Deduced Amino Acid Sequence of an α-Gliadin Gene from Spelt Wheat (Spelta) Includes Sequences Active in Celiac Disease

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ABSTRACT

The complete amino acid sequence of an α-type gliadin from spelt wheat (spelta) has been deduced from the cloned DNA sequence and compared with α-type gliadin sequences from bread wheat. The comparison showed only minor differences in amino acid sequences between the α-type gliadin from bread wheat and the α-type gliadin from spelta. The two sequences had an identity of 98.5%. Larger differences can be found between different α-type gliadin amino acid sequences from common bread wheat. Because all the different classes of gliadins, α, β, γ, and ω, appear to be active in celiac disease, it is reasonably certain that the spelta gliadin is also toxic. We conclude that spelta is not a safe grain for people with celiac disease, contrary to the implications in labeling a bread made from spelta as "an alternative to wheat". Our conclusions are in accord with spelta and bread wheat being classed taxonomically as subspecies of the same genus and species, *Triticum aestivum* L.

Cereal Chem. 76(4):548–551

We have been approached by the leaders of celiac patient organizations in the United States to clarify the situation with regard to spelt (spelta) grain and celiac disease because so many of their members have heard that it is a safe alternative to wheat. We have also had anecdotal reports from them that some celiac patients who have tried spelta have been adversely affected by it.

Spelta is touted as a miracle food by some health food companies in their product brochures and on the product labels. It is sometimes implied that spelta is safe for celiac patients and also for those with wheat allergy. We are not aware of any rigorous scientific evaluation of such claims.

Recently, several scientific studies have been conducted to clarify the issue in regard to the compositional and nutritional claims made for spelta (Abdel-Aal et al 1995, 1998; Ranhotra et al 1995, 1996a,b). Although small compositional differences were found for the small numbers of samples in these studies, these differences are probably not significantly greater than what would be found if widely differing varieties of ordinary bread wheat were grown at widely different locations and in different crop years, so that environmental (soil, fertilizer, weather) and genetic differences were varied over their full range. Ranhotra et al (1996b), using a commercial antibody test for gluten, found that all spelt varieties tested positively. Abdel-Aal et al (1998) concluded that spelt wheats were not superior to modern bread and durum wheats in chemical composition.

Spelta is, in fact, wheat even though it is often referred to as if it were a separate species (e.g., as *Triticum spelta* L.). Genetically, it is simply one of several closely related, fully interfertile subspecies of the hexaploid wheat, *T. aestivum* L. Common bread wheat belongs to the same species. As a subspecies, spelta is then named *T. aestivum* ssp. *spelta* (L.) Thell. (MacKey 1966, van Slageren 1994), and common bread wheat is named *T. aestivum* ssp. *aestivum* (Morris and Sears 1967, van Slageren 1994). Like bread wheat, spelta carries the AABBDD genomes. [See also URL http://www.ksu/wgrc/Germplasm/Taxonomy/taxvsl.html. Sponsored by Kansas State University, Manhattan, KS.] The complex loci coding for gliadin proteins are closely homologous to those of bread wheat (Lafiandra et al 1989), and the proteins of spelt wheat varieties are similar to those of bread wheat cultivars in their gel electrophoretic patterns at acid pH, although some minor differences have been noted (Federmann et al 1992, Harsch et al 1997).

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Publication no. C-1999-0526-02R.

Celiac disease is a condition in which susceptible individuals suffer enteropathy upon eating storage proteins from wheat, rye, and barley (Mäki and Collin 1997). Until recent years, oats have generally been considered harmful, but several recent, high-quality studies have found no harm from oats to patients with celiac disease or with dermatitis herpetiformis, a closely related skin condition (Janatuinen et al 1995, Srinvasan et al 1996, Hardman et al 1997, Reunala et al 1998). The current knowledge of toxic sequences in various grain proteins has been reviewed by Kasarda (1997).

