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(54) **Title**: WHEAT ANTIGENS AND PEPTIDES FOR DIAGNOSIS OF WHEAT INDUCED HYPERSENSITIVITY

(57) **Abstract**: The present invention relates to the field of different wheat hypersensitivities, particularly with antigens and peptides for discrimination of different forms of these diseases. The invention relates to the identification of novel wheat allergens and the use thereof in therapy and diagnosis of celiac disease, dermatitis herpetiformis, and IgE-mediated allergy. Furthermore, the present invention provides the use of known peptides and proteins in therapy and diagnosis. The invention also relates to methods for diagnosis and treatment of celiac disease, dermatitis herpetiformis,and IgE-mediated allergy.

Wheat antigens and peptides for diagnosis of wheat induced hypersensitivity

Field of the invention

The present invention relates to the field of different forms of wheat hypersensitivities, particularly with antigens and peptides for discrimination of different forms of these diseases.

Background

According to Gell and Coombs classification (which is a classification of immune mechanisms of tissue injury), four types of hypersensitivity exist: type I, immediate hypersensitivity reactions, mediated by interaction of IgE antibody and antigen and release of histamine and other mediators; type II, antibody-mediated hypersensitivity reactions, due to antibody-antigen interactions on cell surfaces; type III, immune complex, local or general inflammatory responses due to formation of circulating immune complexes and their deposition in tissues; and type IV cell-mediated hypersensitivity reactions, initiated by sensitized T lymphocytes either by release of lymphokines or by T-cell–mediated cytotoxicity.

Wheat (*Triticum aestivum*) can cause different forms of hypersensitivities. It can cause three distinct IgE-mediated allergies, wheat pollen allergy, Baker's Asthma and wheat food allergy. The wheat pollen allergy belongs to the group of grass pollen allergies. Baker's asthma is a respiratory allergy which is caused by wheat flour; it is an important occupational disease that often affects bakers, millers or confectioners. Wheat induced food allergy is very common and occurs after ingestion of wheat containing food, leading to diverse clinical manifestations including eczema, urticaria, gastrointestinal symptoms, conjunctivitis and many other symptoms (1). Additional to the IgE mediated wheat allergy there exists a hypersensitivity to wheat, the celiac disease, which is characterized by IgA antibodies and T-cell reactivity against wheat proteins and development of auto reactive IgA antibodies against several intestinal proteins (2, 3). It is an inflammatory hypersensitivity to wheat which causes villous atrophy in the small intestine and leads to symptoms like chronic diarrhoea or constipation, malnutrition, anaemia, fatigue, growth retardation and migraine (4).

Since wheat (*Triticum aestivum*) and wheat products are a major element in nutrition, avoidance of wheat products is currently the only therapy for patients suffering from wheat induced hypersensitivities. Antigen specific approaches would require a detailed knowledge and availability of the hypersensitivity causing protein. To date there is a lack of defined proteins and peptides to be used as diagnostic tool to discriminate between the different forms

of hypersensitivities to wheat. Therefore, precise diagnosis still relies on specific inhalation challenge in case of respiratory allergy to wheat flour, double-blind placebo-controlled food challenge (DBPCFC) in case of suspected food allergy, and diet followed by rechallenge and/or intestinal biopsy for celiac disease. Constantin et al (5) identified specific recombinant wheat flour allergens which are recognized by Baker's asthma patients, but not by wheat food allergic patients. They showed the usefulness of micro-arrayed recombinant allergens in contrast to natural extracts. However, the panel of allergens was incomplete and there is therefore a need to identify more antigens and peptides that are involved in wheat food allergy or celiac disease and to establish methods and diagnostic tests to differentiate patients suffering from the different forms of wheat hypersensitivities. In addition, there is a need to use such wheat antigens and peptides for treatment of wheat mediated hypersensitivities.

Summary of the invention

The object of the present invention is to overcome the problems related to the prior art as described above. The present invention provides polypeptides and nucleic acid sequences, which are related to different forms of wheat hypersensitivities, and which may be used for therapy and diagnosis of different forms of wheat hypersensitivities.

According to one aspect of the present invention, an isolated polypeptide is provided that comprises the amino acid sequence according to any one of SEQ ID NO: 26-50, 62-86, and 89-110.

In one embodiment, the polypeptide is characterised in that it is isolated from wheat or recombinantly produced. Alternatively, the polypeptide may be produced by chemical synthesis.

A further embodiment of the present invention provides an isolated nucleic acid molecule encoding the polypeptide as described above. For example, the nucleic acid may have the nucleotide sequence according to any one of SEQ ID NO: 1-25.

According to another aspect of the present invention, the polypeptide as described above, or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, is for use in therapy or diagnosis.

More particulary, the polypeptide or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, is for use in therapy or diagnosis of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy. Dermatitis herpetiformis is a skin disease, which is associated with celiac disease.

The present invention further provides an isolated polypeptide comprising the amino acid sequence according to any one of SEQ ID NO: 51-61, 87 and 88, or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, for use in therapy or diagnosis.

More specifically, such an isolated polypeptide comprising the amino acid sequence according to any one of SEQ ID NO: 51-61, 87 and 88 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, is for use in therapy or diagnosis of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy. Further, according to one embodiment, the use in therapy comprises tolerance induction or prophylactic treatment.

According to yet another aspect, the present invention provides a pharmaceutical composition comprising a polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110, or a hypoallergenic form of said polypeptide that is modified to abrogate or attenuate its T cell-, IgA- or IgE-binding response, and optionally pharmaceutically acceptable excipients, carriers, buffers and/or diluents.

In one embodiment, the hypoallergenic form of the polypeptide comprised by the pharmaceutical composition is modified by fragmentation, truncation or tandemerization of the molecule, deletion of internal segments, domain rearrangment, substitution of amino acid residues, disruption of disulfide bridges.

A further aspect of the present invention provides a method for producing an allergen composition comprising the step of adding a polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110, or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, to a composition comprising an allergen extract and/or at least one purified allergen component.

Further provided is an allergen composition obtainable with the above-described method.

The present invention also provides a method for *in vitro* diagnosis of celiac disease comprising

- contacting a body fluid or tissue sample from a mammal suspected of having celiac disease with at least one polypeptide having the amino acid sequence according to any one of SEQ ID NO: 62-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide; and
- measuring activated T cells in the sample, such as by use of a lymphocyte proliferation assay, a FACS analysis of the cell activation, or by measuring cytokine release;

wherein the presence of activated T cells is indicative of celiac disease.

T-cell number and function may for example be monitored by assays that detect T cells by an activity such as cytokine production, proliferation, or cytotoxicity (9, 10).

It has previously been described that certain T cells from celiac mucosa produce cytokines with Thl or Th0 profile, particularly interferon-gamma (IFN-γ). This cytokine, particularly in combination with TNF-alpha, might be involved in several pathological features of the celiac lesion (10).

In a similar scenario, it has been shown that IFN-γ derived from T cells facilitates allergen penetration through respiratory epithelium cells and thereby augment allergic inflammation (11). Further, it has been shown that IFN-γ-containing culture supernatants from peripheral blood mononuclear cells stimulated by a certain autoantigen caused disintegration of respiratory epithelial cell layers and apoptosis of skin keratinocytes. This damage could be inhibited with a neutralizing anti-IFN-γ antibody (12).

Consequently, the present invention also provides a method for *in vitro* diagnosis of celiac disease comprising

- contacting leukocytes from a mammal suspected of having celiac disease with at least one polypeptide having the amino acid sequence according to any one of SEQ ID NO: 62-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, in a medium;
- contacting a cell sample from said mammal with the medium; and
- measuring the presence of interferon-gamma or other cell-damaging substance(s) in the cell sample;

wherein the presence of interferon-gamma or other cell-damaging substance(s) is indicative of celiac disease.

In an embodiment, the leukocytes producing cell-damaging substances are lymphocytes, such as different types of T cells.

In an embodiment, the medium, in which leukocytes are brought into contact with the polypeptide(s), is a body fluid or tissue sample, and before bringing the cell sample into contact with the body fluid or tissue sample, a supernatant is prepared from the body fluid or tissue sample, and the cell sample is contacted with the supernatant. The presence of interferon-gamma or other cell-damaging substance(s) in the cell sample is then measured.

In a preferred embodiment of this method, the cell sample comprises intestinal epithelial cells.

The cell damage resulting from the effect of the cell-damaging substance(s) may include disintegration of cell layers and apoptosis.

The invention further provides a method for *in vitro* diagnosis of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy, comprising

- contacting a body fluid or tissue sample from a mammal suspected of having celiac disease or IgE-mediated allergy with at least one polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide; and
- detecting the presence, in the sample, of IgA or IgE antibodies specifically binding to said polypeptide or polypeptides;

wherein the presence of such antibodies specifically binding to said polypeptide or polypeptides is indicative of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy.

According to a preferred embodiment of the invention, the IgE-mediated allergy is wheat food allergy.

According to a further aspect, a diagnostic kit is provided for performing the methods of the invention, comprising a polypeptide having the amino acid sequence according to any one of

SEQ ID NO: 26-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, or a pharmaceutical composition as described above.

Definitions

All words and terms used in the present specification are intended to have the meaning usually given to them in the relevant art. However, for the sake of clarity, a few terms are specifically clarified below.

The expression "a fragment or variant of a polypeptide sharing epitopes for antibodies with said polypeptide" has the meaning as defined in WO2008/079095.

Brief description of the drawings

Fig. 1. cDNA and deduced amino acid sequences of LMW Glutenin GluB3-23 and C175. The C-terminal part shown in bold letters is the clone 175 sequence.

Fig. 2. Nucleotide sequence of Glu-B1 aligned with amino acid sequence of the IgE-reactive clone 43.

Fig. 3. Nucleotide sequence of Glu-B1 aligned with amino acid sequence of the IgE-reactive clone 82.

Fig. 4. The nucleotide sequence and deduced amino acid sequence of the clone 84-derived allergen.

Fig. 5. Deduced amino acid sequences of IgE reactive cDNA clones coding for wheat allergens.

Fig. 6. Amino acid sequences of IgE reactive wheat epitopes.

Fig. 7a. Domain structure of the natural GluB3-23, the recombinant GluB3-23 and C175.

Fig. 7b and 7c: Mass spectrometry (MS) of the purified C175 and GluB3-23. The mass/charge ratio is shown on the x-axis and the intensity is displayed on the y-axis and is shown in arbitrary units.

Fig. 8a. Domain structure of the natural Glu-B1 and the recombinant proteins

Fig. 8b. Mass spectrometry (MS) of the purified mal 43 (clone 43). The mass/charge ratio is shown on the x-axis and the intensity is displayed on the y-axis and is shown in arbitrary units.

Fig. 8c. Mass spectrometry (MS) of the purified mal 82 (clone 82). The mass/charge ratio is shown on the x-axis and the intensity is displayed on the y-axis and is shown in arbitrary units.

Fig. 9a. The C-terminal acidic extension domain and a part of the thionin domain are identified as IgE epitope-containing portion.

Fig. 9b. Mass spectrometry of the recombinant allergen α-purothionin. The mass/charge ratio is shown on the x-axis and the intensity is displayed on the y-axis as a percentage of the most intensive signal obtained in the investigated mass range.

Fig. 10. IgE reactivity of patients suffering from wheat food allergy. Dot blotted purified recombinant proteins (GluB3-23 and C175), aqueous wheat seed (WSE) extract and human serum albumin (HSA) were incubated with sera from patients suffering from wheat food allergy. Bound IgE Abs were detected with ^{125}I labeled anti human IgE Abs and visualized by autoradiography.

Fig. 11. IgE reactivity of patients suffering from wheat food allergy. Dot blotted purified recombinant proteins (GluB3-23, C175, mal 43 and mal 82), aqueous wheat seed (WSE) extract and human serum albumin (HSA) were incubated with sera from patients suffering from wheat food allergy. Bound IgE Abs were detected with ^{125}I labelled anti human IgE Abs and visualized by autoradiography. Frequencies of recognition are displayed in the right margin.

Fig. 12. IgE reactivity of patients suffering from wheat food allergy. Wheat seed extract, HSA, purified alpha purothionin were dotted onto nitrocellulose membrane strips and incubated with sera from wheat food allergic patients. Bound IgE antibodies were detected with ^{125}I-labelled anti-human IgE antibodies and visualized by autoradiography.

