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Identification and Analysis of Multivalent Proteolytically Resistant Peptides from Gluten: Implications for Celiac Sprue

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Abstract

Dietary gluten proteins from wheat, rye and barley are the primary triggers for the immuno-pathogenesis of Celiac Sprue, a widespread immune disease of the small intestine. Recent molecular and structural analyses of representative gluten proteins, most notably α- and γ-gliadin proteins from wheat, have improved our understanding of these pathogenic mechanisms. In particular, based on the properties of a 33-mer peptide, generated from α-gliadin under physiological conditions, a link between digestive resistance and inflammatory character of gluten has been proposed. Here we report three lines of investigation in support of this hypothesis. First, biochemical and immunological analysis of deletion mutants of α-2 gliadin confirmed that the DQ2 restricted T cell response to the α-2 gliadin are directed towards the epitopes clustered within the 33-mer. Second, proteolytic analysis of a representative γ-gliadin led to the identification of another multivalent 26-mer peptide that was also resistant to further gastric, pancreatic and intestinal brush border degradation, and was a good substrate of human transglutaminase 2 (TG2). Analogous to the 33-mer, the synthetic 26-mer peptide displayed markedly enhanced T cell antigenicity compared to monovalent control peptides. Finally, *in silico* analysis of the gluten proteome led to the identification of at least 60 putative peptides that share the common characteristics of the 33-mer and the 26-mer peptides. Together, these results highlight the pivotal role of physiologically generated, proteolytically stable, TG2-reactive, multivalent peptides in the immune response to dietary gluten in Celiac Sprue patients. Prolyl endopeptidase treatment was shown to abolish the antigenicity of both the 33-mer and the 26-mer peptides, and was also predicted to have comparable effects on other proline-rich putatively immunotoxic peptides identified from other polypeptides within the gluten proteome.

INTRODUCTION

Celiac Sprue, also known as Celiac Disease or gluten-sensitive enteropathy, is a widespread autoimmune disease of the small intestine caused by the intake of gluten proteins from common food sources such as wheat, rye and barley. In genetically susceptible patients the exposure of gluten to the small intestine induces a CD4+ T cell mediated inflammatory response, leading to destruction of the intestinal villous structure [1–3]. Clinical symptoms of Celiac Sprue include fatigue, chronic diarrhea, abdominal distension, weight loss due to malabsorption, and extraintestinal symptoms including anemia, psychiatric syndromes and neurological symptoms [3]. Untreated Celiac Sprue is also associated with an elevated risk of infertility, bone disorders and intestinal malignancies [4, 5]. Despite of the wide prevalence (0.5–1%) and serious clinical

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implications of the disease, currently the only effective therapy is strict dietary abstinence from these common food grains.

The principal toxic components of wheat gluten belong to a family of closely related proline and glutamine rich proteins called gliadins. When genetically predisposed individuals who express the human leukocyte antigen (HLA) DQ2 (or DQ8) are exposed to certain gliadin epitopes [6–9], these epitopes are presented on the surfaces of antigen presenting cells, which in turn stimulate proliferation of gluten-responsive DQ2 (or DQ8) restricted CD4+ T cells in the small intestinal mucosa of Celiac Sprue patients. To date at least 17 distinct DQ2 restricted T cell epitopes have been identified from gluten proteins found in disease associated grains such as wheat, rye, barley and possibly oats [8–15]. Interestingly, most of these epitopes have to undergo a transglutaminase 2 (TG2) mediated deamidation of certain glutamine to glutamate residues to elicit a T cell response [6, 7].

The identification of an exceptionally immunoreactive 33-mer peptide, LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF (underlined Q residues correspond to transglutaminase deamidation sites) from α-2 gliadin led us to propose that highly antigenic gluten epitopes share at least four properties [12]. First, all antigenic gluten epitopes identified so far are located in proline-rich regions of gliadin [16]. Proline-rich peptides are protected from proteolysis by gastric, pancreatic and intestinal brush border membrane enzymes, so they have an opportunity to build up to high concentrations in the small intestine. The structural basis for gastrointestinal stability of certain gluten peptides has been investigated [10, 12, 17]. Second, the most inflammatory gluten epitopes are also preferred substrates for human transglutaminase 2 (TG2). The molecular logic for TG2 catalyzed deamidation of gluten epitopes has been elucidated [18, 19] Third, the DQ2 molecule appears to have unique features enabling the molecule to bind and present proline rich gluten peptides with glutamate residues to CD4+ T cells in the intestinal mucosa [8, 20, 21]. Finally, it was also proposed that highly antigenic gluten epitopes occur within the context of long, multivalent peptides [12].

As a further test of this hypothesis, in this study we sought to verify that the 33-mer peptide from α-2 gliadin is indeed the principal contributor to this protein's immunotoxicity. Using experimental and computational approaches, we also attempted to identify analogous peptides from other sub-families of gliadin proteins such as γ-gliadins, as well as other families of grains such as barley and rye. The results of these studies are reported here.

In addition to providing a new perspective on Celiac Sprue pathogenesis, the link between digestive resistance and gluten toxicity has also presented an opportunity for countering the toxic effects of gluten in Celiac Sprue patients through the use of an oral prolyl endopeptidase (PEP). For example, a PEP from *Flavobacterium meningosepticum* was shown to complement pancreatic proteases and the brush border enzymes to detoxify selected peptides from gluten [12, 17] as well as whole gluten [22]. Identification of additional highly inflammatory peptides such as the 33-mer can therefore be expected to expand the panel of primary screening targets for isolation or engineering of new therapeutically useful PEPs.

MATERIALS AND METHODS

The peptides LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF, and FLQPQQPFPQQPQQPYPQQPQQPFPQ were synthesized by solid-phase peptide synthesis. Trypsin (T-1250), chymotrypsin (C-4754), carboxypeptidase (C-0261) (all from bovine pancreas), elastase from porcine pancreas were from Sigma (St. Louis, MO). Prolyl endopeptidase from *Flavobacterium meningosepticum* was obtained from US Biological (Marble Head, MA). Enzyme substrates were purchased from Sigma except for Z-Gly-Pro-p-nitroanilide (Bachem, Switzerland). All chemicals were from Sigma unless otherwise noted.

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Preparation of recombinant gliadins

Gliadin genes including α-2 gliadin and γ-5 gliadin were cloned in pET28a plasmid (Novagen) and introduced via transformation into *E. coli* BL21(DE3) (Novagen). Transformed cells were grown in LB media containing 50 μg/ml of kanamycin at 37 ºC until OD_{600} reached 0.8. The expression of protein was induced with the addition of 0.4 mM isopropyl β-D-thiogalactoside (Sigma) and the cultures were further incubated at 37ºC for 20 hours. The cells were centrifuged at 3600 rpm for 30 minutes. The pellet was resuspended in 15 ml of disruption buffer (200 mM sodium phosphate; 200 mM NaCl; 2.5 mM DTT; 1.5 mM benzamidine; 2.5 mM EDTA; 2 mg/L pepstatin; 2 mg/L leupeptin; 30% v/v glycerol) and lysed by sonication (Branson Sonifier, 1 min; output control set to 6). After centrifugation at 45000g for 45 min, the supernatant was discarded and the pellet containing gliadin protein was resuspended in 50 ml of 7M urea in 50 mM Tris Cl (pH = 8.0). The suspension was again centrifuged at 45000g for 45 min and the supernatant was harvested for purification. The supernatant was incubated with 1 ml of nickel-nitrilotriacetic acid resin (Ni-NTA; Qiagen) overnight and then batch-loaded on a column with 2 ml of Ni-NTA. The column was washed with 7M urea in 50 mM Tris Cl (pH = 8.0) and protein was eluted with 200 mM imidazole, 7 M urea in 50 mM Tris Cl (pH = 4.5). The fractions containing gliadin were pooled together and concentrated 100-fold using a 10,000 MWCO concentrator (Amicon). A final concentration of 70% ethanol solution and two volumes of 1.5M NaCl were added to precipitate the protein overnight at 4 ºC. The precipitate was collected by centrifugation at 45000 g for 30 min, rinsed in water, and re-centrifuged. The final purification step was developed with reverse-phase HPLC. The Ni-NTA purified protein fractions were pooled in 7 M urea buffer and injected onto a Vydac (Hesperia, CA) polystyrene reverse-phase column (i.d. 4.6 mm × 25 cm) with the starting solvent (30% of solvent B: 1:1 HPLC-grade acetonitrile/isopropanol : 0.1% TFA). Solvent A was an aqueous solution with 0.1% TFA. The separation gradient extended from 30–100% of solvent B over 120 min at a flow rate of 0.8 ml/min. The protein solution was dried by lyophilization.

Mutagenesis of gliadin

Deletion mutations were engineered in the α-2 gliadin gene in pET28a in two steps. First, two polymerase chain reactions were performed to amplify the flanking gene fragments to the DNA piece to be deleted. Then the two PCR pieces were ligated to omit the deleted DNA. In order for the ligation in the second step to occur, the primers were designed to include an EcoRI restriction site on the ends of the two ligating DNAs with the minimal mutation of the wild-type sequence. For the deletion of fragment Gln35→Gln104 (Fig. 2), the following primers were designed: for the DNA flanking from the left, 5'-CATATGGTTAGAGTTCCAGTGCCAC-3' (sense), 5'-CTGAATTCCTGGAAATTGTTGTTGTTGTACCAATGG-3' (antisense); for the DNA flanking from the right, 5'-CGAATTCAACCACAACAACCAATTTCGCAGCAGCAG-3' (sense), 5'-CAAGCTTCATCGATAGTTAGTACCGAAGATG-3' (antisense). The following primers were synthesized for the deletion of fragment Leu57→Phe89 (Fig. 2): for the DNA flanking from the left, 5'-CATATGGTTAGAGTTCCAGTGCCACAATTG-3' (sense), 5'-CTGAATTCGTGGTTGTTGTGATGGAAATGGTTGCGG-3' (antisense); for the DNA flanking from right, 5'-CGAATTCAACAACCATATCCACAATCGCAACCACAG-3' (sense), 5'-CAAGCTTCATCGATAGTTAGTACCGAAGATG-3' (antisense). Residue Pro91 was converted to Ile to accommodate restriction sites. The PCR reactions were carried out using Pfu polymerase, the α-2 gliadin pET28a plasmid template, the sense and antisense primers. The reaction mixture (50μl) also contained 5μl of DMSO, 200μM of dNTP mix. The temperature profiles were 94°C for 5 min, annealing using a temperature step-down feature to decrease from 80°C to 65°C in 15 cycles, and then 72°C for 1 min in 20 cycles. The amplified

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PCR products were gel purified using a QIAquick gel extraction kit (Qiagen) and ligated using T4 ligase at 4°C overnight.

Proteolysis of Gliadins

Purified recombinant α-2-gliadin, its mutants, and γ-5-gliadin in lyophilized forms were weighed and dissolved in 0.01M hydrocholoric acid. Proteins were incubated at 37°C with pepsin (protease to protein w/w ratio is 1:100, Sigma) at pH 2.0 for 20 min. The reaction mixture was then pH adjusted to 7.0 in 50mM phosphate buffer and supplemented with trypsin (1:100, Sigma), chymotrypsin (1:100, Sigma), elastase (1:500, Sigma) and carboxypeptidase (1:100, Cortex Biochem) at 37°C for various time periods.

Preparation of Rat Brush Border Enzymes (BBM) and BBM Digestion of Peptides

The rat jejunum was removed from the small intestine of adult female rats and further processed as previously described with slight variations [23]. The jejunum was rinsed with 0.9% NaCl. Mucosal scrapings (~1 g) were homogenized in 10 ml of 5 mM EDTA in 5 mM histidine imidazole, pH 7.6 in a Dounce homogenizer and type B pestle (15 vertical strokes). The homogenate was centrifuged at 45000g for 30 min. The pellet was rehomogenized (15 vertical strokes) in 15 ml of 5 mM histidine imidazole, 0.5 mM EDTA, 250 mM sorbitol, 12.5 mM NaCl, pH 7.6. The homogenate was further diluted to 25 ml with the same buffer and centrifuged at 45000g for 30 min. The pellet was resuspended in 10 ml of 50 mM mannitol, 2 mM Tris-HCl, pH 7.1, and homogenized with two 30-s bursts of a Polytron homogenizer at Setting 4. Then $CaCl_2$ was added to a final concentration of 10 mM, and the suspension was stirred at 4 °C for 30 min and centrifuged at 2000g for 10 min. The supernatant containing the partially purified BBM enzymes was harvested by centrifuging at 45000g for 30 min. The BBM pellet was stored in 1 ml of 5 mM histidine imidazole, 0.5 mM EDTA, 100 mM NaCl, pH 7.1. Marker enzyme assays were performed. Total protein concentration was determined by the Lowry method. Specific activities of alkaline phasphatase, dipeptidyl peptidase IV (DPP IV), aminopeptidase N (APN) were determined as previously described [10].

Stable peptic products identified from previous gastric/pancreatic digestions were synthesized (New England Peptides) and purified. BBM digestion mixtures of these peptides consisted of 100 μM of peptide, 10 mM Cbz-tryptophan (internal control for quantification), and a final concentration of BBM proteins of 12.5 ng/μl at 37 °C from 30 min up to 20 hours. The identification of the digestive products was obtained with LC-MS methods described above.

Deamidation by Tissue Transglutaminase

Human TG2 was expressed and purified according to previous work [11]. The activity of the TG2-catalyzed deamidation reaction was analyzed using a spectrophotometric method.

