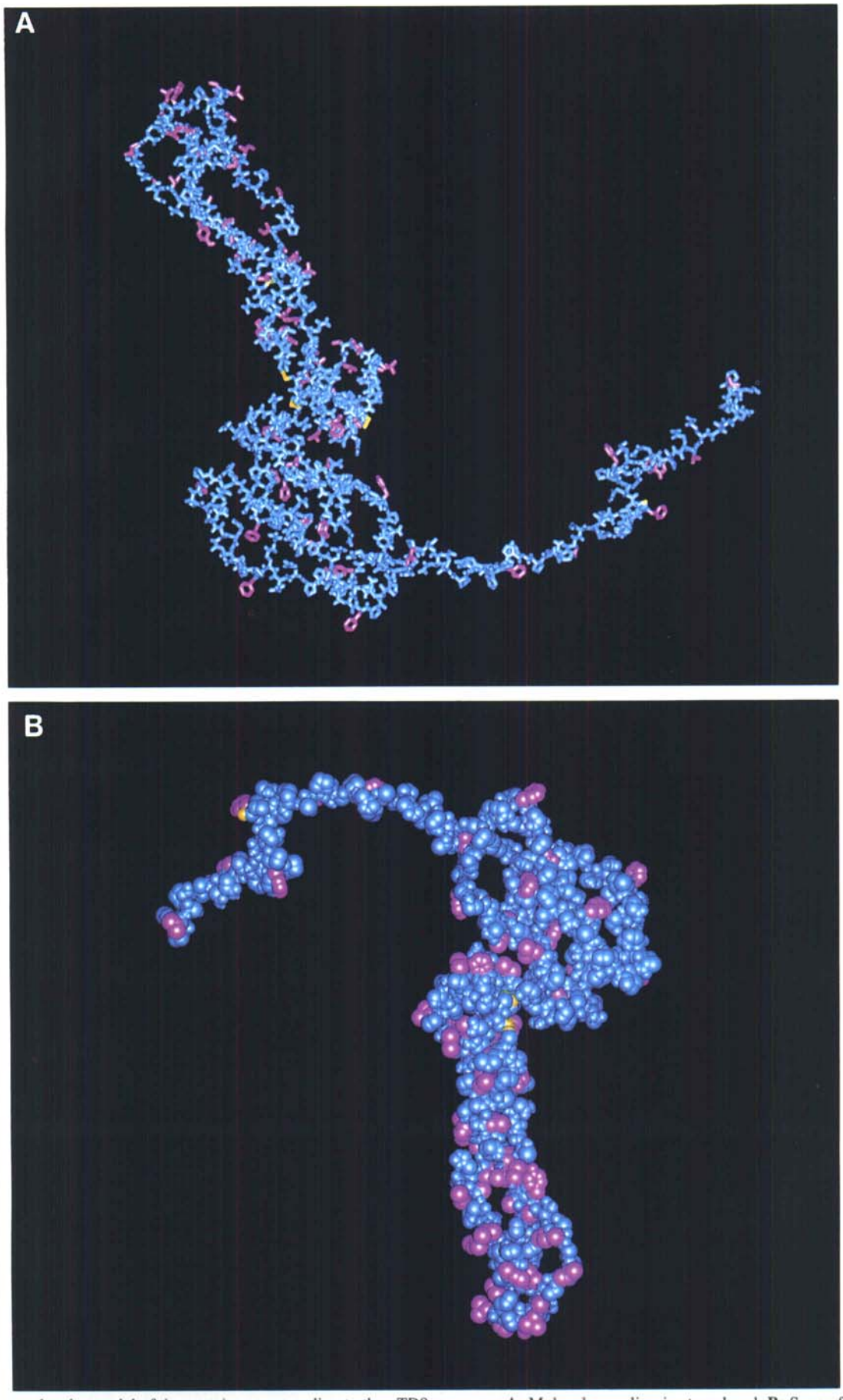


Fig. 8. Computer molecular model of the protein corresponding to the pTD9 sequence. **A:** Molecule as a licorice type bond. **B:** Space-filling form (Van der Waals radii). Most atoms are blue, nonpolar side chains are magenta, and sulfur atoms of cysteine residues are yellow. N-terminus of the molecule is at lower-right in **A** and at upper left in **B**.

Langdon (type γ-42) (D'Ovidio et al 1992) and *T. aestivum* cv. Yamhill (Scheets and Hedgcoth 1988) demonstrate that both durum and common wheat possess highly similar genes. These γ-type glutenin subunits, although strongly homologous with γ-gliadins, do differ in degree of homology in comparison with the sequences that evidently code for monomeric γ-gliadins (Bartels et al 1986, Rafalski 1986). In particular, the sequence equivalent to the pTD9 clone is similar to, but clearly different from, the γ-45 gliadin, which is a true monomeric gliadin (Du Cros 1991). Strong similarity in amino acid sequences also exist between γ-gliadins of wheat and similar proteins of other cereal species, such as γ-hordein and the B_1-hordein of barley, and the γ-secalin of rye (Cameron-Mills and Brandt, 1988). The γ-gliadins may correspond to an ancestral form of sulfur-rich prolamins (Shewry et al 1984).