Fig. 13. Sequence alignment of GluB3-23 with related proteins in rye, barley, oat, spelt and rice. A point indicates identity and a dash displays a gap. At the end of the alignment the identity to GluB2-23 is shown in percentage.

Fig. 14. Multiple sequence alignment of the clone 84-derived allergen alpha purothionin with homologous proteins in other plants. The amino acid sequence (single letter code) of wheat alpha purothionin was aligned with purothionins in wheat (gi|4007850), rye (gi|4007745), barley (gi|246215), oat (gi|21069045), goatgrass (gi|1052551), rice (gi|215768993), sage (gi|77543393), thale cress (gi|21553588), mustard (gi|120564556), pieplant (gi|197312881). Frequencies of recognition are displayed in the right margin.

Fig. 15. IgA reactivity of celiac disease patients to purified wheat proteins. ELISA measurements of IgA reactivity of celiac disease patients' sera and and a control patient's serum to wheat proteins coated onto ELISA plates. After incubation with patients' sera, the bound IgA was detected using mouse anti-human IgA1/A2 as primary antibody and HRP conjugated sheep anti-mouse IgG as detection antibody. The colour reaction was measured at

405 nm. Wheat and control proteins are indicated on the X-axis and the legend on the right-hand corner indicates patients. Abbreviations used: HSA - Human serum Albumin, GG1 - Gamma gliadin 1, GG2 - Gamma gliadin 2, P - Patients, CD - celiac disease positive, GFD – gluten free diet.

Fig. 16. IgA reactivity of celiac disease patients to synthesized gamma gliadin 1 peptides.

Fig. 17. IgG reactivity of celiac disease patients to synthesized gamma gliadin 1 peptides.

Fig. 18. IgA reactivity of Dermatitis herpetiformis patient to recombinant gamma gliadins.

Detailed description of the invention

To date, there is only a limited set of antigens and peptides to be used as diagnostic tool to discriminate between the different forms of wheat induced hypersensitivities. This led the present inventors to look for novel and well-defined wheat antigens and peptides that can be used for the diagnosis of various wheat hypersensitivities, by screening of a wheat cDNA library and use of classical immunochemical approaches and ion exchange chromatography generated gluten fractions, with well characterized patients' sera. The identification of wheat antigens and peptides, the production and characterization of recombinant proteins permits creating tools for diagnosis (development of chips) and for treatment of such wheat induced hypersensitivities.

The examples below illustrate the present invention with the isolation and use of the nucleic acid sequences and polypeptides of the invention. The examples are only illustrative and should not be considered as limiting the invention, which is defined by the scope of the appended claims.

Example 1

Construction and screening of a λgt11 cDNA library from wheat seeds

In order to find new wheat allergens, total RNA from wheat seeds were extracted and a λgt11 cDNA library was constructed as described previously (5). E. coli Y1090 were infected with 7 x 10^5 PFU of recombinant phages and immunoscreened with serum IgE of three patients suffering from wheat food allergy. After pre-adsorption with nitrocellulose filters, containing λgt11 phages, the 1:10 serum dilution was added to the filters prepared from the already titrated phage clones. Bound IgE antibodies were detected with 1:10 diluted ^{125}I-labeled α-human IgE and visualized by autoradiography. The IgE-reactive phage clones were selected for further re-cloning and their DNA was PCR-amplified using Platinum PCR SuperMix

(Invitrogen) with λgt11 primers and sequenced (VBC-Biotech). The obtained sequences were compared with sequences submitted to the GenBank database at the National Center for Biotechnology Information (NCBI) to find homologous proteins. In some cases we obtained only IgE reactive epitopes without identifying a corresponding protein. The list of all IgE reactive clones is shown in **Table 1**.

Example 2

Expression and purification

clone 175 and Glub3-23

The clone 175 sequence (SEQ ID NO: 1) containing 537 nucleotides and the corresponding full sequence GluB3-23 (SEQ ID NO: 51) involving 1107 nucleotides and six His codons were cloned into pET17b *E.coli* expression vectors. The pET 17b-C175 and the pET 17b-GluB3-23 construct were transformed into *E.coli* BL21 (DE3). The transformed cells were grown in 1 liter Luria Broth medium containing 100 mg/l ampicillin at 37°C. The cells were grown until an OD_{600} of 0.4-0.6, and then the over expression was induced by addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0,5mM. Afterwards the bacteria were grown for 4 additional hours; cells were harvested by centrifugation and frozen over night at -20°C. A cleared cell lysate was prepared and a NiNTA-chromatography was performed according to QIAexpressionist handbook (QIAGEN, Hilden, Germany). The protein containing fractions were pooled and dialysed against 10 mM NaH_2PO_4. The protein concentration was determined with a BCA Assay Kit (Novagen).

clones 43 and 82

The clone 43 sequence (SEQ ID NO: 2) containing 828 nucleotides and the clone 82 sequence (SEQ ID NO: 3) involving 588 nucleotides plus six His codons were cloned into pMal-c4x *E.coli* expression vectors (GeneScript USA Inc.). The pMAL-c4x-43 and pMAL-c4x-82 constructs were transformed into *E.coli* BL21 (DE3) and grown in 1 liter Luria Broth + glucose medium containing 100mg/l ampicillin at 37°C. The cells were grown until an OD_{600} of 0.4-0.6, and then the over expression was induced by addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0,5 mM. Afterwards the bacteria were grown for 4 additional hours; cells were harvested by centrifugation and frozen over night at -20°C. A cleared cell lysate was prepared and a NiNTA-chromatography was performed according to QIAexpressionist handbook (QIAGEN, Hilden, Germany). The

protein containing fractions were pooled and dialysed against 10mM NaH_2PO_4. The protein concentration was determined with a BCA Assay Kit (Novagen).

α-purothionin

The clone 84-derived allergen was expressed as a recombinant protein with a C-terminal hexahistidine tag in *E. coli* BL21 (DE3) cells. The pET 17b-*α-purothionin* construct was transformed into Bl21 (DE3) cells. The transformed *E. coli* cells were grown in 250ml LB medium containing 250µl (100mg/ml) ampicillin at 37°C to an optical density (600nm) of 0.6 and protein expression was induced by addition of 125 µl (1M) isopropyl-beta-D-thiogalactosidase (IPTG). *E. coli* cells were harvested after 4 hours by centrifugation at 3500rpm for 15 min at 4°C. The protein was purified by nickel affinity chromatography from the soluble fraction (Quiagen, Hilden, Germany). The allergen was dissolved and stored in 10 mM NaH_2PO4 buffer pH 4.0 at -20°C. The concentrations of the purified allergens were determined by BCA assay (Pierce, Rockford, IL).

Example 3

Characterization of the recombinant proteins

C175 and GluB3-23

Sequence analysis showed that GluB3-23 is an s-LMW glutenin that contains eight cysteine residues for building intramolecular disulphide bonds for stability and intermolecular disulphide bonds with other LMW and HMW glutenin subunits to form macropolymers. The natural protein consists of a signal peptide, an N-terminal region, a repetitive domain and three C-terminal regions. The recombinant C175 protein is comprised by the three C-terminal regions and the hexa-histidine tag (**Fig. 1**). The recombinant Glub3-23 contains all regions of the natural Glub3-23 without the signal peptide and plus a hexa-histidine-tag (**Fig. 7a**).

For the recombinant C175 protein a molecular weight of 20.8kDa and a theoretical pI of 8.81 was calculated and for the recombinant GluB3-23 a molecular weight was assessed at 40.33kDa and the theoretical pI at 8.73. The purity and molecular mass was controlled by SDS-PAGE and Coomassie Brilliant Blue staining (Fling, Bradford). C175 provided a clear band at approximately 21kDa and GluB3-23 at 40kDa. To achieve information about the polymerization behavior of the proteins SDS PAGE silver staining was performed according to BIO RAD silver stain Plus Handbook under reducing and non reducing conditions. For reducing conditions, a sample buffer containing β-Mercaptoethanol was used and samples were boiled at 95°C for 5 minutes; for non reducing conditions, a sample buffer without β-

Mercaptoethanol was used. Under non reducing conditions C175 provided bands at approximately 20kDa, 40kDa and 250kDa, which indicates that C175 forms di-, tri- and polymers by disulfide bonds. Glub3-23 also forms polymers, shown by bands at approximately 40kDa and 250 kDa under non reducing conditions.

Mass spectrometry was performed as described previously (6). In **fig. 7b** and **fig. 7c** mass spectrometry (MS) of the purified C175 and GluB3-23 is shown. The peak with the highest intensity indicates the protein size. C175 shows the peak at 21021.430Da and GluB3-23 at 40321.094Da, which correlates with the calculated mass.

mal43 and mal82

The recombinant proteins corresponding to clones 43 and 82 featured an N-terminal maltose binding protein tag (MBP-tag) shown in **fig. 8a**, resulting from the pMAL-c4x vector. For mal43 a molecular weight of 73.3kDa and a theoretical pI of 5.81 and for mal82 a molecular weight of 64.6kDa and a theoretical pI of 5.99 were calculated. The sequence analysis of the full length HMW Glu-B1 (corresponding to SEQ ID No: 52) showed, that the protein is an x-type HMW protein with four cysteine residues for disulphide bond forming. The natural Glu-B1 contains a signal peptide, an N-terminal non repetitive domain, a large repetitive domain and a C-terminal non repetitive domain. The recombinant mal43 consists of the MBP, a part of the repetitive domain and the hexa-histidine tag. The recombinant mal82 is made up of the MBP, a part of the repetitive region, the C-terminal non repetitive region and the hexa-histidine tag (**Fig. 8a**). In **fig. 2**, the nucleotide sequence of Glu-B1 is aligned with the deduced amino acid sequence of clone 43, and in **fig. 3**, the nucleotide sequence of Glu-B1 is aligned with the deduced amino acid sequence of clone 82.

The purity and molecular mass was controlled by SDS-PAGE and Coomassie Brilliant Blue staining (Fling, Bradford). Mal43 provided a clear band at approximately 73kDa and mal82 at 64kDa. Mass spectrometry was performed as described previously (6). In **fig. 8b** and **fig. 8c** mass spectrometry (MS) of the purified mal43 and mal82 is shown. The peak at 73695.525Da displays the size of mal43. In **fig. 8c** the peak at 65220.872 shows the molecular weight of mal82. These results correlate with the calculated molecular weights.

α-purothionin

The comparison of the deduced amino acid sequences of the IgE-reactive phage clone 84 with published sequences showed that it is a wheat α-purothionin. The structural gene of α-purothionin includes regions encoding a typical signal peptide, a thionin domain (5 kDa) and

a C-terminal acidic extension. The isolated nucleotide sequence of the clone 84-derived allergen (**fig. 4**) shows IgE-reactivity, and this IgE epitope was related to the C-terminal acidic extension domain and a part of the thionin domain (**fig. 9a**). The deduced amino acid sequence for the clone 84-derived allergen has a calculated molecular weight of 12.7 kDa and an isoelectric point (pI) of 6.27. The results of mass spectrometry analysis of purified recombinant protein corresponded with the deduced molecular weight of 12742Da (**fig. 9b**). The purity of the proteins was checked by 14% SDS–PAGE and Coomassie Blue staining (Fling, Bradford) and their identity was confirmed by Western blotting using a monoclonal anti-His tag antibody (Novagen). A Coomassie brilliant blue-stained 14% SDS-PAGE demonstrated the purity and migration of the recombinant allergen α-purothionin at 18 kDa.

Example 4

IgE reactivity of the recombinant proteins

GluB3-23 is a major allergen in wheat dependent food allergy

IgE reactivity of wheat food allergic patients to GluB3-23 and C175 was tested by dot blot analysis shown in **fig. 10** and **fig. 11**. 0.5µg of purified recombinant proteins (GluB3-23 and C175), 2µg aqueous wheat seed (WSE) extract and 0,5µg of human serum albumin (HSA) were dotted onto nitrocellulose (Whatman Protran nitrocellulose membrane, Sigma Aldrich) strips and after blocking with buffer A (50mM sodium phosphate buffer, pH7,4, 0,5% w/v BSA, 0,5%v/v Tween-20, 0,05% w/v NaN_3) incubated with 1:10 diluted sera from patients suffering from wheat food allergy. Bound IgE Abs were detected with 1:10 diluted ^{125}I labeled anti human IgE Abs and visualized by autoradiography.