Liquid chromatography-coupled mass spectrometry analysis

To identify the stable hydrolysis products from each enzymatic digestion, the reaction mixtures were analyzed by LC-MS (SpectraSystem, ThermoFinnigan) using a reversed-phase column (Vydac 259VHP54115). The mobile phase flow rate was 0.2 ml/min, beginning with 0% acetonitrile (in water, 0.1% formic acid and 0.025% trifluoroacetic acid) and achieving 95% acetonitrile over 40 min. The LC-separated peptides were further fragmented and detected by electrospray ionization mass spectrometry. The compounds defined by their m/z values were detected by their ionization intensities. The amino acid sequences of the proteolytic products were determined from the MS-MS fragmentation pattern.

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Gluten specific T cells and T cell proliferation assays

Gluten reactive T cell lines (TCL) and T cell clones (TCC) were established from intestinal biopsy samples of DQ2 positive Celiac patients mainly as described [8]. The following TCLs and TCCs were used in this study: TCL432.1.3 [12], TCL437.1.1 [12] , TCL432.1.4 [12], TCL422.02.4.2 [12], TCL462.1, TCL480.1, TCC430.1.134 [15], TCC387.3 and TCC387.19 [15]. Antigen presenting cells (DR3-DQ2 homozygous, Epstein-Barr virus transformed B-lymphoblast cell lines) were irradiated 75 gray, seeded at 7.5 x 10^4 cells per well, incubated overnight with deamidated α-2 peptides or γ-35 peptides of interest, with or without prolyl endopeptidase (PEP) and BBM treatment in U-bottomed 96 well plates, before addition of T cells (2.5 to 5 x 10^4 cells per well). T cell proliferation was measured as [^{3}H] thymidine incorporation after 48–72 h.

Gluten Protein Database Analysis

Gluten protein sequences (α-gliadins, α gliadin precursors, γ-gliadins, glutenins, hordeins, secalins and avenins) were obtained from NCBI protein and nucleotide databases. Computational prediction of proteolytic fragments by chymotrypsin, trypsin and pancreatic elastase was performed with GCG SeqWeb software (Accelrys, CA). Chymotrypsin cleavage was set to occur at the C-terminal of F, W, Y, M, L; trypsin cleavage is after K and R; pancreatic elastase cleavages after A, G, S, V; carboxypeptidase A (non-specific but prefers aromatic and long side-chain amino acids) combined with carboxypeptidase B (preferentially cleaves K and R) were predicted to cleave from C-terminal end of a polypeptide until the PX sequence. Homology alignment of proteins and their expected stable peptides was performed with BLASTP provided by NCBI. TG2 glutamine deamidation sites were predicted according to the recognition motifs that identifies non-terminal Gln in QXP, or QXP(F,Y,W,M,L,I,V) or QXX(F,Y,W,M,L,I,V) but not QP or QXXP [18]. DQ2 binding motifs were predicted as follows: P1 – F,Y,W, I, L,M,S,P or Q; P2 – not P; P3 – any; P4 – not P; P5 – any; P6 – P, E, Q or D; P7 – not P; P8 – any; P9 – not P [24].

RESULTS

Expression, Purification and Digestion of Recombinant α-2 Gliadin

The yield (2–3 mg/L) and purity of the Ni-NTA purified gliadin protein were adequate for further studies. Purified α-2 gliadin was subjected to proteolysis under conditions simulating gastric and pancreatic conditions, as described in the Experimental Methods section. The resulting peptide fragments were analyzed by LC/MS/MS. The resistant QLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF (and its N-terminal pyroglutamate counterpart) was present in the reaction products and calculated to be in stoichiometric amount with the protein prior to digestion.

α-2 Gliadin in T Cell Proliferation

The mixture of proteolyzed fragments from recombinant α-2 gliadin was used to test six individual DQ2-restricted T cell lines (TCL432.1.3, TCL437.1, TCL432.1.4, TCL422.02.4.2, TCL462.1 and TCL480.1) in a proliferation assay. The digested recombinant α-2 gliadin peptides induce a potent T cell proliferation response comparable to what we have previously observed with the 33-mer (an example can be seen in Figure 4). The EC_{50} for the proteolyzedmixture of α-2 gliadin peptides was found to be comparable to the 33-mer (Table 1). This suggested that the 33-mer sequence in the α-2 gliadin protein contributed most, if not all, of this gliadin's antigenic properties.

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α-2 Gliadin Mutants

Earlier studies involving a panel of overlapping synthetic peptides suggested that a cluster of three overlapping epitopes, designated αI, αII and αIII gliadin epitopes, are the primary immunogenic sequences in the α-2 gliadin protein [12, 25]. As an alternative strategy for assessing the immunological importance of these epitopes, two deletion mutants for α-2 gliadin were constructed (Figure 1). Deletion mutant α-1234 was designed to exclude the 33-mer sequence only, whereas the deletion mutant α-1278 excludes the 33-mer sequence and additional N-terminal residues QQPFPPQQPYPQPQPFPSQQPY and C-terminal residues RPQQPYPQSQPQYSQ, sequences analogous to known T cell epitopes DQ2-γ-III and DQ2-γ-VI [26] and possibly including a disease-specific T cell epitope [27].

Gastric/pancreatic digestions were performed on wild-type α-2 gliadin as well as both deletion mutants. The resulting proteolytic mixtures were compared via reversed phase HPLC, as shown in Fig. 2A for the wild-type protein and in Fig. 2B for deletion mutant α-1234. The 33-mer fragment at retention time of 16 min was present in the wild-type digestion mixture, but not in the mutant digestion mixture. The remaining peptides could be found in both mixtures. Peptide fragments at retention times of 14–15 min from the deletion mutant were found in lower quantities compared to those from the wild-type gliadin, presumably due to a more thorough digestion of these shorter peptides. Similar results were also obtained in the case of deletion mutant α-1278 (data not shown).

In order to determine whether the remaining peptides from the two digested mutants retain T cell stimulatory capacity, these peptide mixtures were assayed against four patient-derived polyclonal T cell lines. As seen in Figure 3, the α-2 gliadin deletion mutants elicit virtually no response in the polyclonal T cell lines tested of these DQ2 positive Celiac Sprue patients, suggesting that the 33-mer peptide is in fact the sole source of α-2 gliadin toxicity. Aside from the 3 epitopes present in the 33-mer (α-I, α-II and α-III), there is another α-gliadin epitope, FRPQQPYPQ (Glia-α20, see supplementary Table S1). Since the Phe residue of the 9-mer core region of this epitope is part of the 33-mer sequence, deletion mutants void of the 33-mer would be unlikely to stimulate Glia-α20-specific T cells.

Prolyl endopeptidase activity

Earlier studies have shown that a prolyl endopeptidase (PEP) enzyme is able to accelerate breakdown of the synthetic 33-mer peptide. In light of the above results, we wished to confirm if comparable PEP mediated detoxification of the entire α-2 gliadin protein could also be achieved. Recombinant α-2 gliadin was pretreated with gastric and pancreatic proteases. The digestive products were then treated with rat brush border enzymes for further breakdown. Ordinarily, the small intestinal brush-border membrane enzymes rapidly break down the products of gastric and pancreatic digestion into single amino acids, dipeptides or tripeptides [28]. However, their action on the relatively long, proline-rich products of peptic/pancreatic digestion of gluten is at best sluggish. Whereas the brush border enzymes failed to detoxify pretreated recombinant α-2 gliadin (Figure 4A), *Flavobacterium* PEP addition was able to detoxify the α-2 gliadin protein in a dose-dependent fashion (Figure 4B).

γ-5 Gliadin

Recombinant γ-5 gliadin (GenBank accession number AJ416339 [16]) was expressed and purified. The protein was proteolyzed with gastric and pancreatic enzymes under physiological conditions. In comparison to the α-2 gliadin, the peptide fragments are not as stable as the 33-mer (data not shown). The main observable peptide products were separated and determined by LC/MS/MS. The most stable fragment was the 26-mer FLQPQQPFPQQPQQPYPQQPQQPFPQ (γ-5 (26–51); Figure 5), another Pro- and Gln- rich peptide containing multiple T cell epitopes.

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Like the 33-mer, this peptide was a good substrate of human TG2, as judged by a standard kinetic assay [18] (data not shown). It also contains multiple copies of known T cell epitopes (Figure 6). In T cell proliferation assays, the TG2-treated 26-mer peptide was compared with its smaller peptide fragments, including PEQPFPEQPEQ (DQ2-γ-VI epitope, core epitope is underlined), FPEQPEQPYPEQ (epitope DQ2-γ-III), TG2-treated LQPQQPFPQQPQQPYPQQPQ (containing both DQ2-γ-III and DQ2-γ-VI), and TG2-treated QFPQTQQPQQPFPQPQQTFP (epitope DQ2-γ-VII).[15] In all cases, the 26-mer peptide exhibited greater antigenicity compared to its shorter derivatives (Figure 7). The EC_{50} value of the 26-mer is ca. 5-fold less than the single DQ2-γ-VI epitope PEQPFPEQPEQ, 30-fold less than the DQ2-γ-III epitope FPEQPEQPYPEQ, and 2–3 fold less than the longer peptides LQPQQPFPQQPQQPYPQQPQ or QFPQTQQPQQPFPQPQQTFP. To evaluate whether the antigenicity of the 26-mer could be reduced or eliminated with exogenous PEP, the peptide was co-treated with rat-derived brush-border-membrane enzymes and PEP. After a 2 h incubation, the antigenicity (as measured by the increase of EC_{50}) was reduced by 10 to 100 fold in all of the T cell clones tested (Figure 7D).

Computational Analysis of Gluten Proteins in the Protein Sequence Database

Although our experimental analysis thus far has focused on one representative α-gliadin and one representative γ-gliadin protein, wheat gluten is a complex mixture of hundreds of homologous gliadin and glutenin proteins. (Similarly, barley and rye contain hordein and secalin proteins, respectively, which are homologous to gliadins and are toxic to Celiac Sprue patients.) Therefore, guided by our experimental data, we performed a thorough search of the NCBI protein and nucleotide database to identify other potentially metastable and inflammatory peptides that might be expected to be released as a result of duodenal breakdown of dietary gluten from wheat, rye or barley by the major pancreatic proteases (trypsin, chymotrypsin, elastase, and carboxypeptidases A and B). For the purposes of this analysis, the effect of pepsin on gluten proteins was not considered, due to variability in gastric residence time and food texture; however, since pepsin cleavage specificity is similar to chymotrypsin in gluten (data not shown) [29], there is reasonable confidence in the comprehensiveness of the analysis.

Overall, the sequences of 157 gluten proteins were analyzed, including α-gliadins, α gliadin precursors, γ-gliadins, wheat glutenins, secalins and hordeins. Since peptide size is a major factor that influences the subsequent proteolytic kinetics of the products of pancreatic protease digestion, we mostly focused our analysis on peptides with 18 or more residues. A few exceptions (accession numbers CAA26449, AAQ63849, AAQ63848, AAQ63847, AAQ63842) correspond to hordeins and secalins, whose longest peptide fragments contain only 17 amino acids (Table S1).

Of the 157 proteins analyzed, 128 proteins revealed the presence of one or more putative proteolytic product with 18 or more residues. 58 proteins have two such peptides encoded within their sequence, whereas 35 proteins have three or more such peptides. Out of a total of 271 such peptides, 147 peptides (from 100 gluten proteins) are longer than 25 residues, and 34 peptides (from 34 proteins) are longer than 35 residues (i.e. ca. 10% of the length of a typical gliadin polypeptide). In addition to their length, the high proline content of these peptides is presumably critical to their proteolytic resistance; the average peptide in Table S1 is comprised of ~30% Pro residues.

The potential T cell antigenicity of these peptides can be appreciated by the fact that 51% of the identified peptides contain one or more epitopes known to be recognized by inflammatory T cells from Celiac Sprue patients (Table S1(A)). Furthermore, analogous to the 33-mer from α-2 gliadin and the 26-mer from γ-5 gliadin, 30% of the identified peptides are potentially multivalent (based on known epitopes). An extreme example is the 1507333A γ-gliadin (also

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designated as sequence M36999), which is predicted to yield a 68-residue peptide spanning at least 4 putative epitopes, PFPQPQQTF (homologous to the known DQ2-α-I epitope PFPQPQLPY), QQPQQPFPQ (DQ2-γ-VII epitope), FPQQPQLPFPQQ (homologous to the known DQ2-γ-III epitope QQPQQPYPQ), and PFPQPQQPQ (also homologous to DQ2-α-I). Indeed, 20-mer synthetic peptides tiling across the entire amino acid sequence of M36999 have been synthesized and assayed against multiple polyclonal T cell lines from DQ2 positive Celiac patients [16]. All peptides containing the above four epitopes were found to be antigenic. Another very long peptide was found in a γ-gliadin (AAK84772) as a 92-mer. It contains at least four T- cell epitopes (4 copies of the known DQ2-γ-VII epitope QQPQQPFPQ), and has yet to be tested for bioavailability and T cell activity. Altogether, of the 21 Celiac-specific gluten epitopes reported to date (Table S1(A)), 14 can be observed in one or more of the putative proteolytically stable peptides identified through our computational analysis. These findings provide a vivid testimony to our earlier proposal that the proteolytic stability and pro-inflammatory character of gluten are intimately correlated.

