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Research paper

Molecular characterization of the IgE-binding epitopes in the fast ω-gliadins of *Triticeae* in relation to wheat-dependent, exercise-induced anaphylaxis

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ABSTRACT

Fast ω-gliadins were minor components of wheat storage proteins but a major antigen triggering allergy to wheat. Sixty-six novel full-length fast ω-gliadin genes with unique characteristics were cloned and sequenced from wheat and its relative species using a PCR-based strategy. Their coding regions ranged from 177 bp to 987 bp in length and encoded 4.28 kDa to 37.56 kDa proteins. On the base of first three deduced amino acids at the N-terminal, these genes could be classified into the six subclasses of SRL-, TRQ-, GRL-, NRL-, SRP- and SRM-type ω-gliadin genes. Compared by multiple alignments, these genes were significantly different from each other, due to the insertion or deletion at the repetitive domain. An analysis of the IgE-binding epitopes of the 66 deduced fast ω-gliadins demonstrated that they contained 0–24 IgE-binding epitopes. The phylogenetic tree demonstrated that the fast ω-gliadins and slow ω-gliadins were separated into two groups and their divergence time was 21.64 million years ago. Sequence data of the fast ω-gliadin genes assist in the study of the origins and evolutions of the different types of ω-gliadins while also providing a basis for the synthesis of monoclonal antibodies to detect wheat antigen content.

1. Introduction

The major storage proteins in wheat endosperm are classically divided into polymeric glutenins and monomeric gliadins (Anderson et al., 2009; Qi et al., 2009; Li et al., 2014; Wan et al., 2014). They have been extensively studied because of their effects on dough quality. The glutenins consist of high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) (Luo et al., 2005; Li et al., 2008, 2010). The gliadins are monomeric proteins that account for approximately 40%–50% of the protein content in wheat flour (Xie et al., 2010; Wan et al., 2014). Their coding genes are located at the short arms of the group 1 chromosome (*Gli-1* loci) and group 6 chromosome (*Gli-2* loci) in common wheat (Wan et al., 2013, 2014). Based on the electrophoretic mobilities through an acidic polyacrylamide gel, gliadins are classified into four different fractions: α-, β-, γ- and ω-gliadins (Chen et al., 2008; Xie et al., 2010).

The ω-gliadin genes belong to a multiple gene family, and multiple copies are possessed in wheat, comprising approximately 5%–10% of the proteins in wheat flour (Altenbach and Kothari, 2007). The structure of ω-gliadin typically consists of relatively short N-terminal and C-terminal domains flanking a highly repetitive domain (Anderson et al., 2009). The ω-gliadins are further separated into the ω_1, ω_2 and ω_5 subgroups, which are encoded by *Gli-A1*, *Gli-D1* and *Gli-B1*, respectively (Wan et al., 2014). These subgroups can be distinguished by the amino acid sequences at the N-terminus and include ARQL or KELQ in ω_1-gliadins, AREL in ω_2-gliadins and SRLL in ω_5-gliadins (DuPont et al., 2000, 2004; Wan et al., 2014). The ω-gliadins are also divided into slow ω-gliadins and fast ω-gliadins based on their electrophoretic mobility in SDS-PAGE (Abdel-Ala et al., 1996). Slow ω-gliadins are composed of $\omega_{1,2}$-gliadins, and fast ω-gliadins contain ω_5-gliadin and its homologous proteins (Abdel-Ala et al., 1996; DuPont et al., 2004).

Abbreviations: aa, amino acids; bp, base pair(s); CD, celiac disease; *E. coli*, *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay; HMW-GS, high molecular weight glutenin subunit; IPTG, Isopropyl β-D-1-thiogalactopyranoside; InDels, insertions and deletions; kDa, kilodalton(s); LMW-GS, low molecular weight glutenin subunit; MEGA, molecular evolutionary genetics analysis; PCR, olymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-PA-gel electrophoresis; WDEIA, wheat-dependent exercise-induced anaphylaxis.

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Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a rare but life-threatening disease in adults (Matsuo et al., 2004, 2005). The clinical symptoms may vary considerably among individuals, including urticaria, angioedema, hypotension, and even shock (Matsuo et al., 2004, 2005; Battais et al., 2005). The ω_5-gliadins of wheat have been shown to be the most active protein in triggering WDEIA. There are several peptides referred to as IgE-binding epitopes in the repetitive domain of the gliadins. These peptides can combine with specific IgEs distributed in the blood of the patient, and anaphylaxis then occurs (Palosuo et al., 2001; Matsuo et al., 2004, 2005; Battais et al., 2005). The immunodominant IgE-binding epitopes presented in the repetitive domain have been identified, and the most immunoreactive epitope was found to be QQIPQQQ (Matsuo et al., 2004).

To date, the full length of only one fast ω-gliadin gene has been cloned from common wheat (Matsuo et al., 2004). In this work, we report the isolation of fast ω-gliadin genes from the hexaploid wheat and detect their variation. The distribution of IgE-binding epitopes of the fast ω-gliadins is also discussed. Moreover, the gene sequence data provide a better understanding of the evolutionary relationships of the ω-gliadin gene superfamily.

2. Material and methods

2.1. Plant materials

The materials used in this paper included *Triticum monococcum* (2n = 2X = 14, AA), *Aegilops speltoides* (2n = 2X = 14, SS), *Aegilops searsii* (2n = 2X = 14, SSSS), *Aegilops tauschii* (2n = 2X = 14, DD), *Triticum turgidum* var. *dicoccoides* (2n = 4X = 28, AABB) and *Triticum aestivum* (2n = 6X = 42, AABBDD) showed in Table 1. These plants were grown in greenhouse under the same condition.