Sera were obtained from populations of patients suffering from wheat food allergy. Patients were selected according to positive case history, positive Skin Prick Test (SPT), double blind or open food challenge or CAP-test (Phadia, Uppsala, Sweden) to wheat.

In **fig. 10** it is demonstrated that 27.3% of the patients show IgE reactivity to C175 and 54.5% of these populations show IgE reactivity to the full length protein GluB3-23. In **fig. 11** it is demonstrated that 73.1% of another population of patients show IgE reactivity to C175 and 80.8% show IgE reactivity to the full length allergen GluB3-23. According to WHO/IUIS Allergen Standardization Committee definition (www.allergen.org) a major allergen has to be recognized by 50% of patients. Therefore, LMW GluB3-23 is a major allergen in wheat food allergy and a promising allergen for diagnosis and possibly for therapy.

Furthermore we showed that most of the epitopes for IgE recognition are localized on the N-terminal part of GluB3-23. An inhibition dot blot was performed with patients' sera. The sera

were pre incubated with 10µg of recombinant GluB3-23, C175 or Bet v 1. Bound IgE Abs were detected with ^{125}I-labelled anti human IgE Abs and visualized by autoradiography, the dot intensity was measured by a gamma counter. **Table 2** shows the inhibition calculated in percentage which demonstrates that the C-terminal part of GluB3-23, C175 has a low potential to inhibit IgE binding to GluB3-23 binding.

Glu-B1 is at least a minor allergen in wheat dependent food allergy

The recombinant high molecular weight proteins mal43 and mal82, representing partial proteins of Glu-B1, were tested in dot blots with sera from patients from a population shown in **fig. 11**. Sera were obtained from patients suffering from wheat food allergy. Patients were selected according to positive case history and positive Skin Prick Test (SPT) or CAP-test (Phadia, Uppsala, Sweden) to wheat. 0.5µg of purified recombinant proteins (mal 43 and mal82), 2µg aqueous wheat seed (WSE) extract and 0.5µg of human serum albumin (HSA) were dotted onto nitrocellulose (Whatman Protran nitrocellulose membrane, Sigma Aldrich) strips and after blocking with buffer A incubated with 1:10 diluted patients sera. Bound IgE Abs were detected with 1:10 diluted ^{125}I labeled anti human IgE Abs and visualized by autoradiography. 30.8% of the wheat food allergic patients showed IgE reactivity to these allergens (**fig. 11).** On the basis of WHO/IUIS Allergen Standardization Committee definition (www.allergen.org), an allergen recognized by 10% of the patients is a minor allergen.

α-purothionin

The IgE reactivity of dot-blotted recombinant wheat α-purothionin was tested with serum IgE antibodies from patients suffering from wheat food allergy (**Table 3**). Each of these patients, showing IgE-reactivity to α-purothionin exhibited IgE reactivity to dot-blotted wheat seed extract. 23% of patients from one population (n=13) and 29% of the patients from another population (n=24) reacted to the recombinant α-purothionin. (**fig. 12**).

Example 5

Sequence alignments and different crop extracts

GluB3-23 and C175

In order to find out if the GluB3-23 wheat allergen has homologues in other crop sorts, an amino acid sequence alignment with rye (*Secale sylvestre*), barley (*Hordeum brevisubulatum*), oat (*Avena sativa*), spelt (*Triticum aestivum subsp. Spelta*), and rice (*Oryza sativa*) was performed shown in **fig. 13**. Rye shows an identity of 76%, barley 64%, oat 48%,

spelt 46% and rice 40% to GluB3-23 in wheat *(Triticum aestivum)*. The most conserved domains were the signal peptide and the C-terminal domains. Subsequently aqueous extracts of the different crops were prepared. 15 grams of the crop were homogenized, 32 ml H_2O and 32 µl Phenylmethylsulfonylfluorid (PMSF) were added and stirred for 4 hours at 4°C. The extracts were centrifuged to remove unsolvable particles. The aqueous extracts were loaded on a preparative 12.5% SDS PAGE and a protein molecular weight marker (PageRuler Plus; Prestained Protein Ladder, Fermentas) was used as standard and proteins were blotted onto nitrocellulose membrane (Whatman Protran nitrocellulose membrane, Sigma Aldrich). The membranes were blocked in Buffer A (50mM sodium phosphate buffer, pH 7.4, 0.5% w/v BSA, 0.5%v/v Tween-20, 0.05% w/v NaN_3) after that they were incubated over night with rabbit preimmune serum or with rabbit Abs raised against C175 or GluB3-23 diluted 1:10000 in buffer A. Then sera were discarded and the membrane was washed three times with buffer A. The bound primary antibodies were detected with ^{125}I labelled anti rabbit IgG Abs (BSM diagnosic, Vienna, Austria), diluted 1:1000 in buffer A and visualized by Kodak XOMAT films with intensifying screens (Kodak, Heidelberg, Germany).

The membranes were incubated with the two different rabbit antibodies against C175 and GluB3-23 in order to identify the cross-reactive parts of the allergen. The bound C175 Abs and GluB3-23 Abs, respectively, were detected by ^{125}I labeled anti rabbit IgG Abs and visualized by autoradiography. GluB3-23 homologues could be detected in all extracts whereas the C175 antibody was not able to detect homologues in oat, and in all other extracts the reaction was weaker than with GluB3-23 antibodies.

α-purothionin

In order to study the cross-reactivity of the clone 84-derived allergen, a multiple sequence alignment of the clone 84-derived allergen α-purothionin with homologous proteins in other plants showed that the protein is also very common in other plant species. The amino acid sequence (single letter code) of clone 84-derived α-purothionin was aligned with purothionins in wheat (gi|4007850), rye (gi|4007745), barley (gi|246215), oat (gi|21069045), goatgrass (gi|1052551), rice (gi|215768993), sage (gi|77543393), thale cress (gi|21553588), mustard (gi|120564556), pieplant (gi|197312881) (**fig. 14**). The clone 84-derived allergen α-purothionin shares the highest degree of sequence identity with α-purothionins from rye (85%), barley (49%) and also exhibits sequence identities of more than 30% with α-purothionins from several other plant sources (e.g.,oat 49%, goatgrass 44%, rice 40%, sage 37%). Nitrocellulose-blotted extracts were probed with rabbit antibodies specific for alpha

purothionin and for control purposes, with the corresponding pre-immune serum. α-purothionin specific antibodies were shown to detect the allergen in SDS-protein extracts from other plant species mentioned above like rye and barley.

Example 6

Protein expression during wheat seed maturation

GluB3-23 and C175

Wheat (*Triticum aestivum*) seed SDS extracts were prepared 7, 10, 15, 20, 25, 30, 35 days after pollination and from mature wheat seeds according to Constantin et al (5). The extracts were separated by gel electrophoresis and blotted onto nitrocellulose membrane. The membrane was incubated with rabbit antibodies raised against GluB3-23. The bound anti GluB3-23 antibodies were detected with ^{125}I labeled anti rabbit IgG antibodies and visualized by autoradiography. It was clearly demonstrated that GluB3-23 accumulates in the wheat seed during maturation.

Example 7

In vitro digestion assays

GluB3-23 and C175

The stability of allergens in digestion assays indicates that a protein, parts of which are not totally digested and are detectable by protein-specific antibodies, belongs to the food allergens (7). Gastric and duodenal in vitro digestion was performed with aqueous wheat seed extracts as described previously (7); with the modification that for the duodenal digestion the commercial enzyme tablet Pankreoflat-Dragee (Solvay Pharma, Hannover, Germany) was used. The digested proteins were detected with rabbit antibodies raised against C175 and GluB3-23.

In gastric as well as in duodenal digestion assays it was demonstrated that only the anti GluB3-23 antibody was able to detect bands after digestion. The C175 part of GluB3-23 was digested in gastric digestion assay after 5 minutes, whereas GluB3-23 N-terminal parts could be detected after 120 minutes digestion. The duodenal digestion assay resulted in a similar pattern. C175 fragments could not be detected after 2 minutes of digestion but parts of GluB3-23 could be detected after 45 minutes of duodenal digestion. Consequently, it was demonstrated, that the N-terminal part of GluB3-23 is the stable indigestible fragment.

Example 8

Gliadins were extracted from wheat grains with 70% ethanol following the Weiss et al (8) procedure. The extracted gliadins were then solubilised by dialysing against Buffer A containing 50 mM Tris buffer pH 4.0 and 4 M urea. The solubilised gliadin was passed through Sulfopropyl (SP) sepharose equilibrated with Buffer A, connected to the FPLC (Fast Protein Liquid Chromatography) machine. The flow through fractions were collected and labelled as FT SP. The column was washed with Buffer A and the bound proteins in the SP column were eluted using Buffer B containing 50 mM Tris pH 4.0, 4 M urea and a salt gradient of 0-500 mM NaCl and these fractions were labelled as Elu SP. A part of the FT SP fraction was dialysed in Buffer C containing 50 mM Tris pH 10.0 and 4 M urea and passed through the Diethyl aminoethyl (DEAE) sepharose column equilibrated with the same buffer. The flow through fractions were collected and labelled as FT DEAE. The column washed with Buffer C and the bound proteins were eluted with Buffer C containing a gradient of 0-500 mM NaCl. The elution fractions were labelled as Elu DEAE.

Example 9

Identification of celiac disease specific wheat protein and peptide antigens

Whole wheat extract, whole gliadins and the four fractions FT SP, Elu SP, FT DEAE and Elu DEAE, obtained as described in Example 8 above, were separated by single dimension reducing SDS gel electrophoresis and blotted onto nitrocellulose membrane and probed with serum IgA from well characterized celiac and non celiac patients. Proteins in the FT SP and FT DEAE fractions were apparently highly specific for the disease since the non-celiac patients and patients on wheat free diet showed less reactivity to the proteins in the FT SP and FT DEAE fractions but were more reactive to other fractions and in general to the whole wheat extract and whole gliadin extract (data not shown).

Example 10

Specificity to celiac disease

Whole wheat extract, gliadins, the four fractions of gliadins, aqueous soluble wheat proteins and the SDS soluble glutenins were separated by electrophoresis and blotted onto nitrocellulose membrane and probed with antibodies generated in rabbit against a clone identified to be involved in wheat allergy. The FT fractions (FT SP and FT DEAE) had significantly better specificity for celiac disease, i.e. most of the proteins in this fraction showed positive IgA reactivity only to CD patients' sera and not to sera of CD patients on

gluten free diet and healthy controls, whereas the Elu fractions (Elu SP and Elu DEAE) contained material which also showed IgA reactivity with sera from healthy control persons or celiac disease patients on diet (data not shown).

Example 11

Identification of peptides and proteins by mass spectrometry

The four fractions were digested with Pepsin/trypsin enzyme mixture according to standard protocols and the peptides obtained were identified by ESI-LC/MS mass spectrometry (HCT ULTRA from Bruker Daltonics). It was found that gamma gliadins were enriched in the FT SP fraction and in the FT DEAE fraction. All the peptides obtained are shown in **Table 4a** (SEQ ID NO:s 62-86 and 108-110).

Example 12

Expression and purification of recombinant gamma gliadin 1 and gamma gliadin 2

Gamma gliadin 1 sequence (SEQ ID NO: 87) and gamma gliadin 2 (SEQ ID NO: 88) (**Table 4b**) were cloned into pET27b *E.coli* expression vector to be expressed as recombinant proteins containing additional 6x Histidine residues at its C terminal end. The pET 27b- GG1 and the pET 27b-GG2 constructs were transformed into *E.coli* BL21 (DE3). The transformed cells were grown in 1 liter Luria Broth medium containing 50mg/l Kanamycin at 37°C. The cells were grown until an OD_{600} of 0.6, and the protein expression was induced by addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM and incubation for 12 hours at 30°C. The cells were harvested by centrifugation and resuspended in lysis buffer (50 mM Tris pH 8.0, 500 mM NaCl, 10% glycerol) and PMSF (phenylmethanesulfonylfluoride) was added shortly before lysis. The cells were then lysed using ULTRA-TURRAX (IKA) disperser and the suspension was centrifuged at high speed to extract the inclusion bodies. The pellet containing the recombinant proteins as inclusion bodies was solubilized in 8M urea buffer and the 6xHistidine tagged GG1 and GG2 purified under denaturing conditions using Ni-Nta chromatography performed according to QIAexpressionist handbook (QIAGEN, Hilden, Germany). The fractions containing the purified proteins were dialyzed stepwise against 50 mM Tris pH 8.0, 100 mM NaCl, 10% glycerol buffer to remove urea and stored as aliquots in -20°C. The protein concentration was determined with a BCA Assay Kit (Novagen).