It is also interesting to note that not all gluten proteins appear to contain long (i.e. proteolytically stable), epitope bearing peptides. For example, several α-gliadins and α-gliadin precursors are predicted to release either a 19-residue peptide fragment QQQPFPPQQPYPQPQPFPS that lacks any intact T cell epitope, or considerably shorter putative monovalent peptides such as RPQQPYPQPQPQY (α-10, not shown in the Supplementary Tables) and QPFPRPQLPYPQPQPF (residues 60–75 in α-7 gliadin). Through the use of pepsin or chymotrypsin digested recombinant α-gliadins, some of these proteins have been shown to exhibit greatly diminished T cell antigenicity [25]. Presumably this reflects the higher susceptibility, and consequently reduced half-life, of the corresponding physiologically generated peptides in the duodenum to intestinal brush border membrane proteolysis. Similarly, proteolytically stable but non-antigenic peptides may also be pathogenically interesting, as discussed below. For example, although α-1 and α-6 gliadin are predicted to release peptide QQPYPQPQPFPPQLPYPQTQPFPPQQPYPQPQPQYPQPQQPI, there is no known T cell epitope in this sequence [25].

Careful analysis of Table S1 also reveals some relatively minor but noteworthy discrepancies between our experimental and computational data. For example, experimental studies with recombinant α-2 and γ-5 gliadin polypeptides has led to the identification of the 33-mer and 26-mer peptides, whereas in silico analysis as outlined above reveals the closely related but distinct putative sequences, QPFPQPQLPYPQPQLPYPQPQLPYPQPQPF and QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ, respectively. In both cases the observed discrepancies arise due to the relatively unpredictable specificity of chymotrypsin. Apparently, chymotrypsin is unable to cleave N-terminal residues (LQL from the 33-mer and FL from the 26-mer), either due to the strong secondary structure content of the peptides [12] or due to end effects. Conversely, chymotrypsin is able to cleave the C-terminal TQQPQ from the computationally predicted analog of the 26-mer, presumably due to its lower but well-known specificity for Gln in the S1 site. Notwithstanding these differences, the strong overall agreement between the experimental and computational results validates our in silico method for identifying long, proteolytically resistant, and multivalent peptides.

In addition to screening the putative peptides in Table S1 for experimentally established T cell epitope sequences, we also screened for putative TG2 deamidation sites and other likely but as yet unproven DQ2-binding epitopes (Table S2). For this, we used TG2 [18] and DQ2 [15, 24] recognition algorithms (see Materials and Methods for details) that have recently been proposed in the literature. Over 100 as yet unreported 9-residue sequences, which represent the core region of T cell epitopes of typically 10-14 residues with TG2 deamidation sites at the P4, P6 or P7 positions, were identified from the sequences of the proteolytically stable fragments in Table S1. The core regions of new putative DQ2 epitopes are tabulated in Table

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2, and the complete data set is summarized in Table S2. Notably, this in silico method was able to identify many new epitopes in hordeins and secalins (from barley and rye, respectively), which are known to be toxic grains for Celiac Sprue patients but have not been studied extensively so far. At the same time, a few discrepancies between experimental and bioinformatic data are apparent. For example, although two new epitopes were predicted in the peptide QQPYPQPQPFPPQLPYPQTQPFPPQQPYPQPQPQYPQPQQPI from α-1 and α-6 gliadin (9-mer core sequences: PQPQPQYPQ and QYPQPQQPI), Proteolytic digests of the α-1 and α-6 gliadin failed to stimulate a limited number of polyclonal T cell lines. The precise reason for this discrepancy remains to be established, and could either reflect a cryptic proteolytic site in the peptide sequence, or it may highlight the need for further testing of these gliadins against a wider panel of T cell lines from Celiac Sprue patients.

Finally, as a control, we performed equivalent *in silico* analysis on several other sequenced families of dietary proteins that are non-toxic to Celiac Sprue patients, including avenins (from oats) bovine myoglobin (from meat), chicken ovalbumin (from eggs), bovine casein and lactoglobulin (from milk). Remarkably, none of these dietary proteins were predicted to release peptide products longer than 10 amino acids as a result of pancreatic proteolysis (Table S3).

DISCUSSION

The recent discovery of an unusually long and potent T cell proliferation agent, the 33-mer peptide from α-2 gliadin, highlighted the importance of understanding the molecular fate of gluten under physiological conditions. In particular, the role of proteolytic resistance in gluten antigenicity became clear. The 33-mer peptide has become a useful probe for DQ2 binding [30, 31], for peptidase development [32] and for studying the transport of peptide and Celiac pathogenesis at the intestinal level [33]. The goals of the current work were therefore two-fold. On one hand we sought to verify the centrality of the 33-mer in the immunotoxicity of α-2 gliadin via deletion mutagenesis. On the other hand, we wished to identify similar peptides from other gluten proteins, especially the closely related γ-gliadins.

Our studies verified that the 33-mer peptide is the single crucial antigenic component in α-2 gliadin. The digestion mixture of α-2 gliadin was comparably potent to an equivalent amount of the synthetic 33-mer peptide, and 10–20 times more potent than any individual T cell epitope present in the 33-mer sequence. Two deletion mutants of α-2 gliadin lacking the 33-mer were engineered, one excluding only the 33-mer sequence and the other deleting the 33-mer as well as additional C-terminal residues. The resulting proteins were no longer toxic. Taken together with earlier data, the 33-mer was thus confirmed as the major Celiac-toxic segment of α-2 gliadin.

The existence of other long, proteolytically resistant, antigenic peptides in gluten proteins was anticipated from early *in silico* analysis using the 33-mer sequence as a probe [14]. To obtain direct experimental evidence for this proposal, here we have identified a 26-mer peptide, FLQPQQPFPQQPQQPYPQQPQQPFPQ, from γ-5 gliadin. This peptide is not as resistant to pancreatic proteolysis as the 33-mer peptide, presumably due to its PQ↓Q repeats, which are weakly susceptible to chymotrypsin cleavage. Like the 33-mer however, the 26-mer peptide is highly resistant to intestinal brush border membrane proteolysis. It is also multivalent; thus far three epitopes, EQPFPEQPE (DQ2-γ-VI), EQPEQPYPE (DQ2-γ-III), EQPEQPFPQ (DQ2-γ-VII) have been identified in the 26-mer [16]. Similar to the 33-mer, the intact 26-mer is more antigenic compared to its smaller monovalent counterparts. Finally, analogous to the 33-mer, the 26-mer is susceptible to PEP breakdown, as illustrated in Fig. 7D.

In an attempt to integrate available biochemical and immunological information to derive a global picture of the physiological immunotoxicity of dietary gluten for Celiac Sprue patients,

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we conducted an in-depth computational analysis of the gluten proteome. Our analysis revealed that long, proteolytically resistant fragments were widespread within the α-gliadin, γ-gliadin, glutenin, hordein and secalin protein families of wheat, rye and barley. In contrast, analogous investigations on sequences of avenins (from oats) and a range of proteins present in bovine myoglobin, chicken ovalbumin, bovine casein and lactoglobulin (from meat, eggs or milk) showed no peptide products longer than 10 amino acids (Table S2). Their susceptibility to proteolysis corresponds to their low proline content (an average of 6% in avenins, 2% in myoglobin, 3% in ovalbumin, 12% in casein and 4% in lactoglobulin). Only avenin and casein proteins produced digestion fragments of 8–10 residues. Notably, these avenin fragments contain T cell epitopes recognized by intestinal T cells of oats-intolerant Celiac Sprue patients [14]. Gluten proteins known to be highly antigenic, such as α-2 gliadin, γ-5 gliadin and the M36999 γ-gliadin, appear to release long, proteolytically stable, multivalent peptides as a result of duodenal proteolysis. In contrast, gluten proteins with limited T cell antigenicity seem to be incapable of generating such long, epitope-bearing peptides. Due to the rapid intestinal brush-border membrane proteolysis of peptides shorter than 10 amino acids [12], the antigenic peptides released from these gluten proteins are likely to be rapidly destroyed by the peptidases of the intestinal brush border membrane.

The putative existence in gluten of long, proteolytically stable peptides that lack any known T cell antigenicity bears some reflection. Perhaps this suggests that the epitope repertoire of gluten responsive T cells in Celiac Sprue patients has not been thoroughly mapped as yet. Alternatively, there may exist non-T cell mediated mechanisms that contribute to the overall gluten enteropathy in Celiac Sprue patients. A few such mechanisms have been proposed based upon the identification of gluten peptides that lack T_H1 reactivity, but are capable of triggering inflammatory reactions in intestinal biopsies derived from Celiac Sprue patients [34–37]. For example, the 13-residue sequence, LGQQQPFPPQQPY, is found in putative physiological peptides (LG)QQQPFPPQQPYPQPQPFPS among α-gliadins, and is known to elicit an innate immune response. However, in no case has a primary molecular target been identified that interacts with the gluten peptide. It is possible that identification of such targets would require use of the physiological forms of these peptides. Finally, gluten sensitivity is occasionally diagnosed in patients who do not have Celiac Sprue [38]. It is conceivable that one or more of these proteolytically resistant peptides plays a crucial role in eliciting a pathological response in those patients. Regardless of the precise mechanism of pathogenicity, peptide length and a high proline content are likely to be common denominators of gluten intolerance. If so, the use of an oral prolyl endopeptidase may be appropriate therapy for such patients, as proposed recently [14, 17, 18, 20, 21].

The limitations of the above computational analysis of the physiological fate of gluten must be acknowledged. For example, such analysis does not consider the effect of gastric pepsin on food. Similarly, it does not incorporate complexities resulting from the extensive disulfide crosslinked structure of glutenin, or other secondary structural features of gluten peptides that influence their proteolytic susceptibility. Ultimately, a thorough analysis of gluten proteolysis using experimental proteomic methods will be required in order to obtain a quantitatively accurate picture of the physiological process of gluten digestion. Notwithstanding these limitations, our results have clearly reinforced the notion that protease stability and immunotoxicity of gluten are intimately correlated. This has implications for protein engineering of wheat strains with reduced antigenicity for Celiac Sprue patients [26, 39], and for the development of high-throughput screening tools for identifying and engineering therapeutically useful enzymes [10, 12, 22, 32, 40].

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Synopsis

The link between digestive resistance and inflammatory character of gluten in Celiac Sprue was further validated. Deletion mutations confirmed the central role of the 33-mer in T cell response. Proteolysis of γ-gliadin resulted in a 26-mer with enhanced T cell antigenicity. *In silico* analysis of the gluten proteome found over 60 putative physiological, stable, TG2-reactive, and multivalent peptides. Prolyl endopeptidase abolished the antigenicity of the 33-mer and the 26-mer peptides.

Acknowledgements

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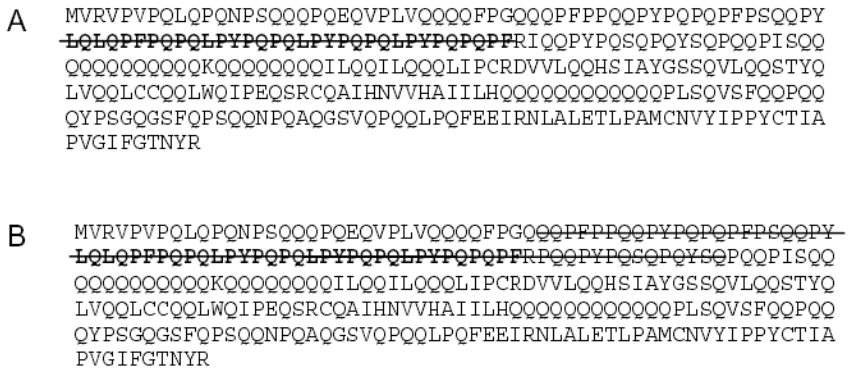

Figure 1.

Protein sequences of the α-2 gliadin deletion mutants. (A) Deletion mutant A1234. (B) Deletion mutant A1278. The peptide sequence in red is the proteolytically resistant 33-mer.

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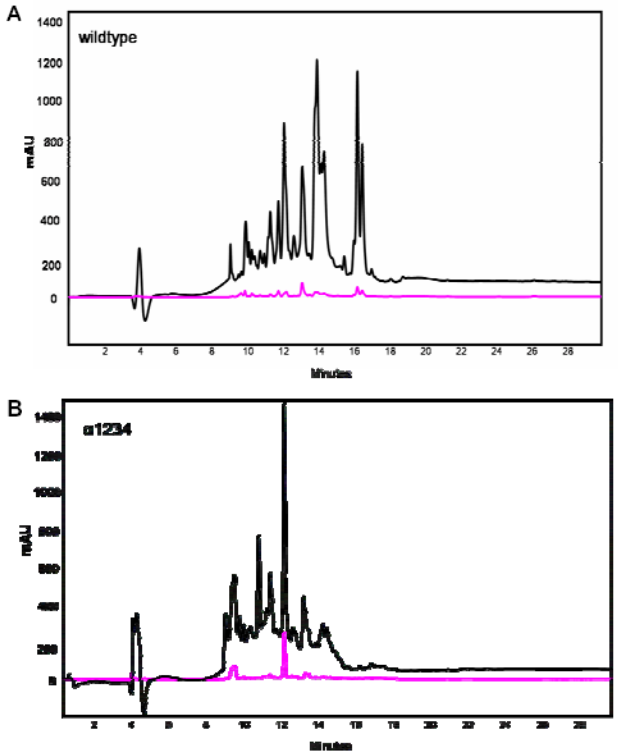

Figure 2.
Gastric/pancreatic proteolysis of the wild-type α-2 gliadin (A) and its deletion mutant α1234 (B), illustrated by HPLC with UV detection at 215 nm (black traces) and 280 nm (magenta traces). The 33-mer resulted from the wildtype digestion eluted at a retention time of approximately 16 minutes, which is missing in the deletion mutant digestion.