At the more extreme end of the spectrum of response to wheat, rye, and barley storage proteins in celiac disease, the intestinal mucosa of susceptible individuals is damaged by eating these grains and acquires a flattened appearance. The mucosal damage results in malabsorption of almost all nutrients, and symptoms can be diverse as a consequence (Howdle and Losowsky 1992, Mäki and Collin 1997). The fundamental mechanism by which the ingestion of wheat gluten proteins and their equivalents in rye and barley trigger a pathophysiological response that eventually may lead to intestinal damage is unknown, but an abnormal immune response to certain gliadin amino acid sequences has been the favored hypothesis in recent years (van de Wal et al 1998).

Although there seems little basis for the claims that spelt is a satisfactory grain for celiac patients, these claims continue to be made (bread made from spelta has been found in health food stores labeled as an alternative to wheat). To provide further proof that spelta is not safe for celiac patients, we have cloned and sequenced the complete gene coding for a spelta α-type gliadin to compare the corresponding protein sequence with that of a wheat α-type gliadin (A-gliadin) (Kasarda et al 1984) that is almost certainly toxic in celiac disease. We also compare the spelta gliadin sequence with the sequences of small synthetic peptides that have been tested for toxicity in celiac disease.

MATERIALS AND METHODS

DNA Source, PCR Amplification, and Southern Blot Analyses

DNA was extracted from the spelt wheat accession number ATRI 2021/SKL (var. arduini Mazz.) obtained from the Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, Germany. Polymerase chain reaction (PCR) analyses were performed in a final reaction volume of 100 μL using 100-300 ng of genomic DNA, 2.5 units of Taq DNA polymerase (Boehringer, Germany), 1× Taq PCR buffer (Boehringer, Germany), 250 ng of each of the two primers, and 200 μM of each deoxyribonucleotide. Amplification conditions were for 30 cycles at 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min. A final step at 72°C for 7 min was also performed. Oligonucleotides used as primers were synthesized on the basis of an α-gliadin sequence previously published (Anderson et al 1984, Reeves and Okita 1987) and have the following

sequences: UTV23F) 5′ CACTTGTAAGTAGTCGCCACCA 3′, UTV24R) 5′ GTTAGTACCGAAGATGCCAA 3′. Aliquots (10 µL) of the amplification products were fractionated on 1.5% agarose gel in 1× TBE buffer (Tris-borate-ethylenediaminetetraacetic acid) following standard procedures (Sambrook et al 1989). After gel electrophoresis, the DNA was blotted onto a nylon membrane (Amersham, UK) using standard procedures (Sambrook et al 1989) and hybridized under high-stringency conditions with the pTU1 clone that contains the coding region of an α-gliadin gene from *T. urartu* (D'Ovidio et al 1992). The pTU1 clone was labeled with digoxigenin by PCR according to the procedure reported by D'Ovidio and Anderson (1994).

Cloning an α-Gliadin Sequence and Nucleotide Sequence Analysis

The single amplification product obtained with primers UTV23F and UTV24R was purified from agarose gel and ligated into the *Sma*I dephosphorylated site of the pUC18 plasmid vector using the SureClone ligation kit (Pharmacia Biotech, Sweden). After transformation into the *Escherichia coli* strain NM522, the recombinant colonies were analyzed to verify the presence of the α-gliadin fragment. Several recombinant clones contained an insert with the expected size and one of these, pTS63, was used for nucleotide sequence analysis.

Sequencing analysis was conducted using the dideoxy primer-extension method (Sanger et al 1977). The resulting nucleotide sequence was analyzed using the PC-gene computer program (Intelligenetics, Inc., Mountain View, CA).

TABLE I
Toxic Amino Acid Sequences from *Triticum aestivum* α-Gliadin Compared with *T. spelta* α-Gliadin Sequences and an Oat Avenin Sequence

Result	Source	Sequence[a]	Reference
A	*T. aestivum*	LGQQQPFPPQQPYPQPQPF	Sturgess et al 1994
B	*T. spelta*	LGQQQPFPPQQPYPQPQPF	Current study
C	*T. aestivum*	LGQQQPFPPQQPY	Marsh et al 1995
D	*T. spelta*	LGQQQPFPPQQPY	Current study
E	Oat	qqQQQPFvqQQqmflqpll	Egorov 1988

[a] Lower case single letter coding used for some of the amino acids of the oat avenin sequence indicates differences in comparison with the wheat and spelta gliadin sequences (results A and B). Single letter code for amino acids: A, alanine; R, arginine; N, asparagine; D; aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine.