Example 13

IgA reactivity to wheat food antigens

100 µl of the wheat proteins GG1 (Gamma gliadin 1), GG2 (Gamma gliadin 2), α-purothionin, GluB3-23, C-175, Mal 82 and Mal 43 of a concentration of 5 µg/ml were coated overnight at 4°C onto Nunc Maxisorp Elisa plates. The remaining free binding sites were blocked with 1% BSA in PBST for 2 hours at room temperature. 100 µl of sera from celiac disease patients, celiac patients on diet and negative controls were added at a dilution of 1:100 in 0.5% BSA in PBST buffer and incubated for 12 hours at 4°C. The plates were washed 5 times in PBST buffer and 100 µl of mouse anti-human IgA_1/A_2 (BD Biosciences) diluted to 1:1000 in 0.5% BSA/PBST buffer was added and incubated at room temperature for 5 hours. The plates were then washed 5 times with PBST and 100 µl of sheep anti-mouse IgG conjugated with Horse radish peroxidise(HRP) (Amersham) antibodies were added and incubated at 37°C for one hour. The plates were washed 3 times with PBST and the antibodies were detected using the HRP-ABTS(2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) detection system and colour reaction was measured at 405 nm in an ELSA plate reader. The values generated are plotted as a graph, see **fig. 15**. Recombinant GG1 and GG2 show high IgA reactivity , α-puthionin shows negative IgA reactivity, and the LMW glutenin GluB3-23 and C-175 and HMW-glutenins (Mal 82 and Mal 43) show moderate IgA reactivity. The results show that the recombinant proteins GG1 and GG2 are highly specific for IgA reactivity. Absence of IgA reactivity to α-purothionin shows its specificity as an IgE binding allergen. Hence it is a very specific candidate protein for IgE reactivity.

Example 14

Synthesis of overlapping gamma gliadin peptides for mapping T cell and B cell epitopes

19 GG1 peptides were synthesized by FMOC technique, spanning the entire length of the GG1 protein and overlapping each other by five amino acid residues, and each peptide being 18-26 amino acid residues in length (**Table 5**, SEQ ID NO: 89-107). After synthesis, the peptides were purified by HPLC using gradient of 0-100% Acetonitrile with 0.1% TFA. The purified peptides were analyzed by MALDI for correct molecular mass and the pure fractions were freeze dried and stored at -20° C until further use.

Example 15

IgA reactivity of celiac disease patients to synthetic GG1 peptides

ELISA plates were coated with 100 ul of 5 µg/ml of the 19 overlapping peptides, rGG1, alpha-gliadin peptide p56-75 and deamidated alpha gliadin peptide p56-75 (Q65E) and probed with serum-IgA from CD patients, CD patients on gluten free diet and healthy controls and detected using mouse anti-human IgA1/A2 and sheep anti-mouse IgG- HRP labelled antibody. Human serum albumin (HSA) was used as control (n = 8 CD patients, 2 CD patients on gluten free diet and 3 healthy controls). Serum IgA reactivity from celiac disease patients showed that the N-terminal region, rich in proline and glutamines had higher IgA reactivity than the c terminal region of the protein, which is poor in proline and glutamine (**fig 16**). Each of the synthetic GG1 peptides no. 2-7, 9, 13 and 18 (i.e. SEQ ID NO: 90-95, 97, 101 and 106, respectively) showed specific IgA reactivity. Comparison of IgA reactivity of the GG1 peptides with a known immunodominant epitope alpha gliadin P56-75 and deamidated alpha-gliadin peptide P56-75 (Q65E) showed that peptide 4 (SEQ ID NO: 92) and peptide 6 (SEQ ID NO: 94) had better sensitivity, as well as peptide 7 (SEQ ID NO: 95) and peptide 9 (SEQ ID NO: 97).

Example 16

IgG (total) reactivity of celiac disease patients to synthetic GG1 peptides

ELISA plates were coated with 100 ul of 5µg/ml of the 19 overlapping synthetic peptides and rGG1 and probed with serum from CD patients, CD patients on gluten free diet and healthy controls and detected using mouse anti-human IgG (total) and sheep anti-mouse IgG- HRP labelled antibody. Human serum albumin (HSA) was used as control (n= 8 CD patients, 2 CD patients on gluten free diet and 3 healthy controls). Reactivity to peptides in the N terminal region was higher than the C terminal region. The sensitivity and specificity of the assay using IgG was low (**fig. 17**).

The celiac disease specific proteins and peptides disclosed in the above examples 14-16 are useful for antibody-based diagnosis, both IgA and IgG testing, preferably IgA-based testing.

Example 17

Dermatitis herpetiformis patient's serum IgA reactivity to recombinant Gamma gliadins

ELISA plates were coated with 100 µl of 5 µg/ml rGamma gliadin 1, rGamma gliadin 2, α-gliadin peptide p56-75 (α-gli peptide), deamidated α-gliadin peptide (D) and Human serum albumin. The bound proteins were probed with sera from Dermatitis herpetiformis (DH) patients and healthy controls. IgA reactivity to the proteins was detected using mouse anti-

human IgA1/A2 and sheep anti-mouse IgG- HRP labelled antibody. (n= 12 DH patients and 2 healthy controls). Recombinant GG1 and GG2 showed higher IgA reactivity to sera from DH patients than to healthy controls **(Fig 18)**. This result suggests that recombinant proteins GG1 and GG2 can be used in the diagnosis of Dermatitis herpetiformis.

Table 1. IgE reactive clones and their corresponding proteins

cDNA clone no.	SEQ ID NO	SEQ ID NO of corresponding amino acid seq.	Corresponding protein	SEQ ID NO corresponding protein
175	1	26	>gi|169666917|gb|ACA63857.1| LMW glutenin subunit [Triticum aestivum]	51
43	2	27	>gi|71084277|gb|AAZ23584.1| HMW glutenin x-type subunit Bx7 precursor [Triticum aestivum]	52
82	3	28	>gi|71084277|gb|AAZ23584.1| HMW glutenin x-type subunit Bx7 precursor [Triticum aestivum]	52
84	4	29	>gi|4007850|emb|CAA65313.1| alpha purothionin [Triticum aestivum]	53
50	5	30	>gi|94315063|gb|ABF14401.1| 1Dx high molecular weight glutenin subunit [Triticum aestivum]	54
118	6	31	>gi|6684164|gb|AAF23507.1|AF216869_1 glutenin, high molecular weight subunit type y precursor [Triticum aestivum]	55
34	7	32	>gi|154268818|gb|ABS72146.1| alpha gliadin [Triticum aestivum]	56
78	8	33	>gi|205363284|gb|ACI04082.1| gamma-gliadin [Triticum aestivum]	57
85	9	34	>gi|194718421|gb|ACF93462.1| gamma-gliadin [Triticum aestivum]	58
16	10	35	>gi|73912496|dbj|BAE20328.1| omega-5 gliadin [Triticum aestivum]	59
39	11	36	>gi|10953877|gb|AAG25638.1| beta-amylase [Hordeum vulgare subsp. vulgare]	60
4	12	37	>gi|89143120|emb|CAJ32654.1| putative avenin-like a precursor [Triticum aestivum]	61
46	13	38	IgE reactive epitope	
67	14	39	IgE reactive epitope	

68	15	40	IgE reactive epitope	
79	16	41	IgE reactive epitope	
95	17	42	IgE reactive epitope	
106	18	43	IgE reactive epitope	
110	19	44	IgE reactive epitope	
117	20	45	IgE reactive epitope	
190	21	46	IgE reactive epitope	
195	22	47	IgE reactive epitope	
72	23	48	IgE reactive epitope	
116	24	49	IgE reactive epitope	
91	25	50	IgE reactive epitope	

Table 2. IgE inhibition

Pre-incubation	Bet v 1		GluB3-23		C175	
	cpm	%inhibition	cpm	% inhibition	cpm	% inhibition
Pat 8	75.0	0.00	31.5	100.0	73.9	3.0
Pat 10	377.6	0.00,0	36.0	100.0	379.1	0.0
Pat 3	829.0	0.00,0	326.0	63.3	650.0	22.6
Pat 11	379.0	0.00,0	58.9	93.8	190.0	55.3
Pat 12	11.5	-	17.7	-	16.2	-

Table 3. Demographic, clinical and serological characterization of patients with wheat-dependent food allergy

				Total IgE	**Wheat-specific IgE**	
Patient	**Age**	**Sex**	**Symptoms**	**(kU/L)**	**(kU/L)**	**Positive SPT results**
I1	12	F	A	562	68,8	w(4)
V1	41		GE, AS, D	252	>100 (6)	w, hdm, r, c
V2	19	M	GE, AS	653	>100 (6)	w, r, gp, e, b, n
G1	14	M	A, AD, AST	636	>200	w(8), ew(9), ey(8)
G2	3	M	A, AD	165	11,7	w(8)
G3	3	M	A, AD	349	8,09	w(4), ew(4.5), ey(3.5)
G4	3	F	U, AD, AST	795	165	w(7.5), ew(6.5), ey(4)
G5	5	M	A, AD, AST	2723	695	w(7)
G6	5	M	AD, AST	2724	48	
G7	12	F	U, AD, AST	4539	>100	w(11)

kU/L: kilounit per liter, A: anaphylaxis, AD: atopic dermatitis, AS: airway symptoms, AST: asthma, D: dyspnea, GE: gastroenteral symptoms, U: urticaria, b: birch, c: cat, e: egg, ew: egg white, ey: egg yolk, gp: grass pollen, hdm: house dust mite, n: nut, r: rye, w: wheat

Table 4a. Peptides identified by mass spectrometry after peptic-tryptic digestion

Fraction	SEQ ID NO	Peptide sequence	Class of gliadins
FT SP	62	AQIPQQLQ	γ -gliadins
	63	PQQQRPFIQPSL	γ -gliadins
	64	LVQGQGIIQPQQPAQLE	γ -gliadins
	65	APFASIVAGIGGQ	γ -gliadins
	69	LVPLSQQQQVGQGILV	γ -gliadins
	70	LPLYQQQQVGQGTLV	γ -gliadins
	71	FLPLSQQQQVGQGSLV	γ -gliadins
	80	LQQPNIAHASSQVSQQSYQLL	γ -gliadins
	108	LSQQQQVGQGSLV	γ -gliadins
	109	LYQQQQVGQGTLV	γ -gliadins
Elu SP	72	LQLQPFPQPQLP	α/β gliadins
	73	FFQPSQQNPQAQGSFQPQQLPQFE	α/β gliadins
	74	FRPSQQNPQAQGSVQPQQLPQF	α/β gliadins
	75	RVPVPQLQPQNPSQQQPQKQ	α/β gliadins
	78	LQQHNIAHGSSQVLQ	α/β gliadins
	76	LQQHNIAHASSQVLQQSTYQLLQ	α/β gliadins
	79	MVRVPVPQLQ	α/β gliadins
	77	LQQHNIAHGSSQVLQESTYQLV	α/β gliadins
	85	LPQQPPFSQQQQPILP	LMW glutenin subunit
	84	LPQQQIPFVHPSILQ	LMW glutenin subunit
	81	FLQPHQIAQLE	LMW glutenins
	82	LAQGTFLQPHQIAQLE	LMW glutenins
	83	FSQQQQLFPQQPSFS	LMW glutenins
	86	LLQQQIPIVHPSILQ	LMW glutenin subunit
	68	LVQGQGIIQPQQPAQLE	γ gliadins
FT DEAE	65	APFASIVAGIGGQ	γ -gliadins
	66	NIQVDPSGQVQALQ	γ -gliadins
	67	NIQVDPSGQVQWLQQ	γ -gliadins
	68	LVQGQGIIQPQQPAQLE	γ -gliadins
Elu DEAE	79	MVRVPVPQLQ	α/β gliadins
	110	LQQHSIAYGSSQVLQ	α gliadins
	70	LPLYQQQQVGQGTLV	γ -gliadins
	80	LQQPNIAHASSQVSQQSYQLL	γ -gliadins

Table 4b. Gamma gliadin 1 (GG1) and gamma gliadin 2 (GG2)

Protein	SEQ ID NO
Gamma gliadin 1 (GG1)	87
Gamma gliadin 2 (GG2)	88

Table 5. Synthesized GG1 peptides

Peptide no.	SEQ ID NO	rGG1 peptide sequence
1	89	MNIQVDPSGQVQWLQQQLV
2	90	QQQLVPQLQQPLSQQPQQTF
3	91	QQPQQTFPQPQQTFPHQPQQQ
4	92	QPQQQVPQPQQPQQPFLQPQQPFPQQ
5	93	PFPQQPQQPFPQTQQPQQ
6	94	QQPQQPFPQQPQQPFPQTQQ
7	95	PFPQTQQPQQPFPQLQQPQQ
8	96	QQPQQPFPQPQQQLPQPQQ
9	97	PQPQQPQQSFPQQQRPFI
10	98	QRPFIQPSLQQQLNPCKNIL
11	99	CKNILLQQSKPASLVSSLWS
12	100	LVSSLWSIIWPQSDCQVMRQ
13	101	QVMRQQCCQQLAQIPQQLQCA
14	102	QLQCAAIHSVVHSIIMQQQQQ
15	103	QQQQQQQQQQGIDIFLPLSQ
16	104	LPLSQHEQVGQGSLVQGQGI
17	105	QGQGI IQPQQPAQLEAIRSLV
18	106	IRSLVLQTLPSMCNVYVPPECS
19	107	PPECSIMRAPFASIVAGIGGQ

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Claims

1. An isolated polypeptide comprising the amino acid sequence according to any one of SEQ ID NO: 26-50, 62-86, and 89-110.