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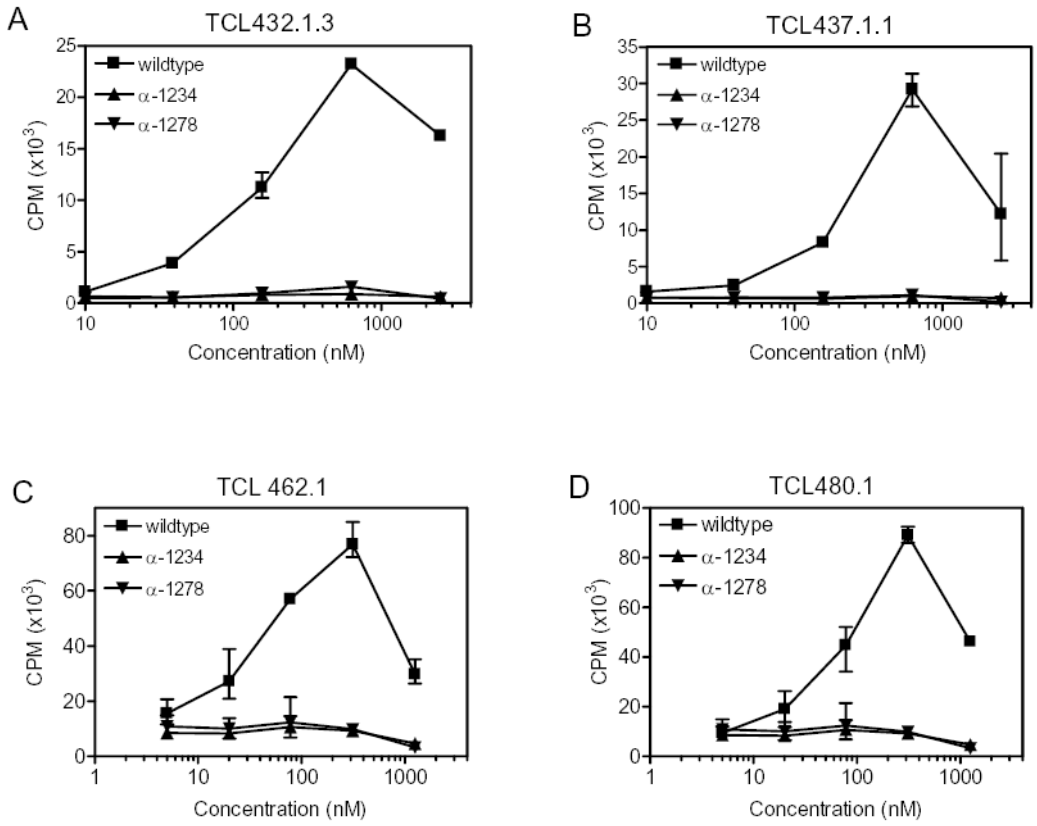

Figure 3.

Stimulation of four HLA-DQ2 restricted T cell lines derived from biopsies of Celiac Sprue patients by the PTCEC (pepsin, trypsin, chymotrypsin, elastase, carboxypeptidase B) and brush-border membranedigestive peptide mixtures from α-2 gliadin deletion mutants α-1234 and α-1278. No T cell stimulation was elicited. Error bars indicate the observed range within the triplicates.

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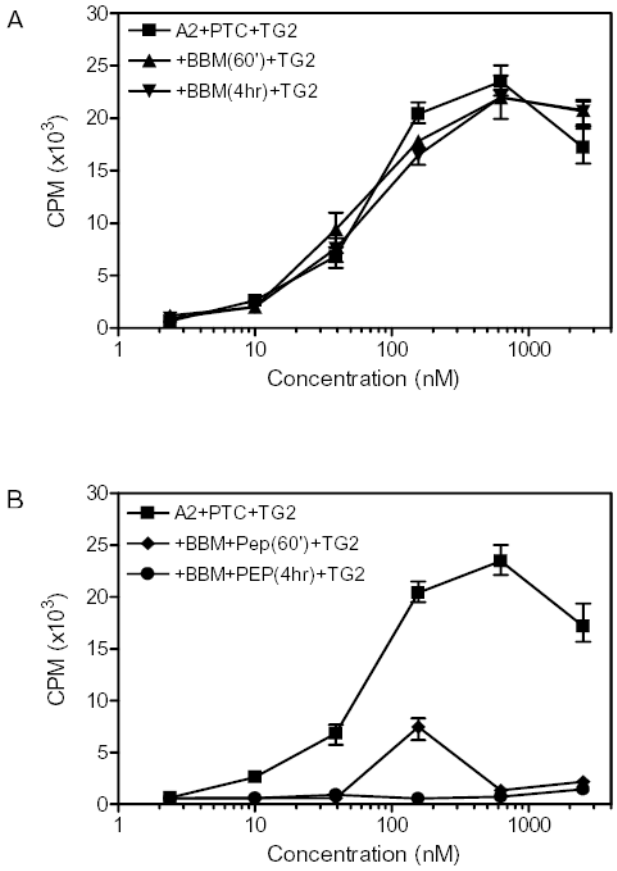

Figure 4.

Peptidase catalyzed breakdown of α-2 gliadin. The results of T cell line TCL 422.02.4.2 are shown and similar results were obtained with the T cell lines TCL432.1.4, TCL462.1 and TCL480.1. (A) Brush border membrane (BBM) treatment of α-2 gliadin did not result in efficient antigenic detoxification; (B) BBM and PEP treatment of α-2 gliadin efficiently abrogated T cell responses.

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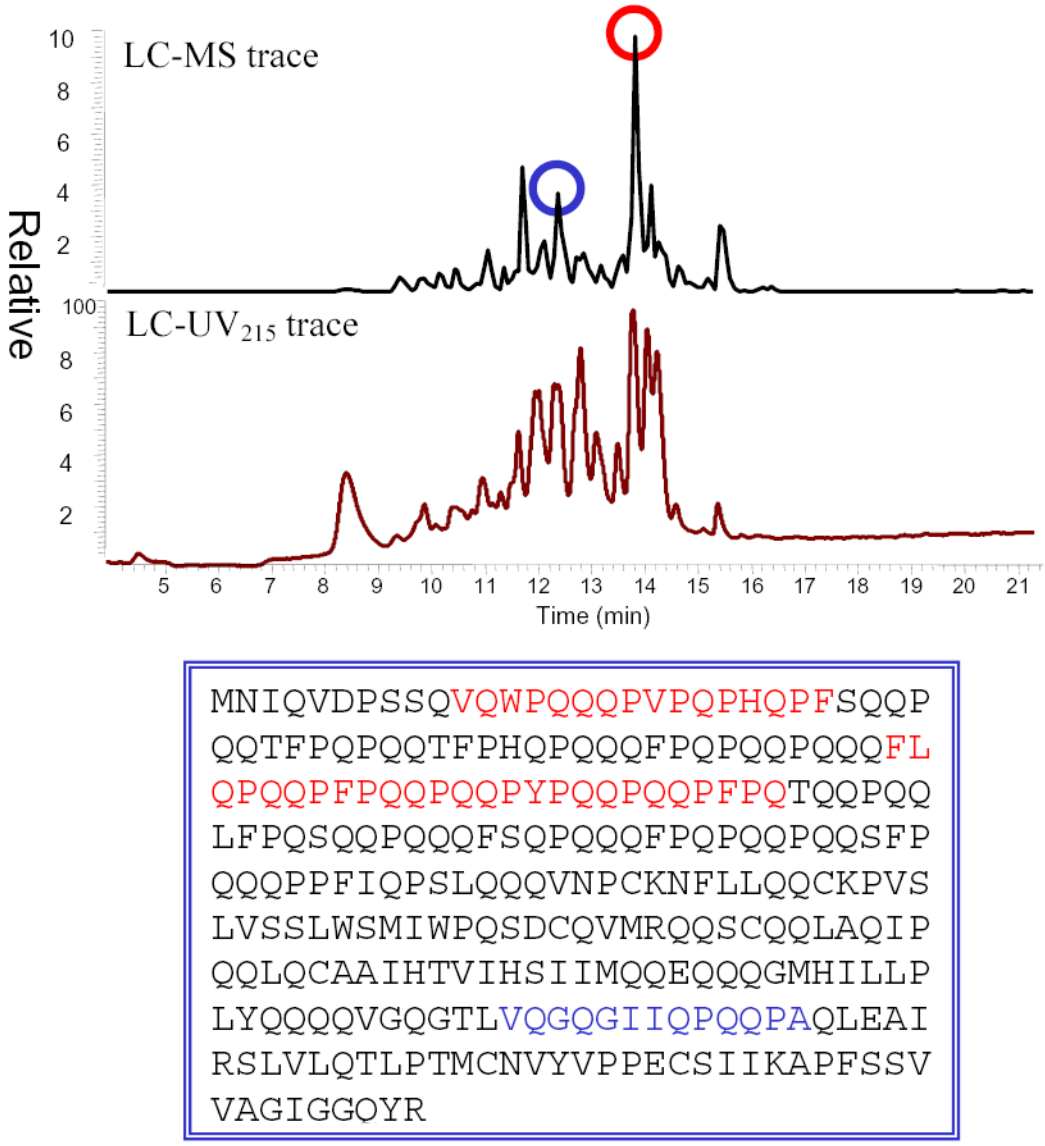

Figure 5.
LC-MS analysis of γ-5 gliadin proteolysis. The gliadin protein was treated with pepsin, trypsin, chymotrypsin, elastase, and carboxypeptidase. The blue- and red- colored peptides in the protein sequence are found present in the product mixture.

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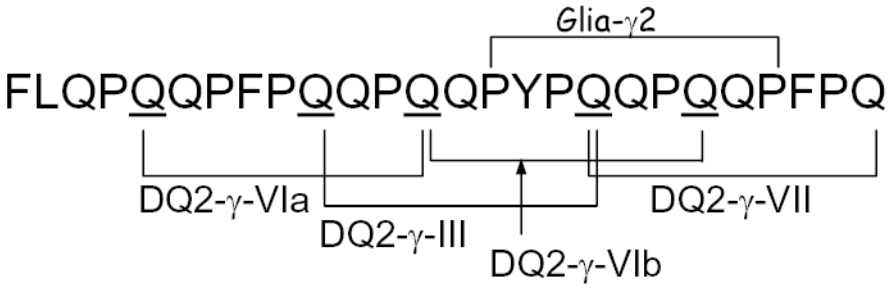

Figure 6.
Known γ-gliadin epitopes are clustered in the 26-mer.

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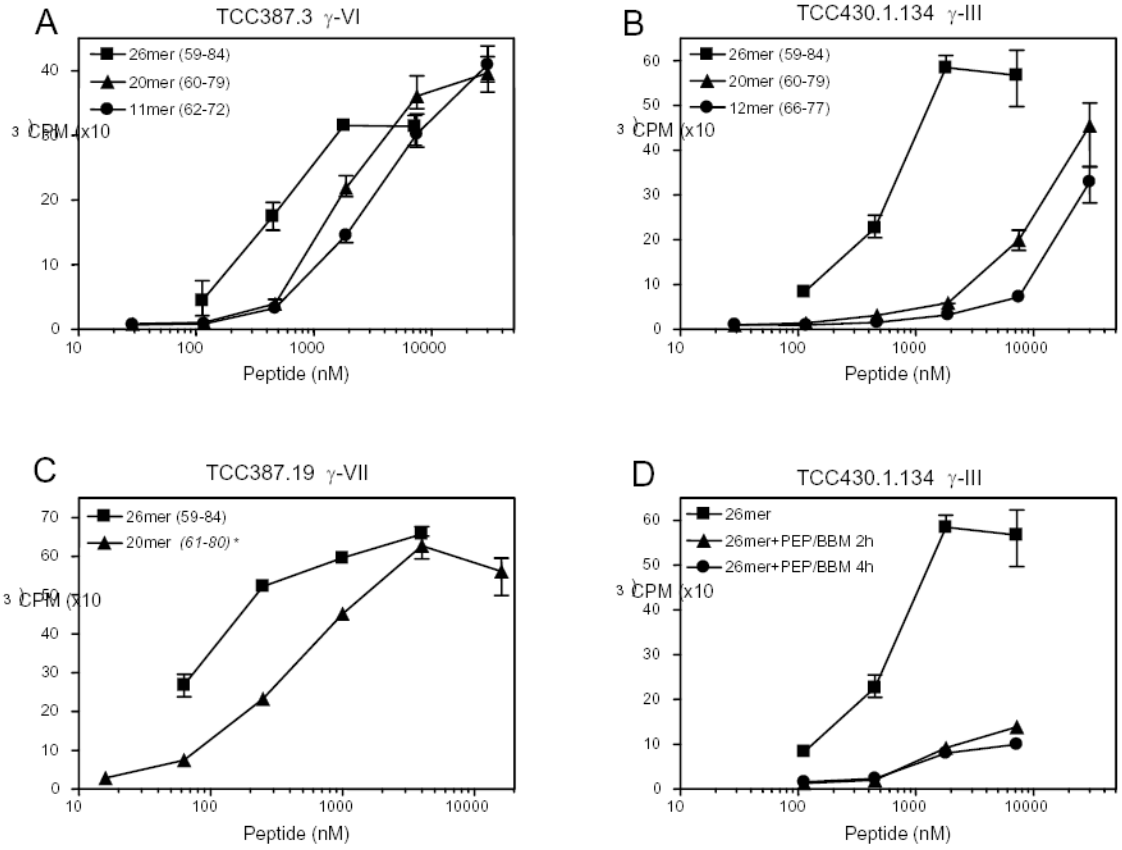

Figure 7.