2.2. RNA extraction and reverse transcription PCR

The total RNAs from the seeds 15 days after flowering were extracted using the RNAprep pure Plant Kit (Tiangen, Beijing, China). Reverse transcription PCR was carried out using the PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Dalian, China).

2.3. Molecular cloning and sequencing

A pair of primers (P1: 5′-GAGCAATAGTAAACACAAATCAAAC-3′ and P2: 5′-GTTAGTCAATGGAGCATAATGTAACG-3′) was constructed to amplify the complete coding sequences of the fast ω-gliadin genes. PCR amplifications were performed in a 50 μL reaction volume containing 0.5 μL of cDNA, 0.5 mM of each primer, 25 μL of 2 × GC buffer I, 0.5 mM dNTP and 2.5 units of LA Taq polymerase (Takara, Dalian, China). The reactions were carried out in a C-1000 thermocycler (Bio-Rad Laboratories, Hercules, CA) using the following reaction protocol: 95 °C for 5 min, 35 cycles at 95 °C for 40 s, 60 °C for 30 s, 72 °C for 90 s and a final extension at 72 °C for 8 min. The PCR products were separated on 2.0% agarose gels. The expected fragments were recovered from the gels and purified. Then the purified products were ligated into the pMD18-T vector (Takara, Dalian, China) and transformed into cells of the *Escherichia coli* JM109 strain using a standard protocol. Colony PCR was carried out to select the positive clones, and the sequencing was performed by a commercial company (Beijing Genomics Institute, China).

Table 1
Plant materials used in this study.

	Species	Genome
S285	*Triticum monococcum*	AA
S288	*Triticum monococcum*	AA
Y179	*Aegilops speltoides*	SS
Y2131	*Aegilops searsii*	S^sS^s
Y446	*Aegilops searsii*	S^sS^s
Ae34	*Aegilops tauschii*	DD
RL5263	*Aegilops tauschii*	DD
Y225	*Aegilops tauschii*	DD
AS4	*Triticum turgidum* var. *dicoccoides*	AABB
AS8	*Triticum turgidum* var. *dicoccoides*	AABB
ES6	*Triticum turgidum* var. *dicoccoides*	AABB
ES9	*Triticum turgidum* var. *dicoccoides*	AABB
Chinese Spring	*Triticum aestivum*	AABBDD
Tibetan semi-wild wheat	*Triticum aestivum*	AABBDD
Xinjiang rice wheat	*Triticum aestivum*	AABBDD
Sumai 3	*Triticum aestivum*	AABBDD
Yannong 19	*Triticum aestivum*	AABBDD
Weimai 8	*Triticum aestivum*	AABBDD
Jimai 22	*Triticum aestivum*	AABBDD
Shannong 664	*Triticum aestivum*	AABBDD

2.4. Bioinformatics analysis

The sequence alignments were carried out based on multiple alignments using the software DNAMAN 6.0.2. A neighbor-joining tree of the genes cloned in this work with other wheat storage protein genes in GenBank was constructed using MEGA 4.0 according to Li et al. (2014). The identification of the IgE-binding epitopes in the fast ω-gliadins was followed according to Matsuo et al. (2004, 2005) and Battais et al. (2005). Only perfect matches were considered.

3. Results

3.1. Cloning of the fast ω-gliadin genes

Because it is difficult to clone the fast ω-gliadin genes using genomic DNA as templates and because numerous storage protein pseudogenes are present in wheat endosperm, the cDNA of the seeds during the grain filling stage was used as the template for amplification in the present work. As shown in Fig. 1, the molecular weight of PCR products were quite different in wheat and its relative species. More than one band were obtained in several PCR products which were ranged from 300 bp to 1000 bp (Fig. 1). All of the fragments were purified and ligated into the pMD18-T vector. After cloning and sequencing, 66 novel fast ω-gliadin genes from wheat and its relative species were obtained, including six types of ω-gliadin genes, according to the first three amino acid residues of the deduced N-terminal sequences. All 66 genes were submitted to GenBank (GenBank number showed in Table 2).

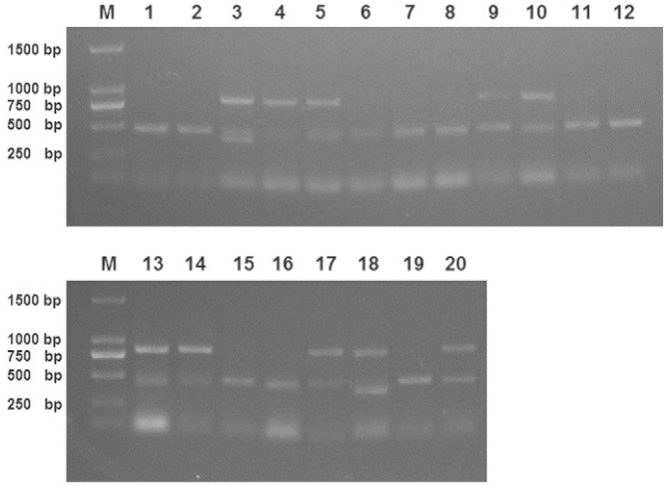

Fig. 1. PCR amplification of fast ω-gliadin genes in wheat and its relative species. M: DNA ladder; lane 1: S285; lane 2: S288; lane 3: Y179; lane 4: Y2131; lane 5: Y446; lane 6: Ae34; lane 7: RL5263; lane 8: Y225; lane 9: AS4; lane 10: AS8; lane 11: ES6; lane 12: ES9; lane 13: Chinese Spring; lane 14: Tibetan semi-wild wheat; lane 15: Xinjiang rice wheat; lane 16: Yannong 19; lane 17: Sumai 3; lane 18: Weimai 8; lane 19: Jimai 22; lane 20: Shannong 664.