Commonly, gliadin components contain an even number of cysteines, all of which form intramolecular disulfide bonds (Bartels et al 1986, Rafalski 1986). However, the nucleotide sequence of Scheets and Hedgcoth (1988) showed the existence of γ-gliadins (which, as indicated in this article, should be called γ-type glutenins) possessing an odd number of cysteine residues, in particular a ninth cysteine residue located at position 26 from the N-terminus. Because the other eight cysteines appear to be generally homologous to those of classical γ-gliadins, it has been proposed that the extra cysteine near the N-terminus forms an intermolecular disulfide bond, thus incorporating this particular protein into the glutenin polymer fraction. Moreover, it appears likely that, being able to form only a single intermolecular disulfide bond, this γ-type glutenin is likely to act as a terminator of growing polymer chains and cause the molecular weight distribution of the glutenin polymer system to be shifted downwards. The size of the glutenin polymers may play an important role in influencing gluten properties (Dachkevitch and Autran 1989)

The pTD9 clone described here encodes a γ-type glutenin that is quite similar to the one described by Scheets and Hedgcoth (1988), especially in having an extra cysteine residue at position 26, which would probably make it a chain terminator. In support of this possibility, we purified a γ-type glutenin subunit from a residue glutenin fraction of Lira biotype 45 that showed sequence identity through 29 residues with the equivalent sequence of the pTD9 clone, including the cysteine residue at position 26. The deduced molecular weight from SDS-PAGE (38,000) of the purified γ-type glutenin subunit is not greatly different from the molecular weight of the γ-type glutenin subunit encoded by the pTD9 clone (32,330). Mobility on SDS-PAGE may be affected by conformational structure, so it is possible that the protein we purified corresponds to that coded by pTD9. Our results provide additional support for the possibility that gliadin-like proteins may behave as glutenin subunits when they can form intermolecular disulfide bonds—either because they have an odd number of cysteine residues so that at least one cysteine cannot link up with another to form an intramolecular disulfide bond or, in a protein with an even number of cysteines, because conformational structure prevents two or more cysteines from forming intramolecular disulfide bonds (Lew et al 1992). Köhler et al (1993) found an intermolecular disulfide bond between two peptides, one derived from a LMW glutenin subunit and the other from a γ-type glutenin in the bread wheat cultivar Rektor, which supports this hypothesis, even though the cysteine residue involved does not correspond to residue 26 and has not been recognized so far in DNA-based sequences.

Arbitrarily, the model shown in Figure 8 may be considered to consist of two or three domains. The tightly folded C-terminal domain, which contains all four intermolecular disulfide bonds, is quite clearly defined. Figure 8A shows the molecule in a licorice-type bond model that illustrates overall chain structure more clearly than the space-filling model (Van der Waals radii for all atoms) shown in Figure 8B. The C-terminal domain, which is likely to be the same for both γ-type glutenins and γ-gliadins, has a diameter of about 20Å and a length of about 90Å. The nonpolar side chains (magenta color in Fig. 8) occupy a significant amount of the surface, but this aspect of the model is especially speculative. The force field used in the calculations has terms for electrostatic interactions, including hydrogen bonding, but does not deal at all with entropy-determined hydrophobic associations of side chains arising from interactions with water molecules (Kasarda et al 1994). Thus, in the real molecule, the nonpolar side chains might tend to cluster more and be less accessible to solvent water.

There is a relatively short N-terminal domain of about 60 residues that appears to be somewhat extended and flexible. This extension might result partly from electrostatic repulsions of four histidine residues arrayed between residues 37–57, although shielding by salt ions or complexation with a metal atom such as copper might permit this region to condense into a more compact form. This extended domain contains the cysteine at position 26 in the sequence, which we expect will form a single intermolecular disulfide bond. The sulfur atom of cysteine 26 can be seen in Figure 8A and B. The histidine side chains should be primarily in the positively charged form at pH 6.4, the calculated pI. Two of the histidine residues appear only in the N-terminal domain of γ–type glutenin subunits with a cysteine at residue 26.

The portion of the protein from residue 60–140 might be considered an intermediate domain. Here the chain folds back on itself in a series of loose loops that appear rather disklike in three-dimensional view. The disk is shown in Figure 8 with its major axis approximately in the plane of the photo. There was a fairly natural tendency for the loops to form as a result of proline positioning in the sequence, even before any energy minimization or dynamic calculations were performed. These loops may not be representative of the real molecule, in that the series of phenylalanine side chains (characteristic of repeat structures) might tend to cluster somewhat through hydrophobic interactions to produce a more compactly folded domain.

Overall, the dimensions of the modeled molecule are not in very good agreement with those found by scanning tunneling microscopy, and small-angle X-ray scattering studies (Thomson et al 1992) for a γ–gliadin. Thomson et al (1992) reported a diameter of 34 Å and a length of 92 Å. The elongated C-terminal domain had dimensions of about 20 Å × 90 Å. The remainder of the molecule would almost double that length, if the model of Figure 8 were viewed literally, although this part of the polypeptide chain would not have a rigid conformation. Dimensions similar to those of Thompson et al (1992) would result for our model if the N-terminal half of the molecule folded back on the C-terminal domain as though hinged in the middle to form a relatively compact structure.

The model we have constructed must be considered highly speculative insofar as it is based only on sequence information, without any knowledge of atom coordinates or constraints such as might be obtained by X-ray diffraction of crystals or by solution nuclear magnetic resonance studies, albeit with apparently correct disulfide bond assignments. The tight folding in the C-terminal half of the molecule required for formation of the four intramolecular disulfide bonds makes the shape of the molecule in this domain probable. We think there is considerable value in the ability to visualize possible structures through modeling, which should provide the basis for testable hypotheses about the structure of γ-type glutenin subunits and γ-gliadins. The model gives us, for example, an indication of the relative inaccessibility of the disulfide bonds in the C-terminal domain, and the relative accessibility of the cysteine at position 26, which is likely to interact with similar cysteine residues of other glutenin subunits to form intermolecular disulfide bonds. The development of any atom constraints from physical studies, such as nuclear magnetic

resonance, would enable us to improve the model considerably. The current model is, then, in the absence of any detailed structural information, a starting point for working toward a valid structural model.

ACKNOWLEDGMENTS

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