T cell stimulation assay of the 26-mer fragment from γ-5 gliadin. T cell clones were tested with the 26-mer and control shorter peptides. (A) TCC387.3 (reactive to the DQ2-γ-VI epitope) was tested with TG2-treated 26-mer, TG2-treated LQPQQPFPQQPQQPYPQQPQ (“20mer”);, and the reference mono-epitope peptide PEQPFPEQPEQ (“11mer”), (B) TCC430.1.134 (reactive to the DQ2-γ-III epitope) was tested with TG2-treated 26-mer, TG2-treated LQPQQPFPQQPQQPYPQQPQ (“20mer”), and the reference mono-epitope peptide FPEQPEQPYPEQ (“12mer”); (C) TCC387.19 (reactive to DQ2-γ-VII epitope) was tested with the TG2-treated 26-mer and TG2-treated QFPQTQQPQQPFPQPQQTFP (“20mer”); (D) TCC430.1.134 (reactive to DQ2-γ-III) was stimulated with TG2-treated 26-mer, with and without rat intestinal brush border membrane (BBM) and prolyl endopeptidase (PEP) incubation. Numbers in the brackets refer to the positioning of the peptides within the γ-5 gliadin. * This peptide is derived from the 1507333A γ-gliadin (M36999) and identical to sequence 61–80.

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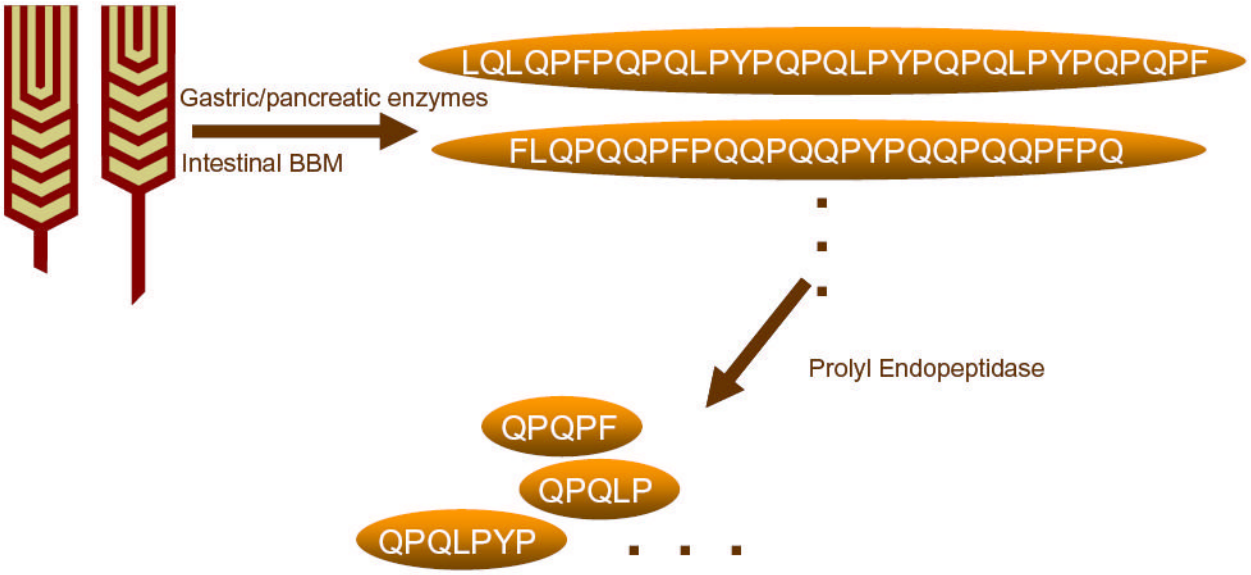

Graphic.

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Table 1

EC_{50} values of the α-2 gliadin peptide mixture, the 33mer and a control peptide for the epitope FPQPQLPYPQP.

	α -2 gliadin	**33-mer**	**Control peptide**
EC_{50} (nM)	180 ± 50	175 ± 40	1000

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Table S1 (A)

Known prolamin epitopes recognized by intestinal T cells of Celiac Sprue patients.

T cell epitope	Source	Native sequence of 9-mer core region of epitope[4]		Ref.
α–I	α-gliadin, recombinant[1]	PFPQPQLPY	Crystal[5]	Arentz-Hansen, 2000
α–III	α -gliadin, recombinant[1]	PYPQPQLPY	TCC[6]	Arentz-Hansen, 2002
α–II	α -gliadin, recombinant[1]	PQPQLPYPQ	DQ-TCC[7]	Arentz-Hansen, 2000
Glia-α 20	α–gliadin, peptide[2]	FRPQQPYPQ	TCC[6]	Vader 2002,Vader, 2003
γ–I	γ–gliadin, natural[3]	PQQSFPQQQ	DQ-TCC[7]	Sjöström, 1998
γ–II (Glia-γ 30)	γ–gliadin, natural[3]	IQPQQPAQL	DQ-TCC[7]	Vader 2002, Vader, 2003; Qiao, 2005
γ–III	γ–gliadin, recombinant[1]	QQPQQPYPQ	DQ-TCC[7]	Arentz-Hansen, 2002; Qiao, 2005
γ–IV	γ–gliadin, recombinant[1]	SQPQQQFPQ	DQ-TCC[7]	Arentz-Hansen, 2002; Qiao, 2005
γ–VIa	γ–gliadin, peptide[2]	QQPFPQQPQ	TCC[6]	Qiao, 2005
γ–VIb	γ–gliadin, peptide[2]	QQPYPQQPQ	TCC[6]	Qiao, 2005
γ–VIIa	γ–gliadin, peptide[2]	PQPQQQFPQ	TCC[6]	Qiao, 2005
γ–VIIb	γ–gliadin, peptide[2]	QQPQQPFPQ	TCC[6]	Qiao, 2005
Glia-γ 2	γ–gliadin, unknown	PYPQQPQQP		Vader, 2003
Glu-5 (Var1)	Not defined, natural[3]	QIPQQPQQF	TCC[6]	Vader 2002,Vader, 2003
Glu-5 (Var2)	Not defined, natural[3]	QLPQQPQQF	TCC[6]	Vader 2002,Vader, 2003
Glt-17 (Var1)	LMW-glutenin, peptide[2]	PFSQQQQPV	TCC[6]	Vader 2002,Vader, 2003
Glt-17 (Var2)	LMW-glutenin, peptide[2]	PFSQQQQPI	TCC[6]	Vader 2002,Vader, 2003
Hor-α 2/Sec-α 2	Hordein/Secalin[2]	PQPQQPFPQ	TCL[8]	Vader, 2003
Hor-α 9/Sec-α 9	Hordein/Secalin[2]	PFPQPQQPF	TCL[8]	Vader, 2003
Avenin-I (Av-α 9A)	Avenin,[23]	PYPEQQEPF	TCC[6]	Vader, 2003, Arentz-Hansen, 2004
Avenin-II (Av-α 9B)	Avenin[2]	PYPEQQQPF	TCL[8]	Vader, 2003, Arentz- Hansen, 2004

[1] Identified from fragments of recombinant gliadin protein digests.

[2] Identified from panels of synthetic peptides.

[3] Identified from fragments of natural gliadin or gluten protein digests.

[4] Core 9-mer region interacting with DQ2 is shown. T cells usually require additional flanking residues for recognition. Glutamine residues deamidated by tissue transglutaminase are underlined.

[5] Defined by the crystal structure of the peptide DQ2 complex.

[6] Register defined from minimal fragment recognized by specific T cell clones and binding analysis of lysine substituted analogs.

[7] Register defined from minimal fragment recognized by specific T cell clones and compliance with the DQ2 binding motif.

[8] Register defined from recognition by T cell lines and homology with other well characterized epitopes.

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Table S1 (B)

Computational predictions of physiological DQ2- antigenic peptides from gluten proteins in wheat, barley, and rye. These peptide sequences are from pancreatic proteolysis of chymotrypsin, trypsin, elastase and carboxypeptidases. The known DQ-2 restricted T cell epitopes are bolded in the sequences. The α-2 and γ-5 gliadin peptides are colored red. Each peptide's residue positions from the original protein sequence obtained from NCBI Protein database are indicated. The accession number of each protein is indicated in their names.

Name	Position	Peptide Fragments with Chymotrypsin, Trypsin, Elastase, Carboxypeptidases Cleavage Predictions and Known T-Cell Epitopes	# Epitopes
> CAB76964_Alpha-2 Gliadin	60 – 89	QPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	>3
> CAC94871_Gamma-5 Gliadin	28 – 56	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	61 – 89	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
> CAB76961_Alpha-8 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76958_Alpha-4 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76962_Alpha Gliadin-10	none		
> CAB76956_Alpha-7 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76955_Alpha-9 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76957_Alpha-1 Gliadin	35 – 53	QQEQFPPQQPYPHQQPFPS	
	54 – 95	QQPYPQPQPFPPQLPYPQTQPFPPQQPYPQPQPQYPQPQQPI	
> CAB76954_Alpha-6 Gliadin	54 – 95	QQPYPQPQPFPPQLPYPQTQPFPPQQPYPQPQPQYPQPQQPI	
> CAB76960_Alpha-3 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76959_Alpha-5 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> AAA17741_Alpha Gliadin	53 – 71	QQQPFPPQQPYPQPQPFPS	
> CAA10257_Alpha Gliadin	53 – 71	QQQPFPPQQPYPQPQPFPS	
> BAA12318_Alpha Gliadin	33 – 51	QQQPFPPQQPYPQPQPFPS	
> T06500_Alpha Gliadin A-IV Precursor	79 – 101	QPFPQPQLPYPQPQLPYPQPQPF	>3
	53 – 71	QQQPFPPQQPYPQPQPFPS	
> AAA96276_Alpha Gliadin	53 – 70	QQQPFPPQQPYPQPQFPS	
> D22364_alpha-gliadin precursor (clone A735)	53 – 71	QQQPFPPQQPYPQPQPFPS	
	79 – 106	QPFPQPQPFPPQLPYPQPQLPYPQPQPF	2
> P04727_Alpha-gliadin clone PW8142 precursor	50 – 68	QQQQFPPQQPYPQPQPFPS	
> P04725_Alpha-gliadin A-V precursor	53 – 71	QQQQFPPQQPYPQPQPFPS	
	79 – 98	QPFPQPQPFPPQLPYPQPQS	
> AAA96522_Alpha Gliadin	50 – 68	QQQQFPPQQPYPQPQPFPS	
> AAA96524_Alpha Gliadin	none		
> AAA96523_Alpha Gliadin	53 – 71	QQQPFPPQQPYPQPQPFPS	
> AAQ63862_Gamma Gliadin	28 – 51	QQPQQIFPQPQQTFPHQPQQPFPQ	
	53 – 73	QQPQQPFPQPQQQFPQPQQPQ	2
> AAQ63865_Gamma Gliadin	28 – 56	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAQ63864_Gamma Gliadin	28 – 61	QQPQQIFPQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAF42989_Gamma Gliadin	26 – 44	QQPQQTFPQPQQTFPHQPQ	
	59 – 120	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPL	>3
	123 – 143	QQPQQPFPQPQQQLPQPQQPQ	1
> AAK84779_Gamma Gliadin	45 – 63	QQPQQTFPQPQQTFPHQPQ	
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	142 – 162	QQPQQPFPQPQQQLPQPQQPQ	1
> CAB75404_Gamma-1 Gliadin	28 – 46	QQPQQTFPQPQQTFPHQPQ	
	60 – 89	IQPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	1
	91 – 111	QQPQQPFPQPQQQFPQPQQPQ	2
> CAC94868_Gamma-2 Gliadin	28 – 56	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	61 – 89	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> AAK84773_Gamma Gliadin	30 – 78	QWPQQQQPFPQPQQPQQPFPQPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3