2. The polypeptide according to claim 1, characterised in that it is isolated from wheat or recombinantly produced.

3. An isolated nucleic acid molecule encoding the polypeptide according to claim 1 or 2.

4. The nucleic acid according to claim 3 having the nucleotide sequence according to any one of SEQ ID NO: 1-25.

5. The polypeptide according to claim 1 or 2 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, for use in therapy or diagnosis.

6. The polypeptide according to claim 1 or 2 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, for use in therapy or diagnosis of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy.

7. An isolated polypeptide comprising the amino acid sequence according to any one of SEQ ID NO: 51-61, 87 and 88 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, for use in therapy or diagnosis.

8. An isolated polypeptide comprising the amino acid sequence according to any one of SEQ ID NO: 51-61, 87 and 88 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, for use in therapy or diagnosis of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy.

9. The isolated polypeptide according to claim 8, wherein the use in therapy comprises tolerance induction or prophylactic treatment.

10. A pharmaceutical composition comprising a polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110, or a hypoallergenic form of said polypeptide

that is modified to abrogate or attenuate its T cell-, IgA- or IgE-binding response, and optionally pharmaceutically acceptable excipients, carriers, buffers and/or diluents.

11. A pharmaceutical composition according to claim 10, wherein said hypoallergenic form of said polypeptide is modified by fragmentation, truncation or tandemerization of the molecule, deletion of internal segments, domain rearrangment, substitution of amino acid residues, disruption of disulfide bridges.

12. A method for producing an allergen composition comprising the step of adding a polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110, or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, to a composition comprising an allergen extract and/or at least one purified allergen component.

13. An allergen composition obtainable with the method according to claim 12.

14. A method for *in vitro* diagnosis of celiac disease comprising
- contacting a body fluid or tissue sample from a mammal suspected of having celiac disease with at least one polypeptide having the amino acid sequence according to any one of SEQ ID NO: 62-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide; and
- measuring the presence of activated T cells in the sample, such as by use of a lymphocyte proliferation assay, a FACS analysis of the cell activation, or by measuring cytokine release;
wherein the presence of activated T cells is indicative of celiac disease.

15. A method for *in vitro* diagnosis of celiac disease comprising
- contacting leukocytes from a mammal suspected of having celiac disease with at least one polypeptide having the amino acid sequence according to any one of SEQ ID NO: 62-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, in a medium;
- contacting a cell sample from said mammal with the medium; and
- measuring the presence of interferon-gamma or other cell-damaging substance(s) in the cell sample;
wherein the presence of interferon-gamma or other cell-damaging substance(s) is indicative of celiac disease.

16. The method according to claim 15, wherein the cell sample comprises intestinal epithelial cells

17. A method for *in vitro* diagnosis of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy, comprising
- contacting a body fluid or tissue sample from a mammal suspected of having celiac disease, dermatitis herpetiformis, or IgE-mediated allergy with at least one polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide; and
- detecting the presence, in the sample, of IgA or IgE antibodies specifically binding to said polypeptide or polypeptides;
wherein the presence of such antibodies specifically binding to said polypeptide or polypeptides is indicative of celiac disease, dermatitis herpetiformis, or IgE-mediated allergy.

18. A polypeptide according to claim 6 or 8, or a method according to claim 17, wherein the IgE-mediated allergy is wheat food allergy.

19. A diagnostic kit for performing the method according to any one of claims 14-18, comprising a polypeptide having the amino acid sequence according to any one of SEQ ID NO: 26-110 or a fragment or variant thereof sharing epitopes for antibodies with said polypeptide, or a composition according to claim 10 or 11.

Fig. 1

atgaagaccttcctcatctttgctctcctcgccattgtggcgacaagtgccattgcacaa
M K T F L I F A L L A I V A T S A I A Q
atggagaatagccacatccctggtttggagagaccatcgcagcaacaaccattaccacca
M E N S H I P G L E R P S Q Q Q P L P P
caacaaacattatcgcaccaccaacaacaacaacccatccaacaacaaccacaaccattt
Q Q T L S H H Q Q Q Q P I Q Q Q P Q P F
tcacaacagcaaccatgttcacagcaacaacaacaaccattatcgcagcaacaacaacca
S Q Q Q P C S Q Q Q Q Q P L S Q Q Q Q P
ccattttcacaacaacaaccaccattttcgcagcaacaacaacaaccattatcgcagcaa
P F S Q Q Q P P F S Q Q Q Q Q P L S Q Q
caacaaccaccattttcacaacaacaaccaccattttcgcagcaacaacaaccaccattt
Q Q P P F S Q Q Q P P F S Q Q Q Q P P F
tcacaacaacaaccaccattttcgcagcaacaacaaccagttctaccgcaacaaccatca
S Q Q Q P P F S Q Q Q Q P V L P Q Q P S
ttttcacagcaacaactaccaccattttcacagcaacaatcaccgttttcgcaacaacaa
F S Q Q Q L P P F S Q Q Q S P F S Q Q Q
caaatagtactacagcaacaaccaccatttttgcaacaacaacaaccaagtctaccgcaa
Q I V L Q Q Q P P F L Q Q Q Q P S L P Q
caaccaccattttcgcagcaacaacaacaactagttctaccgcaacaacaaataccattt
Q P P F S Q Q Q Q Q L **V L P Q Q Q I P F**
gttcatccatctatcttgcagcagctaaacccatgcaaggtattcctccagcagcaatgc
V H P S I L Q Q L N P C K V F L Q Q Q C
agccctgtggcaatgccacaaagtcttgctaggtcgcaaatgttgcagcagagcagttgc
S P V A M P Q S L A R S Q M L Q Q S S C
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H V M Q Q Q C C Q Q L P Q I P Q Q S R Y
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E A I R A I I Y S I I L Q E Q Q Q V Q G
tccatccaaactcctcagcagcaaccccaacagttgggccaatgtgtttcccaaccccaa
S I Q T P Q Q Q P Q Q L G Q C V S Q P Q
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Q Q S Q Q Q L G Q Q P Q Q Q Q L A Q G T
tttttgcagccacaccagatagctcagcttgaggtgatgacttccattgcgctccgtacc
F L Q P H Q I A Q L E V M T S I A L R T
ctgccaacgatgtgccgtgtcaatgtgccgttgtatagaaccaccactagtgtgccattc
L P T M C R V N V P L Y R T T T S V P F
ggtgttggcaccggagttggttcctactaa
G V G T G V G S Y -

Fig. 2

```
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ggcacgagacaatacgagcagcaacctgtggtgccgtccaaggccggatccttctacccc
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ccaggacaa
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343               G  Q  Q  L  G  Q  G  Q  P  G  Y  Y  P  T  S  Q  Q  360
    tcggaacaagggcagcagccaggacaaggaaaacaaccaggacaaggacaacaagggtac
361  S  E  Q  R  Q  Q  P  G  Q  G  K  Q  P  G  Q  G  Q  Q  G  Y  380
    tacccaacttctccgcaacagtcaggacaagggcaacaactgggacaagggcaaccaggg
381  Y  P  T  S  P  Q  Q  S  G  Q  G  Q  Q  L  G  Q  G  Q  P  G  400
    tactacccaacttctccacagcagtcaggacaaggacaacaatcaggacaaggacaacaa
401  Y  Y  P  T  S  P  Q  Q  S  G  Q  G  Q  Q  S  G  Q  G  Q  Q  420
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421  G  Y  Y  P  T  S  P  Q  Q  S  G  Q  G  Q  Q  P  G  Q  G  Q  440
    tcggggtacttcccaacttctcggcagcagtcaggacaagggcagcagccaggacaagga
441  S  G  Y  F  P  T  S  R  Q  Q  S  G  Q  G  Q  Q  P  G  Q  G  460
    caacagtcgggacaagggcaacaaggtcagcaaccaggacaaggacaacaagcgtactac
461  Q  Q  S  G  Q  G  Q  Q  G  Q  Q  P  G  Q  G  Q  Q  A  Y  Y  480
    ccaacttcttcgcaacagtcaagacaaaggcaacaggcaggacaatggcaacgaccggga
481  P  T  S  S  Q  Q  S  R  Q  R  Q  Q  A  G  Q  W  Q  R  P  G  500
    caagggcaaccagggtactacccaacctctccacagcagccaggacaagagcaacaatca
501  Q  G  Q  P  G  Y  Y  P  T  S  P  Q  Q  P  G  Q  E  Q  Q  S  520
    ggacaagcgcaacaatcaggacaatggcaactagtgtactacccaacttctccgcaacag
```

521 G Q A Q Q S G Q W Q L V Y Y P T S P Q Q 540

ccaggccaattgcaacaaccagcacaagggcaacaaccagcacaagggcaacaatcagca

541 P G Q L Q Q P A Q G Q Q P A Q G Q Q S A 560

caagagcaacagccaggacaagcgcaacaatcaggacaatggcaactagtgtactaccca

561 Q E Q Q P G Q A Q Q S G Q W Q L V Y Y P 580

acttctccgcaacagccaggacaattgcaacaaccagcacaagggcaacaagggtactac

581 T S P Q Q P G Q L Q Q P A Q G Q Q G Y Y 600

ccaacttctccacaacagtcaggacaagggcaacaagggtactacccaacttctccgcaa

601 P T S P Q Q S G Q G Q Q G Y Y P T S 618

cagtcaggacaagggcaacaagggtactacccaacttctccgcaacagtcaggacaaggg

cagcagccaggacaaggacaacagccaagacaagggcaacaagggtactacccaatttct

ccgcagcagtcaggacaagggcaacaaccaggacaagggcaacaaggatactacccaact

tctccgcagcagtcaggacaagggcaacaaccaggacatgagcaacagccaggacaatgg

ctgcaaccaggacaagggcaacaagggtactatccaacttcttcacagcagtcaggacaa

gggcatcaatcaggacaagggcaacaagggtactacccaacttctctgtggcaaccagga

caagggcaacaaccaggacaagggcaacaaggctacgccagcccataccatgttagcgcg

gagtaccaggcggcccgcctaaaggtggcaaaggcgcagcagctcgcggcacagctgccg

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Fig. 3

atggctaagcgcctggtcctctttgcggcagtagtcgtcgccctcgtggctctcaccgcc
gctgaaggtgaggcctctggacaactacaatgtgagcacgagctcgaggcatgccaacag
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ccaggacaagggcaacaactgggacaagggcaaccagggtactacccaacttcgcagcag
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601 P T S P Q Q S G Q G Q Q G Y Y P T S P Q 620

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621 Q S G Q G Q Q G Y Y P T S P Q Q S G Q G 640

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641 Q Q P G Q G Q Q P R Q G Q Q G Y Y P I S 660

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661 P Q Q S G Q G Q Q P G Q G Q Q R Y Y P T 680
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681 S P Q Q S G Q G Q Q P G H E Q Q P G Q W 700
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701 L Q P G Q G Q Q G Y Y P T S S Q Q S G Q 720
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721 G H Q S G Q G Q Q G Y Y P T S L W Q P G 740
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741 Q G Q Q P G Q G Q Q G Y A S P Y H V S A 760
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761 E Y Q A A R L K V A K A Q Q L A A Q L P 780
gcaatgtgccggctggagggcagcgacgcattgtcgaccaggcagtga
781 A M C R L E G S D A L S T R Q - 795