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PTS63     - MKTFLILALLAIVATTATTAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQF   50
                                  ||||||||||||||||||||||||||||||
ALPHA1    -                      VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF   30

PTS63     - LGQQQPFPPQQPYPQPQPFPSQQPYLQLQPFPQPQLPYSQPQPFRPQQPY  100
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
ALPHA1    - LGQQQPFPPQQPYPQPQPFPSQQPYLQLQPFPQPQLPYSQPQPFRPQQPY   80

PTS63     - PQPQPQYSQPQQPISQQQQQQQQQQQQQQQQQQQQQILQQILQQQLIPCMD  150
            ||||||||||||||||||||||||||||||||  ||||||||||||||||
ALPHA1    - PQPQPQYSQPQQPISQQQQQQQQQQQQQQQQQQ--ILQQILQQQLIPCMD  128

PTS63     - VVLQQHNIAHGRSQVLQQSTYQLLQELCCQHLWQIPEQSQCQAIHKVVHA  200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
ALPHA1    - VVLQQHNIAHGRSQVLQQSTYQLLQELCCQHLWQIPEQSQCQAIHNVVHA  178

PTS63     - IILHQQQKQQQQPSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQ  250
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
ALPHA1    - IILHQQQKQQQQPSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQ  228

PTS63     - LPQFEEIRNLALQTLPAMCNVYIPPYCTITPFGIFGTN  288
            ||||||||||||||||||||| ||||| ||||||||
ALPHA1    - LPQFEEIRNLALQTLPAMCNVYIAPYCTIPPFGIFGTN  266
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Fig. 1. Alignment between the deduced amino acid sequence encoded by the pTS63 clone (PTS63) and an α-gliadin sequence (ALPHA1) obtained by direct amino acid sequencing of an α-type gliadin protein (Kasarda et al 1984). The first 20 residues of the PTS63 sequence correspond to a signal sequence (removed in the mature protein). peptide sequence in the N-terminal domain that has shown activity in celiac disease is underlined (ALPHA1 residues 31–49 inclusive). The peptide sequence in the C-terminal domain that shows homology with the E1b protein (ALPHA1 residues 206–217) associated with adenovirous type 12 infection (Kagnoff et al 1984) is also underlined.

RESULTS

Amplification of α-gliadin genes from spelta was obtained by PCR analysis of genomic DNA using specific primers developed from previously published α-gliadin nucleotide sequences (Anderson et al 1984, Reeves and Okita 1987). The region amplified by the primers spans the complete coding region and part of the 5′ flanking region. The analysis of the PCR product on an agarose gel indicated a single DNA fragment ≈1,100 bp. Southern blot analysis using the pTU1 probe showed a strong and specific hybridization signal of the 1,100 bp amplification product. This fragment was cloned, and one of the recombinant clones, pTS63, was sequenced. The sequence of the pTS63 was 1,081 bp long and included 217 bp of the 5′ flanking region along with the complete coding region of 864 bp. The European Molecular Biology Laboratory accession number for the sequence is AJ130948.

The deduced encoded protein is an α-gliadin with 288 amino acids in the polypeptide chain, a calculated molecular weight of 33,203, and a pI of 8.02. Nucleotide and amino acid comparison of the pTS63 with other α-gliadin sequences showed a high degree of homology along the entire sequence (data not shown). Comparison between the deduced amino acid sequence of the pTS63 clone and an α-gliadin sequence obtained by direct amino acid sequencing (Kasarda et al 1984) showed almost perfect homology except for three substitutions and a small deletion. This last difference is localized in the first polyglutamine region, which had two additional glutamine residues in the α-gliadin encoded by the pTS63 clone in comparison (Fig. 1) with the sequence of Kasarda et al (1984), which is designated ALPHA1 in Fig. 1.