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Name	Position	Peptide Fragments with Chymotrypsin, Trypsin, Elastase, Carboxypeptidases Cleavage Predictions and Known T-Cell Epitopes	# Epitopes
	80 – 103	QQPQQPLPQPQQPQQPFPQQQQPL	1
> AAK84780_Gamma Gliadin	1 – 38	QPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	2
	40 – 60	QQPQQPFPQPQQQFPQPQQPQ	2
> M36999_Gamma Gliadin	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	2
> AAQ63868_Hordein (leaf)	none		
> S07975_hordein B3 (clone pB7)	23 – 47	QPQQPQPFPQQPIPQQPQPYPQQPQ	
> CAA60681_hordein B1	20 – 51	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQ	
> 1103203A_hordein	23 – 47	QPQQPQPFPQQPIPQQPQPYPQQPQ	
> P80198_Gamma-hordein 3	36 – 82	QQEPEQPYPQQQPLPQQQPFPQQPQLPHQHQFPQQLPQQQFPQQMPL	1
> BAB78747_Glutenin	41 – 61	HHHQQQPIQQQPHQFPQQQPC	
> BAB78749_Glutenin	none		
> AAS66085_glutenin pGM107	none		
> AAV92073_Glutenin (in press)	none		
> AAV92049_Glutenin (in press)	none		
> AAV92036_Glutenin (in press)	none		
> AAV91997_Glutenin	150 – 168	QQPPFPQQHQQFPQQQIPV	
> AAS10189_Glutenin	none		
> S18236_secalin clone pSec2B	76 – 100	PIQPQQPFPQQPQQPFPQPQQQLPL	2
	101 – 118	QPQQPFPQPQQPIPQQPQ	
	121 – 150	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPL	
	228 – 255	QQPQRPQQPFPQQPQQIIPQQPQQPFPL	1
> S18235_secalin clone pSec1B	63 – 100	QPQQPFPQPQQPTPIQPQQPFPQRPQQPFPQPQQQLPL	2
	121 – 150	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPL	1
	228 – 255	QQPQRPQQPFPQQPQQIIPQQPQQPFPL	
> CAA26449_secalin unnamed	5 – 21	FPLPQQPFPQPQQPFPQ	
> AAB58403_secalin omega	63 – 91	QPQQPFPQPQQPTPIQPQQPFPQQPQQPF	
	121 – 150	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPL	
	187 – 208	QPQQPFPQQPEQIIPQQPQQPS	
	228 – 255	QQPQRPQQPFPQQPQQIIPQQPQQPFPL	1
> AAG35598_secalin omega	68 – 119	PQPQQPYPQQPFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQQFPQQPQQPV	>3
	141 – 159	QQFPQQPQQPFPQQPQQPV	2
	165 – 185	QQPFPQTQQPQQPFPQPQQPQ	1
	138 – 160	QQPQQPFPQPQQPQQPFPQPQPQ	2
> AAV86084_secalinGlutenin	152 – 170	QQPPFPQQHQQFPQQQIPV	
> AAV86085_secalinGlutenin	77 – 95	QQPPFPQQHQQFPQQQIPV	
> AAT74547_omegaGliadin	39 – 56	HQQQPFPQQPYPQQPYPS	
	91 – 116	QPQQPFPQQPQQPQQPFPQPQQPFPW	3
	132 – 154	QPQQPFPQQPQQPFPQPQLPFPQ	2
	271 – 297	FPQQPQQPFPPTTTKPFPQQPQQPFPL	
	234 – 252	QPQQPFPQQPQQPFPQQPQ	2
> AAT01617_omegaGliadin	none		
> AAG17702_omegaGliadin	82 – 116	TQPQQPTPIQPQQPFPQQPQQPQQPFPQPQQPFPW	3
	132 – 154	QPQQPFPQQPQQPFPQPQLPFPQ	2
	171 – 193	QPQQPFPQQPQQPFPQPQQPIPV	2
	235 – 253	QPQQPFPQQPQQPFPQQPQ	2
> S52390_D_hordein precursor	none		
> S18350_hordein	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	1
	107 – 140	QPQQPFPQPQEPIPQQPQQPFPQQPQQPFPQQPQ	2
	208 – 227	QPQPFPQQPQQPLPQPQQPF	
> T06211_C-hordein precursor	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	1
	137 – 160	QQPQQPFPQQPQQPFPQPQQPFPW	3
	217 – 235	QPQQPFPQQPQQPFPQQPQ	2
> S20519_B-hordein	20 – 48	QQQPFPQQPFPQQPQPYPQQPQPYPQQPF	1
	49 – 83	QPQQPFPQQTIPQQPQPYPQQPFPPQQEFPQQPPF	

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Name	Position	Peptide Fragments with Chymotrypsin, Trypsin, Elastase, Carboxypeptidases Cleavage Predictions and Known T-Cell Epitopes	# Epitopes
> S07365_B1-hordein	20 – 59	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQQPFPQQPV	
	60 – 83	PQQPQPYPQQPFPPQQPFPQQPPF	
> P17990_hordeinGamma 1	49 – 69	QQQFPQPQQPFPQQPQQPFPQ	3
	76 – 94	QQPQHQFPQPTQQFPQRPL	
> P06471_B3-hordein	23 – 47	QPQQPQPFPQQPIPQQPQPYPQQPQ	
> P06470_B1-hordein	20 – 59	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQQPFPQQPV	
	60 – 82	QQPQPYPQQPFPPQQPFPQQPPF	
> AAU06229_hordeinB	32 – 78	PWPQQPFPQQPYPQQPQPYPQQPQPYPQQPYPQQPQPYPQQPQPYPS 40	2
> AAU06228_hordeinB	32 – 52	PWPQQPFPQQPYPQQPQPYPQ	1
> AAU06227_hordeinB	32 – 62	PWPQQPFPQQPYPQQPQPYPQQPQPYPQQPY	1
	63 – 85	LPQPQPYPQQPQPYPQQPQPYPS	
> AAQ63849_hordeinGamma3	50 – 66	QQQQLPQQHPFPQQMPL	
> AAQ63848_hordeinGamma3	67 – 83	QQQQLPQQHPFPQQMPL	
> AAQ63847_hordeinGamma3	67 – 83	QQQQLPQQHPFPQQMPL	
> AAQ63842_hordeinGamma3	67 – 83	QQQQLPQQHPFPQQMPL	
> AAP31051_D-hordein	none		
> T04474_B1-hordein	20 – 51	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQ	
> BAA11642_D-hordein	none		
> AAB28161_C-hordein	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	1
	136 – 159	QQPQQPFPQQPQQPFPQPQQPFPW	3
	216 – 234	QPQQPFPQQPQQPFPQQPQ	2
> 1604464A_gammaHordein	48 – 68	QQQFPQPQQPFPQQPQQPFPQ	3
	76 – 94	QQPQHQFPQPTQQFPQRPL	
> AAA92333_C-hordein	92 – 117	QPQPPQQPFPQPQQPNPQQPQQPFPR	
	124 – 146	PQQPQQPFPQQPQQPFPQPQQPF	3
	194 – 210	QQPQQTIPQQPQQPFPL	
	211 – 229	QPQQPFPQQPQQPLPQQPQ	1
	242 – 259	QPQQPFPQPQPFPQEQPQ	
> CAA59104_D-hordein	none		
> CAA26889_hordein unnamed	20 – 59	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQQPFPQQPV	
	60 – 83	PQQPQPYPQQPFPPQQPFPQQPPF	
> CAA42642_hordein unnamed	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	1
	107 – 140	QPQQPFPQPQEPIPQQPQQPFPQQPQQPFPQQPQ	2
	208 – 227	QPQPFPQQPQQPLPQPQQPF	
> CAA48209_hordeinD	none		
> CAA51204_gamma3-hordein	33 – 79	QQEPEQPYPQQQPLPQQQPFPQQPQLPHQHQFPQQLPQQQFPQQMPL	1
> CAI35909_alpha-precursor	68 – 86	QQQPFPPQQPYPQPQPFPS	
> T06282_alpha-precursor	none		
> AAA96525_alpha	none		
> EEWTG_gamma-precursor	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
> P21292_gamma-precursor	60 – 126	HQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	2
> P06659_gamma-precursor	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	82 – 112	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> P04730_gamma	none		
> P08453_gamma-precursor	46 – 67	QQPQQTFPQPQQTFPHQPQQQV	
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	142 – 162	QQPQQPFPQPQQQLPQPQQPQ	1
> P04729_gamma-precursor	none		
> P08079_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> AAQ63863_gamma	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAQ63861_gamma	28 – 57	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQR	
	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAQ63860_gamma	28 – 46	QQPQQTFPQPQQTFPHQPQ	

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Name	Position	Peptide Fragments with Chymotrypsin, Trypsin, Elastase, Carboxypeptidases Cleavage Predictions and Known T-Cell Epitopes	# Epitopes
	60 – 93	QPQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	2
	96 – 116	QQPQQPFPQPQQQFPQPQQPQ	2
> AAQ63859_gamma	36 – 64	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> AAQ63858_gamma	28 – 46	QQPQQTFPQPQQTFPHQPQ	
	61 – 122	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	124 – 144	QQPQQPFPQPQQQLPQPQQPQ	1
> AAQ63857_gamma	28 – 46	QQPQQIFPQPQQTFPHQPQ	
	60 – 85	QPQQPFPQQPQQPFPQTQQPQQPFPQ	3
	88 – 108	QQPQQPFPQPQQQFPQPQQPQ	2
> AAQ63856_gamma	28 – 56	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> JS0402_gamma	46 – 64	QQPQQTFPQPQQTFPHQPQ	
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	142 – 162	QQPQQPFPQPQQQLPQPQQPQ	1
> JA0153_gamma	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	2
> CAA54925_gamma	40 – 107	HHQPQQTFPQPEQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	109 – 129	QQPQQPFPQPQQQFPQPQQPQ	2
> AAN32705_gamma	none		
> CAC94870_gamma-4	28 – 56	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	72 – 89	QPYPQQPQQPFPQTQQPQ	2
> CAC94869_gamma-3	28 – 56	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	60 – 79	LQPQQPFPQQPQQPYPQQPQ	3
> AAK84778_gamma	46 – 64	QQPQQTFPQPQQTFPHQPQ	
	78 – 112	IQPQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	2
	114 – 134	QQPQQPFPQPQQQFPQPQQPQ	2
> AAK84777_gamma	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	2
> AAK84776_gamma-13	46 – 91	QQPQHTFPQPQQTCPHQPQQQFPQPQQPQQPFPQPQQPQQPFPQPQ	2
	94 – 117	QLPFPQQPQQPLPQPQQPQQPFPQ	1
> AAK84775_gamma	1 – 28	PQQPQLPFPQQPQQPFPQPQQPQQPFPQ	2
	30 – 50	QQPQQPFPQPQQQFPQPQQPQ	2
> AAK84774_gamma	47 – 65	QQPQQIFPQPQQTFPHQPQ	
	68 – 159	FPQPQQTFPHQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQPQQPQLP FPQQPQQPFPQPQQPQQPFPQ	>3
	161 – 184	QQPQQPLPQPQQPQQPFPQQQQPL	1
> AAK84772_gamma	47 – 65	QQPQQIFPQPQQTFPHQPQ	
	68 – 159	FPQPQQTFPHQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQPQQPQLP FPQQPQQPFPQPQQPQQPFPQ	>3
	161 – 184	QQPQQPLPQPQQPQQPFPQQQQPL	1
> AAD30440_gamma	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10619_gamma	20 – 37	QTFPHQPQQQFPQPQQPQ	
	48 – 70	HQQPQQPYPQQPQQPFPQTQQPQ	>3
	77 – 103	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC11097_gamma	29 – 76	FPQPQQPQQQFPQPQQPQQPFPQPQQPQLPFPQQPFPQPQQPQQPFPQ	2
	78 – 98	QQPQQPFPQPQQQFPQPQQPQ	2
> CAC11096_gamma	29 – 76	FPQPQQPQQQFPQPQQPQQPFPQPQQPQLPFPQQPFPQPQQPQQPFPQ	2
	78 – 98	QQPQQPFPQPQQQFPQPQQPQ	2
> CAC11099_gamma	17 – 45	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	50 – 78	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11098_gamma	14 – 42	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	47 – 75	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11089_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3

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> CAC11088_gamma	15 – 43	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	48 – 76	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11087_gamma	25 – 53	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	58 – 86	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11080_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11079_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11078_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11065_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11064_gamma	27 – 55	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	60 – 88	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11057_gamma	23 – 51	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	56 – 84	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11056_gamma	17 – 45	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	50 – 78	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11055_gamma	19 – 47	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	52 – 80	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10624_gamma	13 – 35	PQPQQTFPHQQQQQFPQPQQPQ	
	40 – 68	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	75 – 104	QQPQQPYPQQPQQPYPQQPQQPFPQTQQPQ	>3
	111 – 137	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10623_gamma	13 – 34	PQPQQTFPHQPQQQFPQPQQPQ	
	39 – 67	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	74 – 103	QQPQQPYPQQPQQPYPQQPQQPFPQTQQPQ	>3
	110 – 136	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10622_gamma	8 – 36	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	41 – 69	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	76 – 102	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10620_gamma	20 – 37	QTFPHQPQQQFPQPQQPQ	
	48 – 70	HQQPQQPYPQQPQQPFPQTQQPQ	>3
	77 – 103	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10633_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	42 – 70	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10632_gamma	42 – 70	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10631_gamma	7 – 35	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	40 – 68	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10630_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	42 – 65	QPQQPQQPYPQEPQQPFPQTQQPQ	1
	77 – 97	QQPQQQFPQPQQQFPQPQQPQ	1
> CAC10629_gamma	42 – 70	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10628_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	42 – 65	QPQQPQQPYPQEPQQPFPQTQQPQ	1
	77 – 97	QQPQQQFPQPQQQFPQPQQPQ	1
> CAC10627_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	42 – 65	QPQQPQQPYPQEPQQPFPQTQQPQ	1
	72 – 92	QQPQQQFPQPQQQFPQPQQPQ	1
> CAC10626_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	79 – 107	QPQQPFPQQQQQPYPQQPQQPFPQTQQPQ	3
> CAC10625_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	
	79 – 107	QPQQPFPQQQQQPYPQQPQQPFPQTQQPQ	3
> CAC10617_gamma	30 – 60	QKPQQTFPQPQQTFPFPHQPQQQFPQPQQPQ	
	65 – 88	QPQQPQQPYPQQPQQTFPQTQQPQ	2

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Name	Position	Peptide Fragments with Chymotrypsin, Trypsin, Elastase, Carboxypeptidases Cleavage Predictions and Known T-Cell Epitopes	# Epitopes
> CAC10616_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQIPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	98 – 124	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10594_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQIPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	98 – 124	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10593_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQIPQPQQPQ	
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	98 – 124	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> AAD30556_gamma	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> AAF42989_gamma	27 – 45	QQPQQTFPQPQQTFPHQPQ	
	60 – 121	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	123 – 143	QQPQQPFPQPQQQLPQPQQPQ	1
> BAA11251_gamma-precursor	27 – 45	QQPQQTFPQPQQTFPHQPQ	
	59 – 91	PQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	2
	95 – 115	QQPQQPFPQPQQQFPQPQQPQ	2
> 1507333A_gamma	59 – 126	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	128 – 148	QQPQQPFPQPQQQFPQPQQPQ	2
> 1209306A_gamma	none		
> CAA37491_gamma	60 – 126	HQPQQTFPQPEQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	2
> AAA34289_gamma	46 – 64	QQPQQTFPQPQQTFPHQPQ	
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
> AAA34288_gamma	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	82 – 112	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAA34287_gamma	none		
> AAA34286_gamma	none		
> AAA34285_gamma-precursor	none		
> AAA34274_gamma-precursor	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	
	82 – 112	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAA34272_gamma-precursor	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	2

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Table 2

Predicted novel HLA-DQ2 restricted T cell epitopes. The native 9-mer sequences of the core regions are displayed. Typically, the entire T cell epitopes span 10–14 residues and contain glutamate residues converted from glutamine by tissue transglutaminase mediated deamidation.