Fig. 4

```
1   t GGA GCG TCA GTA TCG GCG GAA TTC CTC TCA AGT GGC CTA
        G   A   S   V   S   A   E   F   L   S   S   G   L    13

41    AGC TGC CCT AAA GGC TTC CCT AAA TTG GCC CTT GAG TCC
        S   C   P   K   G   F   P   K   L   A   L   E   S    26

80    AAC TCA GAT GAA CCA GAC ACC ATT GAG TAC TGC AAC TTG
        N   S   D   E   P   D   T   I   E   Y   C   N   L    39

119   GGA TGT AGG TCT TCC GTG TGT GAC TAC ATG GTC AAC GCA
        G   C   R   S   S   V   C   D   Y   M   V   N   A    52

158   GCT GCT GAC GAT GAA GAG ATG AAA CTC TAT GTG GAA AAT
        A   A   D   D   E   E   M   K   L   Y   V   E   N    65

197   TGT GCT GAT GCT TGT GTC AGT TTC TGT AAC GGT GAT GCT
        C   A   D   A   C   V   S   F   C   N   G   D   A    78

236   GGC CTC CCA TCC CTT GAT GCG TAC TGA tgc gtg tcc atg
        G   L   P   S   L   D   A   Y                       86

      gtc tga gat ttc aca ggg caa ggt cgt gtc tca cct ttg

      cgt tca ata aaa ttg gat ccc atc gag agt atc caa acc

      agt gtg tca ccc tgt ttt atg tgt gtg tat gtc cat tcc

      ttg ttc gaa ata aaa gcc atc atg atg gat gcc atg ttg

      ctg cca aaa aaa aaa aaa aaa aaa aaa aaa aaa aaa aaa

      aaa aaa aaa aaa aaa aaa aaa aaa aaa aaa aaa aac cg
```

Fig. 5

clone 175

EPSVSAEFRQQLGQGQQGYYPTSLQPGQKQQAGQGQQSGQGQQGYYPTSPQQSGQGQQPG
QGQPGYYPTSPQQSGQWQQPGQGQQPGQGQQSGQWQLVYYPTSPQQQIVLQQRPPFLQQQ
QPSLPQQPPFSQQQQQLVLPQQQIPFVHPSILQQLNPCKVFLQQQCSPVAMPQSLARSQM
LQQSSCHVMQQQCCQQLPQIPQQSRYEAIRAIIYSIILQEQQQVQGSIQTPQQQPQQLGQ
CVSQPQQQSQQQLGQQPQQQQLAQGTFLQPHQIAQLEVMTSIALRTLPTMCRVNVPLYRT
TTSVPFGVGTGVGSY-

>gi|169666917|gb|ACA63857.1| low molecular weight glutenin subunit [Triticum aestivum]

MKTFLIFALLAIVATSAIAQMENSHIPGLERPSQQQPLPPQQTLSHHQQQQPIQQQPQPFSQQQPCSQQQ
QQPLSQQQQPPFSQQQPPFSQQQQQPLSQQQQPPFSQQQPPFSQQQQPPFSQQQPPFSQQQQPVLPQQPS
FSQQQLPPFSQQQSPFSQQQQIVLQQQPPFLQQQQPSLPQQPPFSQQQQQLVLPQQQIPFVHPSILQQLN
PCKVFLQQQCSPVAMPQSLARSQMLQQSSCHVMQQQCCQQLPQIPQQSRYEAIRAIIYSIILQEQQQVQG
SIQTPQQQPQQLGQCVSQPQQQSQQQLGQQPQQQQLAQGTFLQPHQIAQLEVMTSIALRTLPTMCRVNVP
LYRTTTSVPFGVGTGVGSY

clone 82

WSPSVSAEFPTSPQQSGQGQQGYYPTSPQQSGQGQQGYYPTSPQQSGQGQQPGQGQQPRQ
GQQGYYPISPQQSGQGQQPGQGQQRYYPTSPQQSGQGQQPGHEQQPGQWLQPGQGQQGYY
PTSSQQSGQGHQSGQGQQGYYPTSLWQPGQGQQPGQGQQGYASPYHVSAEYQAARLKVAK
AQQLAAQLPAMCRLEGSDALSTRQ--

>gi|71084277|gb|AAZ23584.1| HMW glutenin x-type subunit Bx7 precursor [Triticum aestivum]

MAKRLVLFAAVVVALVALTAAEGEASGQLQCEHELEACQQVVDQQLRDVSPGCRPITVSPGTRQYEQQPV
VPSKAGSFYPSETTPSQQLQQMIFWGIPALLRRYYPSVTSSQQGSYYPGQASPQQSGQGQQPGQEQQPGQ
GQQDQQPGQRQQGYYPTSPQQPGQGQQLGQGQPGYYPTSQQPGQKQQAGQGQQSGQGQQGYYPTSPQQSG
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QPGYYPTSQQSEQGQQPGQGKQPGQGQQGYYPTSPQQSGQGQQLGQGQPGYYPTSPQQSGQGQQSGQGQQ
GYYPTSPQQSGQGQQPGQGQSGYFPTSRQQSGQGQQPGQGQQSGQGQQGQQPGQGQQAYYPTSSQQSRQR
QQAGQWQRPGQGQPGYYPTSPQQPGQEQQSGQAQQSGQWQLVYYPTSPQQPGQLQQPAQGQQPAQGQQSA
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LQPGQGQQGYYPTSSQQSGQGHQSGQGQQGYYPTSLWQPGQGQQPGQGQQGYASPYHVSAEYQAARLKVA
KAQQLAAQLPAMCRLEGSDALSTRQ

clone 43

SPSVSAEFRNSGQQLGQGQPGYYPTSQQSEQRQQPGQGKQPGQGQQGYYPTSPQQSGQGQ
QLGQGQPGYYPTSPQQSGQGQQSGQGQQGYYPTSPQQSGQGQQPGQGQSGYFPTSRQQSG
QGQQPGQGQQSGQGQQGQQPGQGQQAYYPTSSQQSRQRQQAGQWQRPGQGQPGYYPTSPQ
QPGQEQQSGQAQQSGQWQLVYYPTSPQQPGQLQQPAQGQQPAQGQQSAQEQQPGQAQQSG
QWQLVYYPTSPQQPGQLQQPAQGQQGYYPTSPQQSGQGQQGYYPTSSR

>gi|71084277|gb|AAZ23584.1| HMW glutenin x-type subunit Bx7 precursor [Triticum aestivum]

MAKRLVLFAAVVVALVALTAAEGEASGQLQCEHELEACQQVVDQQLRDVSPGCRPITVSPGTRQYEQQPV
VPSKAGSFYPSETTPSQQLQQMIFWGIPALLRRYYPSVTSSQQGSYYPGQASPQQSGQGQQPGQEQQPGQ
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LQPGQGQQGYYPTSSQQSGQGHQSGQGQQGYYPTSLWQPGQGQQPGQGQQGYASPYHVSAEYQAARLKVA
KAQQLAAQLPAMCRLEGSDALSTRQ

clone 84
G A S V S A E F L S S G L S C P K G F P K L A L E S N S D E P D T I E Y C N
L G C R S S V C D Y M V N A A A D D E E M K L Y V E N C A D A C V S F C N G
D A G L P S L D A Y

>gi|4007850|emb|CAA65313.1| alpha purothionin [Triticum aestivum]
MGSKGFKGVI VCLLILGLVL EQLQVEGKSC CRSTLGRNCY NLCRARGAQK LCAGVCRCKI
SSGLSCPKGF PKLALESNSD EPDTIEYCNL GCRSSVCDYM VNAAADDEEM KLYVENCADA
CVSFCNGDAG LPSLDAY

clone 50
SWSPSVSAEFRNSQQPGQGQQGQQPGQGQQPGQGQPWYYPTSPQESGQGQQPGQWQQPGQ
GQPGYYLTSPLQLGQGQQGYYPTSLQQPGQGQQPGQWQQSGQGQHWYYPTSPQLSGQGQR
PGQWLQPGQGQQGYYPTSPQQPGQGQQLGQWLQPGQGQQGYYPTSLQQTGQGQQS

>gi|94315063|gb|ABF14401.1| 1Dx high molecular weight glutenin subunit [Triticum aestivum]
MAKRLVLFVAVVVALVALTVAEGEASEQLQCERELQELQERELKACQQVMDQQLRDISPECHPVVVSPVA
GQYEQQIVVPPKGGSFYPGATTPPQQLQQRIFWGIPALLKRYYPSVTSPQQVSYYPGQASPQRPGQGQQP
GQGQQSGQGQQGYYPTSPQQPGQWQQPEQGQPGYYPTSPQQPGQLQQPAQGQQPGQGQQGRQPGQGQPGY
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YPTSLQQSGQGQPGYYPTSLQQLGQGQSGYYPTSPQQPGQGQQPGHLQQPAQGQQPEQGQQGQQPGQGQQ
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GQGQPGYYPTSPLQSGQGQPGYYLTSPQQSGQGQQPGQLQQSAQGQKGQQPGQGQQPGQGQQGQQPGQGQ
QGQQPGQGQPGYYPTSPQQSGQGQQPGQWQQPGQGQPGYYPTSPLQPGQGQPVYDPTSPQQPGQGQQPGQ
LQQPAQGQQGQQLAQGQQGQQPAQVQQGQQPAQGQQGQQLGQGQQGQQPGQGQQPAQGQQGQQPGQGQQG
QQPGQGQQPGQGQPWYYPTSPQESGLGQQPGQWQQPGQWQLPGQGQPGYYLTSPLQLGQGQQGYYPTSLQ
QPGQGQQPGQWQQSGQGQHGYYPTSPQLSGQGQRPGQWLQPGQGQQGYYPTSPQQSGQGQQLGQWLQPGQ
GQQGYYPTSLQQTGQGQQSGQGQQGYYSSYHVSVEHQAASLKVAKAQQLAAQLPAMCRLEGGDALSASQ

clone 118
VSIGGFPGQWLQPGQGQQGYYPTSSQQSGQGHQSGQGQQGYYPTSLWQPGQGQQPGQGQQ
GYASPYHVSAEYQAARLKVAKAQQLAAQLPAMCRLEGSDALSTRQ-

>gi|6684164|gb|AAF23507.1|AF216869_1 glutenin, high molecular weight subunit type y precursor [Triticum aestivum]
MAKRLVLFGIVVIALVALTAAEGEASRQLQCERELQESSLEACRQVVDQQLAGRLPWSTGLQMRCCQQLR
DVSAKCRRVAVSQVARQYEQTAVPPKGGSIYPGETTPLQQLQQGIFWGTSSQTVQGYYPSVTSPQQGSYY
PGQASPQQPGQGQQQGKWQEPGQGQQGYYPTSQQQPGQGQQGHYPASQQQPGQGQQGHYPASLQQPGQGQ
QGHYPASLQQPGQGQQTEQPGQMQQPGQGQQIGQGQQPGQGQQIGQGQQIRQGQQPGQGQQGYYQTHPQQ
PGQGQQPGQGQQGYYPTSPQQPGQGQQGHYPGSLRQPGQGQPGQRQQPGQGQQTGQGQQPEQEQQPGQGQ
QGYYPTSPQQPGQGQQPGQGQQGYYPTSLQQPGQGQQPHYPASQQQPGQGQQGHYPTSLLQPGQGQQGHY
PASSLQPGQGQQGHYPASLQQPGQGQQTEQPGQGQQPAQEQQSGQGQQGHYPTSLQQPGQGQPGQRQQPG
QGQQIGQGQQPEQEQQPGQGQPGHYPASVQQPGQGQQTEQTGQGQQPGQGQQPEQEQQPGQGQQGYYITS
LQQPGQGKQLGQWQQPGQGQEGYYPTSPQQPGQGQQGHCPTSRQQPGQAQQPGQGQQIGQAQKPGQGQQG
YYPTSLQQPGQGQQSGQGNQPGQGHQPGQGQQSGQDQQGYDSPCHVSAEQKATSPKVAKAQQPVAQLPTM
CQMEGGDTLSASQ

clone 34
G A S V S A E F Q Q Q Q Q Q Q Q P S S Q V S Y Q Q P Q Q Q Y P S G Q G F F Q
P S Q Q N P Q A Q G F V Q P Q Q L P Q F E E I R N L A L Q T L P A M C N V Y
I P P Y C S T T I A P F G I M S T N Stop

gi|154268818|gb|ABS72146.1| alpha gliadin [Triticum aestivum]
MKTFLILALL AIVATTATIA VRVPVPQLQP QNPSQQQPQE QVPLVQQQQF PGQQQPFPPQ