DISCUSSION

The comparison of spelta and aestivum α-type gliadin sequences in Fig. 1 shows only extremely minor differences in amino acid sequences between the aestivum α-type gliadin and the spelta α-type gliadin. The two sequences have an identity of 98.5%. Larger differences can be found between α-type gliadin amino acid sequences from common bread wheat (Anderson and Greene 1997). Because all the different classes of gliadins, α, β, γ, and ω, appear to cause harm in celiac disease (Ciclitira et al 1984), it is unlikely that the spelta gliadin would not also be toxic.

Sturgess et al (1994) instilled a 19-residue synthetic peptide corresponding to residues 31–49 of an α-type gliadin (identical in both our spelta and aestivum sequences) into the intestines of several patients who had been on a wheat free diet; they followed the effects of the instillation by taking intestinal biopsies and demonstrated changes in the mucosal tissues indicative of toxicity for the peptide. Related studies were conducted by Marsh et al (1995) for a 13-residue synthetic peptide with sequence corresponding to residues 31–43. This latter sequence is included within the residue range of the peptide studied by Sturgess et al (1994). Forsell and Wieser (1995) compared several α-gliadin proteins from wheat and spelta. They demonstrated that α-gliadin components from spelta and aestivum usually had identical N-terminal amino acids in positions 3–56, a region of the molecule that includes the sequences indicated to be toxic by Sturgess et al (1994) and by Marsh et al (1995). Table I compares the sequences of the peptides tested by Sturgess et al (1994) (result A) and Marsh et al (1995, 1997) (result C) with the identical sequences found in our spelta α-gliadin (results B and D) and with the closest contiguous sequence from an oat avenin (Egorev 1988) (result E) as determined by database searching (Kasarda 1997).

Kagnoff et al (1984) proposed that infection by adenovirus type 12 might be an environmental trigger of celiac disease because of a sequence similarity between the E1b protein associated with the viral infection and an α-gliadin from an aestivum cultivar. The sequence in question corresponded to amino acid residues 206–217 of A-gliadin (Kasarda et al 1984). The spelta α-gliadin sequence shown in Fig. 1 has an identical sequence in the corresponding region.

Spelta cultivars differ from aestivum cultivars in that the former lack the free-threshing characteristic of the latter; the glumes adhere tightly to the grain rather than being easily separated from one another. This characteristic misleads some into thinking of spelt as quite different from normal common wheat, but the free-threshing characteristic is controlled by a single gene difference (MacKey 1966). Although aestivum may have arisen from spelta through a mutation that produced the free-threshing characteristic in aestivum cultivars, the reverse has also been suggested (Dvorak et al 1998). In either case, both spelta and aestivum would still be recent in evolutionary time, and the evolutionary precedence question is not of great significance in relation to gluten protein structure. The free threshing character of common bread wheats presumably arose within the last 10,000 years (Morris and Sears 1967), whereas the protein primary structures were largely defined millions of years ago (Shewry et al 1980). In general, one would expect greater differences between tetraploid durum wheat cultivars, used for pasta making, and bread wheat cultivars than for spelta cultivars in comparison with bread wheat cultivars.

Forsell and Wieser (1995) concluded that spelta is not safe for celiac patients on the basis of their N-terminal sequence comparisons. We have extended their findings by providing a complete amino acid sequence for a spelta α-gliadin. We demonstrate that this spelta gliadin is 98.5% identical in sequence to a known aestivum α-gliadin, includes identical sequences that have been demonstrated to be toxic in celiac disease, and is almost certain to be equivalent in toxicity to celiac patients to the component from common bread wheat.

ACKNOWLEDGMENTS

The research was supported in part by the Italian Ministero per l'Università e la Ricerca Scientifica e Tecnologica; funds MURST (60% to R. D'Ovidio) and Road to a Cure (William S. Green, director). We thank Patrick McGuire, University of California, Davis, for helpful discussion in regard to taxonomic classifications.

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[Received February 1, 1999. Accepted April 19, 1999.]