Epitopes	Mainly found in	Epitopes	Mainly found in
QYPQPQQPI	alpha gliadin	PQHQFPQPT	hordein
IFPQPQQTF	gamma gliadin	PQPFPQQPI	hordein
PFPQPQQPQ	gamma gliadin	PQPFPQQPQ	hordein
PFPQPQQQF	gamma gliadin	PQPQEPIPQ	hordein
PFPQPQQQL	gamma gliadin	PQPQQPNPQ	hordein
PFPQPQQTF	gamma gliadin	PQPTQQFPQ	hordein
PFPQQPQQQ	gamma gliadin	PQPYPQQPF	hordein
PFPQQPQQT	gamma gliadin	PQPYPQQPQ	hordein
PFPQQQQPL	gamma gliadin	PQPYPQQPY	hordein
PFPQTQQPQ	gamma gliadin	PQQLPQQQF	hordein
PHQQQQQQF	gamma gliadin	PQQQFPQQM	hordein
PLPQPQQPQ	gamma gliadin	PRPQQPFPW	hordein
PQPQQPQQQ	gamma gliadin	PWPQQPFPQ	hordein
PQPQQQLPQ	gamma gliadin	PYPQQPQPY	hordein
PQQQFPQPQ	gamma gliadin	QEPIPQQPQ	hordein
PQQQFPQTQ	gamma gliadin	QFPQPQQPF	hordein
PQQQIPQPQ	gamma gliadin	QLPQQQFPQ	hordein
PQQQLPQPQ	gamma gliadin	QQPFPPQQE	hordein
PQQQQPFPQ	gamma gliadin	QQPFPQQPF	hordein
PQTQQPQQQ	gamma gliadin	QQPFPQQPI	hordein
QFPQPQQPQ	gamma gliadin	QQPFPQQPV	hordein
QFPQTQQPQ	gamma gliadin	QQPFPQQPY	hordein
QIPQPQQPQ	gamma gliadin	QQPFPQQTI	hordein
QLPFPQQPF	gamma gliadin	QQPLPQQPQ	hordein
QLPFPQQPQ	gamma gliadin	QQPNPQQPQ	hordein
QQPQLPFPQ	gamma gliadin	QQPQHQFPQ	hordein
QQPQQPFPL	gamma gliadin	QQPQLPHQH	hordein
QQPQQPLPQ	gamma gliadin	QQPQQPFPR	hordein
QQPQQQFPQ	gamma gliadin	QQPQQPLPR	hordein
QQQQFPQPQ	gamma gliadin	QQQQLPQQH	hordein
QQQQQQFPQ	gamma gliadin	QQTIPQQPQ	hordein
QQTFPQQPQ	gamma gliadin	PFPQQPYPQ	hordein, omega gliadin
QQTYPQRPQ	gamma gliadin	PQPQQPFPW	hordein, omega gliadin
QRPQQPFPQ	gamma gliadin	QQPIPQQPQ	hordein, secalin
QTQQPQQPF	gamma gliadin	IQPQQPFPQ	secalin
QWPQQQQPF	gamma gliadin	PFPQPQQPT	secalin
PIQQQPHQF	glutenin	PFPQQPQQI	secalin
PQQHQQFPQ	glutenin	PQPQQPIPQ	secalin
QFPQQQIPV	glutenin	PQPQQPTPI	secalin
QQPIQQQPH	glutenin	PQPQQQLPL	secalin
PFPQPQLPF	omega gliadin	PYPQQPFPQ	secalin
PFPQPQQPI	omega gliadin	PYPQQPQQQ	secalin
PQPQLPFPQ	omega gliadin	QFPQQPQQI	secalin
PQPQQPIPV	omega gliadin	QHQQFPQQQ	secalin
PYPQQPYPS	omega gliadin	QQIIPQQPQ	secalin
QQPYPQQPY	omega gliadin	QQPFPQRPQ	secalin
PFPQPQEPI	hordein	QQPQRPEQQ	secalin
PFPQPQQPN	hordein	QQPYPQQPF	secalin
PFPQQPFPQ	hordein	QQQFPQQPQ	secalin
PFPQQPIPQ	hordein	QQTQQPFPL	secalin
PFPQQPQPY	hordein	QRPEQQFPQ	secalin
PIPQQPQPY	hordein	PQPQQPYPQ	secalin, gamma gliadin
PLPRPQQPF	hordein		
PLPQPQQPF	hordein		

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Table S2

Computational predictions of physiological DQ2 antigenic peptides from gluten proteins in wheat, barley, and rye. Peptide sequences are from pancreatic proteolysis of individual gluten polypeptides catalyzed by chymotrypsin, trypsin, elastase and carboxypeptidases. The predicted TG2 deamidated sites are underlined. The DQ2 binding motifs and core region of 9 residues of known T cell epitopes (Table S1(A)) are in bold. For details, see text. Each peptide's residue positions from the original protein sequence obtained from NCBI Protein database are indicated. The accession number of each protein is indicated in their names.

NAME	POSITION	COMPUTATIONAL RESULTS	EP
> CAB76964_Alpha-2 Gliadin	60 – 89	QPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	>3
> CAC94871_Gamma-5 Gliadin	28 – 56	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	61 – 89	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
> CAB76961_Alpha-8 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76958_Alpha-4 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76962_Alpha Gliadin-10	none		
> CAB76956_Alpha-7 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76955_Alpha-9 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76957_Alpha-1 Gliadin	35 – 53	QQEQFPPQQPYPHQQPFPS	
	54 – 95	QQPYPQPQPFPPQLPYPQTQPFPPQQPYPQPQPQYPQPQQPI	3
> CAB76954_Alpha-6 Gliadin	54 – 95	QQPYPQPQPFPPQLPYPQTQPFPPQQPYPQPQPQYPQPQQPI	3
> CAB76960_Alpha-3 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> CAB76959_Alpha-5 Gliadin	34 – 52	QQQPFPPQQPYPQPQPFPS	
> AAA17741_Alpha Gliadin	53 – 71	QQQPFPPQQPYPQPQPFPS	
> CAA10257_Alpha Gliadin	53 – 71	QQQPFPPQQPYPQPQPFPS	
> BAA12318_Alpha Gliadin	33 – 51	QQQPFPPQQPYPQPQPFPS	
> T06500_Alpha Gliadin A-IV Precursor	79 – 101	QPFPQPQLPYPQPQLPYPQPQPF	>3
	53 – 71	QQQPFPPQQPYPQPQPFPS	
> AAA96276_Alpha Gliadin	53 – 70	QQQPFPPQQPYPQPQFPS	
> D22364_alpha-gliadin precursor (clone A735)	53 – 71	QQQPFPPQQPYPQPQPFPS	
	79 – 106	QPFPQPQPFPPQLPYPQPQLPYPQPQPF	2
> P04727_Alpha-gliadin clone PW8142 precursor	50 – 68	QQQQFPPQQPYPQPQPFPS	
> P04725_Alpha-gliadin A-V precursor	53 – 71	QQQQFPPQQPYPQPQPFPS	
	79 – 98	QPFPQPQPFPPQLPYPQPQS	
> AAA96522_Alpha Gliadin	50 – 68	QQQQFPPQQPYPQPQPFPS	
> AAA96524_Alpha Gliadin	none		
> AAA96523_Alpha Gliadin	53 – 71	QQQPFPPQQPYPQPQPFPS	
> AAQ63862_Gamma Gliadin	28 – 51	QQPQQIFPQPQQTFPHQPQQPFPQ	2
	53 – 73	QQPQQPFPQPQQQFPQPQQPQ	>3
> AAQ63865_Gamma Gliadin	28 – 56	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAQ63864_Gamma Gliadin	28 – 61	QQPQQIFPQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAF42989_Gamma Gliadin	26 – 44	QQPQQTFPQPQQTFPHQPQ	1
	59 – 120	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPL	>3
	123 – 143	QQPQQPFPQPQQQLPQPQQPQ	>3
> AAK84779_Gamma Gliadin	45 – 63	QQPQQTFPQPQQTFPHQPQ	1
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	142 – 162	QQPQQPFPQPQQQLPQPQQPQ	>3
> CAB75404_Gamma-1 Gliadin	28 – 46	QQPQQTFPQPQQTFPHQPQ	1
	60 – 89	IQPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	>3
	91 – 111	QQPQQPFPQPQQQFPQPQQPQ	>3
> CAC94868_Gamma-2 Gliadin	28 – 56	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	61 – 89	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> AAK84773_Gamma Gliadin	30 – 78	QWPQQQQPFPQPQQPQQPFPQPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	80 – 103	QQPQQPLPQPQQPQQPFPQQQQPL	>3
> AAK84780_Gamma Gliadin	1 – 38	QPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	40 – 60	QQPQQPFPQPQQQFPQPQQPQ	>3
> M36999_Gamma Gliadin	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	>3
> AAQ63868_Hordein (leaf)	none		
> S07975_hordein B3 (clone pB7)	23 – 47	QPQQPQPFPQQPIPQQPQPYPQQPQ	>3
> CAA60681_hordein B1	20 – 51	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQ	>3
>1103203A_hordein	23 – 47	QPQQPQPFPQQPIPQQPQPYPQQPQ	>3
> P80198_Gamma-hordein 3	36 – 82	QQEPEQPYPQQQPLPQQQPFPQQPQLPHQHQFPQQLPQQQFPQQMPL	>3
> BAB78747_Glutenin	41 – 61	HHHQQQPIQQQPHQFPQQQPC	2
> BAB78749_Glutenin	none		
> AAS66085_glutenin pGM107	none		
> AAV92073_Glutenin (in press)	none		

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NAME	POSITION	COMPUTATIONAL RESULTS	EP
> AAV92049_Glutenin (in press)	none		
> AAV92036_Glutenin (in press)	none		
> AAV91997_Glutenin	150 – 168	QQPPFPQQHQQFPQQQIPV	3
> AAS10189_Glutenin	none		
> S18236_secalin clone pSec2B	76 – 100	PIQPQQPFPQQPQQPFPQPQQQLPL	>3
	101 – 118	QPQQPFPQPQQPIPQQPQ	3
	121 – 150	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPL	>3
	228 – 255	QQPQRPQQPFPQQPQQIIPQQPQQPFPL	>3
> S18235_secalin clone pSec1B	63 – 100	QPQQPFPQPQQPTPIQPQQPFPQRPQQPFPQPQQQLPL	>3
	121 – 150	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPL	>3
	228 – 255	QQPQRPQQPFPQQPQQIIPQQPQQPFPL	>3
> CAA26449_secalin unnamed	5 – 21	FPLPQQPFPQPQQPFPQ	>3
> AAB58403_secalin omega	63 – 91	QPQQPFPQPQQPTPIQPQQPFPQQPQQPF	>3
	121 – 150	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPL	>3
	187 – 208	QPQQPFPQQPEQIIPQQPQQPS	2
	228 – 255	QQPQRPQQPFPQQPQQIIPQQPQQPFPL	>3
> AAG35598_secalin omega	68 – 119	PQPQQPYPQQPFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQQFPQQPQQPV	>3
	141 – 159	QQFPQQPQQPFPQQPQQPV	2
	165 – 185	QQPFPQTQQPQQPFPQPQQPQ	>3
	138 – 160	QQPQQPFPQPQQPQQPFPQPQPQ	3
> AV86084_secalinGlutenin	152 – 170	QQPPFPQQHQQFPQQQIPV	3
> AV86085_secalinGlutenin	77 – 95	QQPPFPQQHQQFPQQQIPV	3
> AAT74547_omegaGliadin	39 – 56	HQQQPFPQQPYPQQPYPS	>4
	91 – 116	QPQQPFPQQPQQPQQPFPQPQQPFPW	>3
	132 – 154	QPQQPFPQQPQQPFPQPQLPFPQ	>3
	271 – 297	FPQQPQQPFPPTTTKPFPQQPQQPFPL	>3
	234 – 252	QPQQPFPQQPQQPFPQQPQ	>3
> AAT01617_omegaGliadin	none		
> AAG17702_omegaGliadin	82 – 116	TQPQQPTPIQPQQPFPQQPQQPQQPFPQPQQPFPW	>3
	132 – 154	QPQQPFPQQPQQPFPQPQLPFPQ	>3
	171 – 193	QPQQPFPQQPQQPFPQPQQPIPV	>3
	235 – 253	QPQQPFPQQPQQPFPQQPQ	>3
> S52390_D_hordein precursor	none		
> S18350_hordein	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	>3
	107 – 140	QPQQPFPQPQEPIPQQPQQPFPQQPQQPFPQQPQ	>3
	208 – 227	QPQPFPQQPQQPLPQPQQPF	>3
> T06211_C-hordein precursor	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	>3
	137 – 160	QQPQQPFPQQPQQPFPQPQQPFPW	>3
	217 – 235	QPQQPFPQQPQQPFPQQPQ	>3
> S20519_B-hordein	20 – 48	QQQPFPQQPFPQQPQPYPQQPQPYPQQPF	>3
	49 – 83	QPQQPFPQQTIPQQPQPYPQQPFPPQQEFPQQPPF	>3
> S07365_B1-hordein	20 – 59	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQQPFPQQPV	>3
	60 – 83	PQQPQPYPQQPFPPQQPFPQQPPF	1
> P17990_hordeinGamma 1	49 – 69	QQQFPQPQQPFPQQPQQPFPQ	>3
	76 – 94	QQPQHQFPQPTQQFPQRPL	3
>P06471_B3-hordein	23 – 47	QPQQPQPFPQQPIPQQPQPYPQQPQ	>3
>P06470_B1-hordein	20 – 59	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQQPFPQQPV	>3
	60 – 82	PQQPQPYPQQPFPPQQPFPQQPPF	1
>AAU06229_hordeinB	32 – 78	PWPQQPFPQQPYPQQPQPYPQQPQPYPQQPYPQQPQPYPQQPQPYPS	>3
>AAU06228_hordeinB	32 – 52	PWPQQPFPQQPYPQQPQPYPQ	>3
>AAU06227_hordeinB	32 – 62	PWPQQPFPQQPYPQQPQPYPQQPQPYPQQPY	>3
	63 – 85	LPQPQPYPQQPQPYPQQPQPYPS	>3
>AAQ63849_hordeinGamma3	50 – 66	QQQQLPQQHPFPQQMPL	1
>AAQ63848_hordeinGamma3	67 – 83	QQQQLPQQHPFPQQMPL	1
>AAQ63847_hordeinGamma3	67 – 83	QQQQLPQQHPFPQQMPL	1
>AAQ63842_hordeinGamma3	67 – 83	QQQQLPQQHPFPQQMPL	1
>AAP31051_D-hordein	none		
>T04474_B1-hordein	20 – 51	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQ	>3
>BAA11642_D-hordein	none		
>AAB28161_C-hordein	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	>3
	136 – 159	QQPQQPFPQQPQQPFPQPQQPFPW	>3
	216 – 234	QPQQPFPQQPQQPFPQQPQ	>3
>1604464A_gammaHordein	48 – 68	QQQFPQPQQPFPQQPQQPFPQ	>3
	76 – 94	QQPQHQFPQPTQQFPQRPL	3
>AAA92333_C-hordein	92 – 117	QPQPPQQPFPQPQQPNPQQPQQPFPR	>3
	124 – 146	PQQPQQPFPQQPQQPFPQPQQPF	>3
	194 – 210	QQPQQTIPQQPQQPFPL	2
	211 – 229	QPQQPFPQQPQQPLPQQPQ	3
	242 – 259	QPQQPFPQPQPFPQEQPQ	
>CAA59104_D-hordein	none		
>CAA26889_hordein unnamed	20 – 59	QQQPFPQQPIPQQPQPYPQQPQPYPQQPFPPQQPFPQQPV	>3
	60 – 83	PQQPQPYPQQPFPPQQPFPQQPPF	1