QPYPQPQPFP SQQPYLQLQP FPQPQLPYPQ PQLPYPQPQL PYPQPQPFRP QQPYPQSQPQ
YSQPQQPISQ QQQQQQQQQQ QKQQQQQQQQ ILQQILQQQL IPCRDVVLQQ HSIAYGSSQV
LQQSTYQLVQ QLCCQQLWQI PEQSRCQAIH NVVHAIILHQ QQQQQQQQQQ QPLSQVSFQQ
PLQQYPLGQG SFQPSLQNPQ AHGSVQPQQL PQFEEIRNLA LQTLPAMCNV YIPPYCTIAP
FGIFGTN

clone 78

P Q Q P F P Q T Q Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q P F P Q Q P Q
Q P F P Q T Q Q P Q Q P F P Q L Q Q P Q Q P F P Q P Q Q Q L P Q P Q Q P Q Q
S F P Q Q Q R P F I Q P S L Q Q Q L N P C K N I L L Q Q C K P A S L V S S L
W S I I W P Q S D C L V M R Q Q C C Q Q L A Q I P Q Q L Q C A A I H S V V H
S I I M Q Q Q Q Q Q Q Q Q Q G M H I F L P L S Q Q Q Q V G Q G S L V Q G Q G
I I Q P Q Q P A Q L E A I R S L V L Q T L P S M C N V Y V P P E C S I M R A
P F A S I V A G I G G Q

>gi|205363284|gb|ACI04082.1| gamma-gliadin [Triticum aestivum]

MNIQVDPSGQ VQWLQQQLVP QLQQPLSQQP QQTFPQPQQT FPHQPQQQVP QPQQPQQPFL
QPQQPFPQQP QQPFPQTQQP QQPFPQQPQQ PFPQTQQPQQ PFPQQPQQPF PQTQQPQQPF
PQLQQPQQPF PQPQQQLPQP QQPQQSFPQQ QRSLIQPSLQ QQLNPCKNIL LQQCKPASLV
SSLWSIIWPQ SDCQVMRQQC CQQLAQIPQQ LQCAAIHSVV HSIIMQQQQQ QQQQQGMHIF
LPLSQQQQVG QGSLVQGQAI IQPQQPAQLE AIRSLVLQTL PSMCNVYVPP ECSIMRAPFA
SIVAGIGGQY R

clone 85

R G Q V Q W P Q Q Q P F R Q P Q Q P F Y Q Q P Q H T F P Q P Q Q T F P H Q P
Q Q Q F P Q P Q Q P Q Q Q F P Q P Q Q P Q Q P F P Q P Q Q A Q L P F P Q Q P
Q Q P F P Q P Q Q P Q Q P F P Q S Q Q P Q Q P F P Q P Q Q P Q Q S F P Q Q Q
Q P L I Q P Y L Q Q Q M N P C K N Y L L Q Q C N P V S L V S S L V S M I L P
R S D C Q V M Q Q Q C C Q Q L A Q I P R Q L Q C A A I H S V V H S I L M Q Q
E Q Q Q G I Q I L R P L F Q L V Q G Q G I I Q P Q Q P A Q Y E V I R S L V L
R T L P N M C N V Y V R P D C S T I N A P F A S I V A G I S G Q

>gi|194718421|gb|ACF93462.1| gamma-gliadin [Triticum aestivum]

MKTLLILTIF AAALTIATAN IQVDPSGQVQ WPQQQPFPQP QPFSQQPQQA FLQPQHTFPL
QPQQVFPQPQ QPQQQFPQPQ QPQQPFPQPQ QPQLPFPQQP QQPFPQPQQP QQPFPQSQQP
QQPFPQPQQQ FPQPQQPQQS FPQQQPPLIQ PYLQQQMNPC KNYLLQQCNP VSLVSSLVSM
ILPRNDCQVM QQQCCQQLAQ IPRQLQCTAI HSVVHAIIMQ QEQQGIQILR PLFQLVQGQG
IIQPQQPAQY EVIRSLVLRT LPNMCNVYVR PDCSTINAPF ASIVAGIGGQ

clone 16

A S V S A E F P Q Q Q F P Q Q Q F H Q Q Q L P Q Q Q F P Q Q Q F P Q Q Q F P
Q Q Q Q F P Q Q Q Q Q L T Q Q Q F P R P Q Q S P E Q Q Q F P Q Q Q F P Q Q P P
Q Q F P Q Q Q F P I P Y P P Q Q S Q E P S P Y Q Q Y P Q Q Q P S G S D V I S
I S G L

>gi|73912496|dbj|BAE20328.1| omega-5 gliadin [Triticum aestivum]

MKTFIIFVLL AMAMNIASAS RLLSPRGKEL HTPQEQFPQQ QQFPQPQQFP QQQIPQQHQI
PQQPQQFPQQ QQFLQQQQIP QQQIPQQHQI PQQPQQFPQQ QQFPQQHQSP QQQFPQQQFP
QQKLPQQEFP QQQISQQPQQ LPQQQQIPQQ PQQFLQQQQF PQQQPPQQHQ FPQQQLPQQQ
QIPQQQQIPQ QPQQIPQQQQ IPQQPQQFPQ QQFPQQQFPQ QQFPQQEFPQ QQQFPQQQIA
RQPQQLPQQQ QIPQQPQQFP QQQQFPQQQS PQQQQFPQQQ FPQQQQLPQK QFPQPQQIPQ
QQQIPQQPQQ FPQQQFPQQQ QFPQQQEFPQ QQFPQQQFHQ QQLPQQQFPQ QQFPQQQFPQ
QQQFPQQQQL TQQQFPRPQQ SPEQQQFPQQ QFPQQPPQQF PQQQFPIPYP PQQSEEPSPY
QQYPQQQPSG SDVISISGL

clone 39

P L Q R S G P E L T I E M I L Q A A Q P K L E P F P F E E H T D L P V Q G L
G G I G G G E V E D P T G G M G G E V Q Q D P T G G M G G E V Q Q D P T G G
M G G E V E D P T G G M G G E L P P T V

>gi|10953877|gb|AAG25638.1| beta-amylase [Hordeum vulgare subsp. vulgare]

VNVKGNYVQV YVMLPLDAVS VNNRFEKGDE LRAQLRKLVE AGVDGVMVDV WWGLVEGKGP
KAYDWSAYKQ LFELVQKAGL KLQAIMSFHQ CGGNVGDAVN IPIPQWVRDV GTCDPDIFYT
DGHGTRNIEY LTLGVDNQPL FHGRSAVQMY ADYMTSFREN MKEFLDAGVI VDIEVGLGPA
GEMRYPSYPQ SHGWSFPGIG EFICYDKYLQ ADFKAAAAAV GHPEWEFPND VGQYNDTPER
TQFFRDNGTY LSEKGRFFLA WYSNNLIKHG DRILDEANKV FLGYKVQLAI KISGIHWWYK
VPSHAAELTA GYYNLHDRDG YRTIARMLKR HRASINFTCA EMRDSEQSSQ AMSAPEELVQ
QVLSAGWREG LNVACENALP RYDPTAYNTI LRNARPHGIN QSGPPEHKLF GFTYLRLSNQ
LVEGQNYANF KTFVDRMHAN LPRDPYVDPM APLPRSGPEI SIEMILQAAQ PKLQPFPFQE
HTDLPVGPTG GMGGQAEGPT CGMGGQVKGP TGGMGGQAED PTSGMGGELP ATM

clone 4

R L L E P V S I G G S A A A R A K F R A P Q Q Q V P V E I M G M V L Q T L P
S M C S V N I P Q Y C T T T P C S T I A P A I Y S I P M T A T C A G G A C

>gi|89143120|emb|CAJ32654.1| putative avenin-like a precursor [Triticum aestivum]

MKTMFLLALL AFTATSAVAQ LYTTCSQGYG QCQQQPQPQP QPQPQMNTCA AFLQQCSQTP
HVQTQMWQAS GCQLVRQQCC QPLAQISEQA RCQAVCSVAQ IIMRQQQGQS FGQPQQQVPV
EIMRMVLQTL PLMCRVNIPQ YCTTTPCSTI TPAIYSIPMT ATCAGGAC

Fig. 6

>clone 72
K G Q Q G Y Y P T S L Q Q P G Q G Q Q G Y Y P T S L Q H T G Q R Q Q P V Q G
Q Q P E Q G Q Q P G Q W Q Q G Y Y P T S P Q R N S S **Stop**

>clone 116
R R Q G Q Q S G Q E Q Q E Q Q I G Q G Q Q P G Q L Q Q P T Q G Q Q G Q Q P G
Q G Q Q G Q Q P G Q G Q Q G Q Q P G Q G Q Q P G Q G Q P G Y Y P T S L Q Q S
G Q G Q Q P G Q W Q Q P G Q G L P I T N S G S D T **Stop**

>clone 91
G A S V S A E F H P G Q A S P Q Q S G Q G Q Q P G Q E Q Q P G Q G Q Q D Q Q
P G Q R Q Q G Y Y P T S P Q Q P G Q G Q Q L G Q G Q P G Y Y P T S R N S S
Stop

>clone 46
T L E P S V S A E F P Q Q Q F P Q P Q Q P Q Q P F P Q Q P Q R N S L Q R I Q
P T A R W E G S F E A A D R G T S A G P A

>clone 67
E P S V S A E F P Q Q P Q Q Q F P Q S Q Q P Q Q P F P Q P Q Q Q F L Q P Q Q
P Q Q S W N S G I P A E R R S L P L P V G L V S T G S A

>clone 68
P S V S A E F P F P W Q P Q Q P F P Q T Q Q S F P L Q P Q Q P F P Q L P P S

>clone 79
L E P S V S A E F Q Q A Q L P F P Q Q P Q Q P F P Q P Q Q P Q Q P F P Q S Q
Q P Q Q P F P Q P Q Q P Q Q S F P Q L Q E F Q L S A G R Y H Y Q L V W C Q R
D P R

>clone 95
E P S V S A E F P F L Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q P F P Q Q
P Q Q P F P Q T Q Q

>clone 106
P F L Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q P F P Q Q P Q Q P F P Q T
Q Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q P F P Q L Q Q P Q Q P F P Q R
I Q P T A R W E G S F E A A D R G T S A G E F Q L S A G R Y H Y Q L V W C Q
R D P R

>clone 110
Q Q F P Q P Q Q P Q Q P F P Q Q P Q Q Q F P Q P Q Q P Q Q P F P Q P Q Q P Q
L P F P Q Q P Q Q P F P Q P Q Q P Q Q P F P Q L Q Q P Q Q P L P Q P Q Q P Q
Q P F R N S S Stop

>clone 117

P S G Q V Q W L Q Q Q L V P Q L Q Q P L S Q Q P Q Q T F P Q P Q Q T F P H Q
P Q Q Q V P Q P Q Q P Q Q P F L Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q
P F P R Q L S A G R Y H Y Q L V W C Q R D P R

>clone 190

P K D S A H R P L G R E L R G G R P R H V G R T G L A N G T G P W G R K R P

>clone 195

P F L Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q P F P Q Q P Q Q P F P Q T
Q Q P Q Q P F P Q Q P Q Q P F P Q T Q Q P Q Q P F P Q L Q Q P Q Q P F P Q R
I Q P T A R G E G S F E A A D R G T S A G E F Q L S A G R Y H Y Q L V W C Q
R D P R