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NAME	POSITION	COMPUTATIONAL RESULTS	EP
>CAA42642_hordein unnamed	84 – 106	QPQQPFPQQPQQPLPRPQQPFPW	>3
	107 – 140	QPQQPFPQPQEPIPQQPQQPFPQQPQQPFPQQPQ	>3
	208 – 227	QPQPFPQQPQQPLPQPQQPF	>3
>CAA48209_hordeinD	none		
>CAA51204_gamma3-hordein	33 – 79	QQEPEQPYPQQQPLPQQQPFPQQPQLPHQHQFPQQLPQQQFPQQMPL	>3
>CAI35909_alpha-precursor	68 – 86	QQQPFPPQQPYPQPQPFPS	
>T06282_alpha-precursor	none		
>AAA96525_alpha	none		
>EEWTG_gamma-precursor	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
>P21292_gamma-precursor	60 – 126	HQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQQPFPQ	>3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	>3
>P06659_gamma-precursor	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	82 – 112	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
>P04730_gamma	none		
>P08453_gamma-precursor	46 – 67	QQPQQTFPQPQQTFPHQPQQQV	1
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	142 – 162	QQPQQPFPQPQQQLPQPQQPQ	>3
>P04729_gamma-precursor	none		
>P08079_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>AAQ63863_gamma	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
>AAQ63861_gamma	28 – 57	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
>AAQ63860_gamma	28 – 46	QQPQQTFPQPQQTFPHQPQ	1
	60 – 93	QPQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	>3
	96 – 116	QQPQQPFPQPQQQFPQPQQPQ	>3
>AAQ63859_gamma	36 – 64	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>AAQ63858_gamma	28 – 46	QQPQQTFPQPQQTFPHQPQ	1
	61 – 122	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	124 – 144	QQPQQPFPQPQQQLPQPQQPQ	>3
>AAQ63857_gamma	28 – 46	QQPQQIFPQPQQTFPHQPQ	2
	60 – 85	QPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	88 – 108	QQPQQPFPQPQQQFPQPQQPQ	>3
>AAQ63856_gamma	28 – 56	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	64 – 94	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
>JS0402_gamma	46 – 64	QQPQQTFPQPQQTFPHQPQ	1
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	142 – 162	QQPQQPFPQPQQQLPQPQQPQ	>3
>JA0153_gamma	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	>3
>CAA54925_gamma	40 – 107	HHQPQQTFPQPEQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	109 – 129	QQPQQPFPQPQQQFPQPQQPQ	>3
>AAN32705_gamma	none		
>CAC94870_gamma-4	28 – 56	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	72 – 89	QPYPQQPQQPFPQTQQPQ	3
>CAC94869_gamma-3	28 – 56	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	60 – 79	LQPQQPFPQQPQQPYPQQPQ	>3
>AAK84778_gamma	46 – 64	QQPQQTFPQPQQTFPHQPQ	1
	78 – 112	IQPQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	>3
	114 – 134	QQPQQPFPQPQQQFPQPQQPQ	>3
>AAK84777_gamma	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	>3
>AAK84776_gamma-13	46 – 91	QQPQHTFPQPQQTCPHQPQQQFPQPQQPQQPFPQPQQPQQPFPQPQ	>3
	94 – 117	QLPFPQQPQQPLPQPQQPQQPFPQ	>3
>AAK84775_gamma	1 – 28	PQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	30 – 50	QQPQQPFPQPQQQFPQPQQPQ	>3
>AAK84774_gamma	47 – 65	QQPQQIFPQPQQTFPHQPQ	2
		FPQPQQTFPHQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQPQQP	>3
		QLPFPQQPQQPFPQPQQPQQP	
	68 – 159	FPQ	
	161 – 184	QQPQQPLPQPQQPQQPFPQQQQPL	>3
>AAK84772_gamma	47 – 65	QQPQQIFPQPQQTFPHQPQ	2
		FPQPQQTFPHQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQQPQQQFPQPQQPQQPFPQPQQP	>3
		QLPFPQQPQQPFPQPQQPQQP	
	68 – 159	FPQ	
	161 – 184	QQPQQPLPQPQQPQQPFPQQQQPL	>3
>AAD30440_gamma	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC10619_gamma	20 – 37	QTFPHQPQQQFPQPQQPQ	2
	48 – 70	HQQPQQPYPQQPQQPFPQTQQPQ	>3
	77 – 103	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
>CAC11097_gamma	29 – 76	FPQPQQPQQQFPQPQQPQQPFPQPQQPQLPFPQQPFPQPQQPQQPFPQ	>3
	78 – 98	QQPQQPFPQPQQQFPQPQQPQ	>3
>CAC11096_gamma	29 – 76	FPQPQQPQQQFPQPQQPQQPFPQPQQPQLPFPQQPFPQPQQPQQPFPQ	>3

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NAME	POSITION	COMPUTATIONAL RESULTS	EP
	78 – 98	QQPQQPFPQPQQQFPQPQQPQ	>3
>CAC11099_gamma	17 – 45	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	50 – 78	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC11098_gamma	14 – 42	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	47 – 75	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC11089_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC11088_gamma	15 – 43	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	48 – 76	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC11087_gamma	25 – 53	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	58 – 86	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC11080_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
>CAC11079_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11078_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11065_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11064_gamma	27 – 55	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	60 – 88	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11057_gamma	23 – 51	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	56 – 84	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11056_gamma	17 – 45	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	50 – 78	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC11055_gamma	19 – 47	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	52 – 80	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10624_gamma	13 – 35	PQPQQTFPHQQQQQQFPQPQQPQ	>3
	40 – 68	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	75 – 104	QQPQQPYPQQPQQPYPQQPQQPFPQTQQPQ	>3
	111 – 137	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10623_gamma	13 – 34	PQPQQTFPHQPQQQFPQPQQPQ	2
	39 – 67	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	74 – 103	QQPQQPYPQQPQQPYPQQPQQPFPQTQQPQ	>3
	110 – 136	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10622_gamma	8 – 36	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	41 – 69	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	76 – 102	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10620_gamma	20 – 37	QTFPHQPQQQFPQPQQPQ	2
	48 – 70	HQQPQQPYPQQPQQPFPQTQQPQ	>3
	77 – 103	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10633_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	42 – 70	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10632_gamma	42 – 70	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10631_gamma	7 – 35	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	40 – 68	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10630_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	42 – 65	QPQQPQQPYPQEPQQPFPQTQQPQ	>3
	77 – 97	QQPQQQFPQPQQQFPQPQQPQ	>3
> CAC10629_gamma	42 – 70	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> CAC10628_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	42 – 65	QPQQPQQPYPQEPQQPFPQTQQPQ	>3
	77 – 97	QQPQQQFPQPQQQFPQPQQPQ	>3
> CAC10627_gamma	9 – 37	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	42 – 65	QPQQPQQPYPQEPQQPFPQTQQPQ	>3
	72 – 92	QQPQQQFPQPQQQFPQPQQPQ	>3
> CAC10626_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	79 – 107	QPQQPFPQQQQQPYPQQPQQPFPQTQQPQ	>3
> CAC10625_gamma	46 – 74	QQPQQTFPQPQQTFPHQPQQQFPQPQQPQ	3
	79 – 107	QPQQPFPQQQQQPYPQQPQQPFPQTQQPQ	>3
> CAC10617_gamma	30 – 60	QKPQQTFPQPQQTFPFPHQPQQQFPQPQQPQ	>3
	65 – 88	QPQQPQQPYPQQPQQTFPQTQQPQ	>3
> CAC10616_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQIPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	98 – 124	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10594_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQIPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	98 – 124	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> CAC10593_gamma	30 – 58	QQPQQTFPQPQQTFPHQPQQQIPQPQQPQ	3
	63 – 91	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
	98 – 124	QQPQQPYPQQPQQPFPQTQQPQQQFPQ	>3
> AAD30556_gamma	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	79 – 107	QPQQPFPQQPQQPYPQQPQQPFPQTQQPQ	>3
> AAF42989_gamma	27 – 45	QQPQQTFPQPQQTFPHQPQ	1

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NAME	POSITION	COMPUTATIONAL RESULTS	EP
	60 – 121	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
	123 – 143	QQPQQPFPQPQQQLPQPQQPQ	>3
> BAA11251_gamma-precursor	27 – 45	QQPQQTFPQPQQTFPHQPQ	1
	59 – 91	PQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQ	>3
	95 – 115	QQPQQPFPQPQQQFPQPQQPQ	>3
> 1507333A_gamma	59 – 126	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	128 – 148	QQPQQPFPQPQQQFPQPQQPQ	>3
> 1209306A_gamma	none		
> CAA37491_gamma	60 – 126	HQPQQTFPQPEQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	>3
> AAA34289_gamma	46 – 64	QQPQQTFPQPQQTFPHQPQ	1
	79 – 140	QPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQQPFPQ	>3
> AAA34288_gamma	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	82 – 112	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAA34287_gamma	none		
> AAA34286_gamma	none		
> AAA34285_gamma-precursor	none		
> AAA34274_gamma-precursor	46 – 74	QQPQQIFPQPQQTFPHQPQQQFPQPQQPQ	>3
	82 – 112	QPFPQQPQQPYPQQPQQPFPQTQQPQQPFPQ	>3
> AAA34272_gamma-precursor	60 – 127	HHQPQQTFPQPQQTYPHQPQQQFPQTQQPQQPFPQPQQTFPQQPQLPFPQQPQQPFPQPQQPQQPFPQ	>3
	129 – 149	QQPQQPFPQPQQQFPQPQQPQ	>3

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Table S3

Computational proteolysis analysis on avenins (from oats) and other dietary proteins including myoglobin, ovalalbumin, casein and lactoglobulin.

Name	Position	Chymotrypsin, Trypsin, Elastase, Carboxypeptidases Prediction	Length
>1411172A_avenin	13–22	QPYPEQQEPF	10
>S06455_avenin-precursor	61–68	DFPITWPW	8
>AAA32716_avenin	32–41	QPYPEQQEPF	10
>AAA32715_avenin	28–38	QPYPEQQQPI	10
>AAA32714_avenin	28–36	QPYPEQQPF	9
>AAA32713_avenin	61–68	DFPITWPW	8
>1502200A_avenin	61–68	DFPITWPW	8
>AAB32025_avenin	9–19	QPYPEQQQPI	10
>MYBO_bovine-myoglobin	none		0
>NP_990483_ovalbumin-chicken	89–97		0
>NP_851351_betaCasein-bovine	51–60		0
	160–170	HQPHQPLPPT	10
>NP_776719_kappaCasein-bovine	22–31	QEQNQEQPI	9
	138–147		0
>NP_776354_betaLactoglobulin-bovine	none		0