Fig. 7a

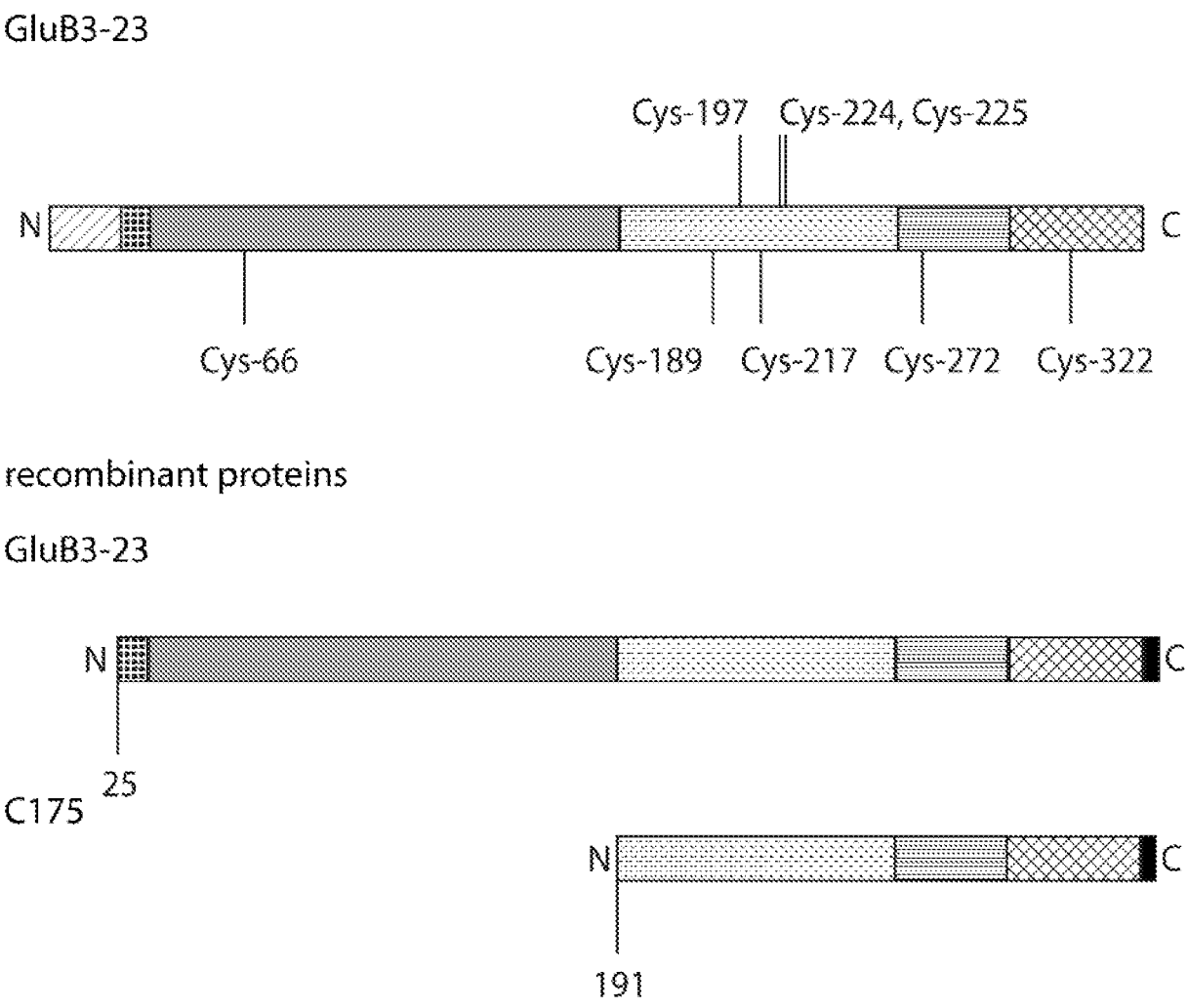

GluB3-23
Cys-197
Cys-224, Cys-225
N
C
Cys-66
Cys-189
Cys-217
Cys-272
Cys-322
recombinant proteins
GluB3-23
N
C
25
C175
N
C
191
signal peptide
C-terminal region I
6x HIS tag
N-terminal region
C-terminal region II
repititive domain
C-terminal region III

Fig. 7b

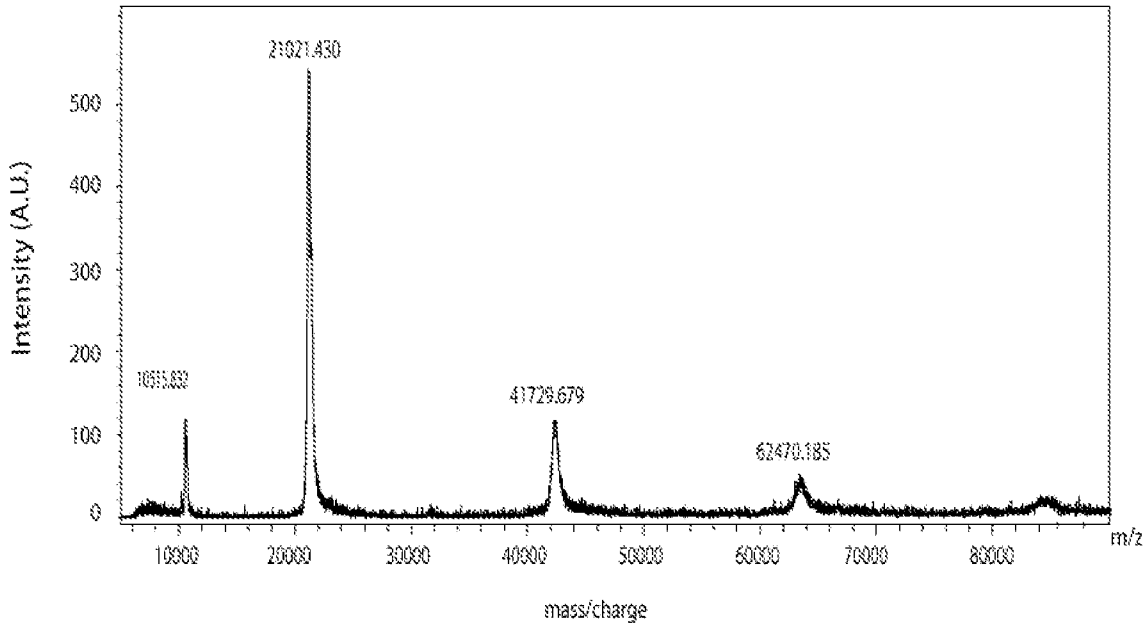

21021.430
10615.852
41729.679
62470.185
Intensity (A.U.)
500
400
300
200
100
0
10000
20000
30000
40000
50000
60000
70000
80000
m/z
mass/charge

Fig. 7c

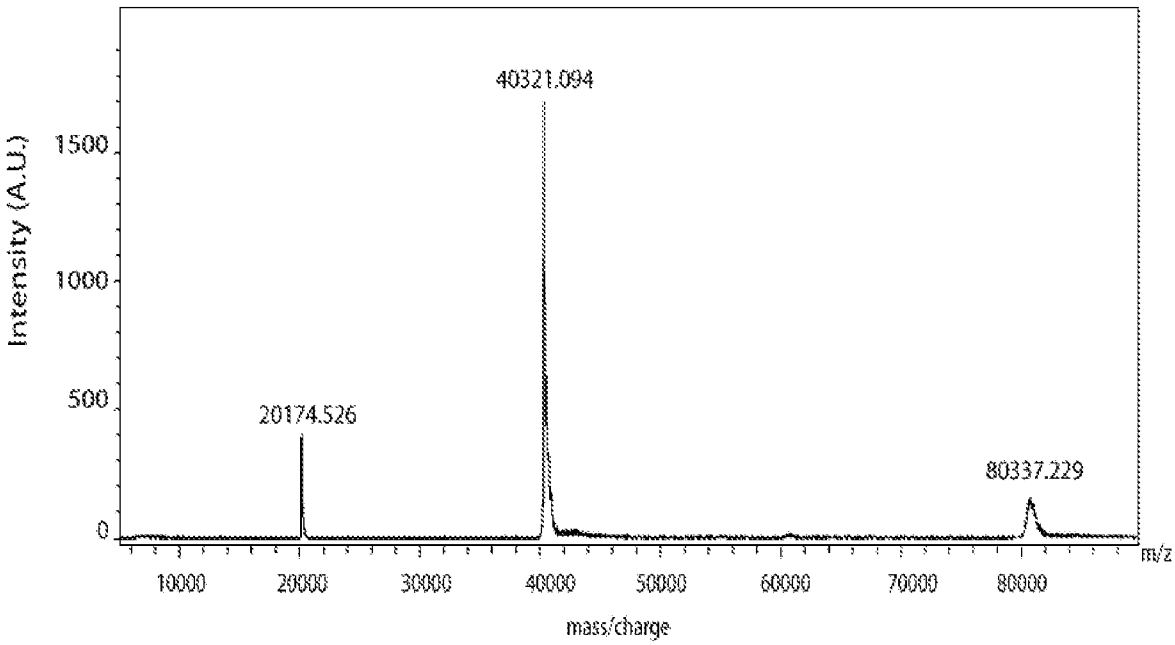

40321.094
20174.526
80337.229
Intensity (A.U.)
1500
1000
500
0
10000
20000
30000
40000
50000
60000
70000
80000
m/z
mass/charge

Fig. 8a

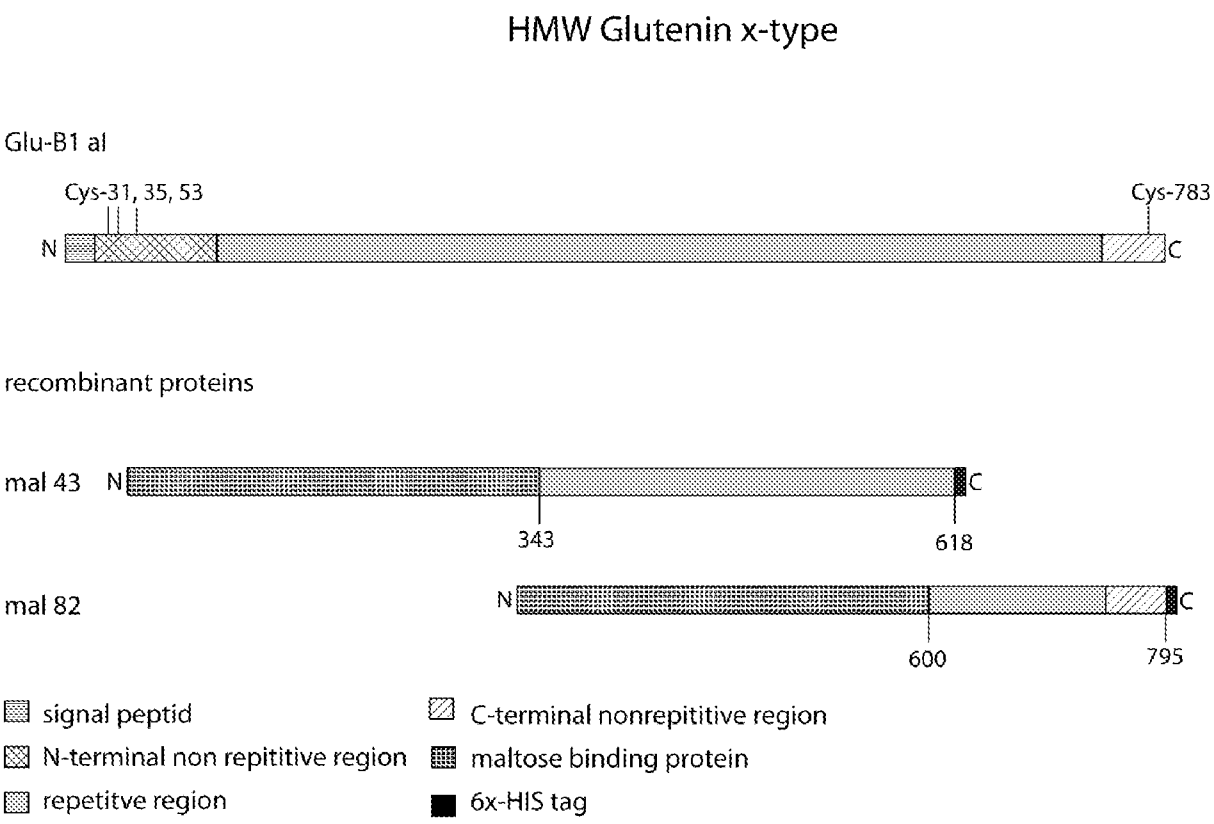

HMW Glutenin x-type
Glu-B1 al
Cys-31, 35, 53
Cys-783
N
C
recombinant proteins
mal 43
N
C
343
618
mal 82
N
C
600
795
signal peptid
N-terminal non repititive region
repetitve region
C-terminal nonrepititive region
maltose binding protein
6x-HIS tag

Fig. 8b

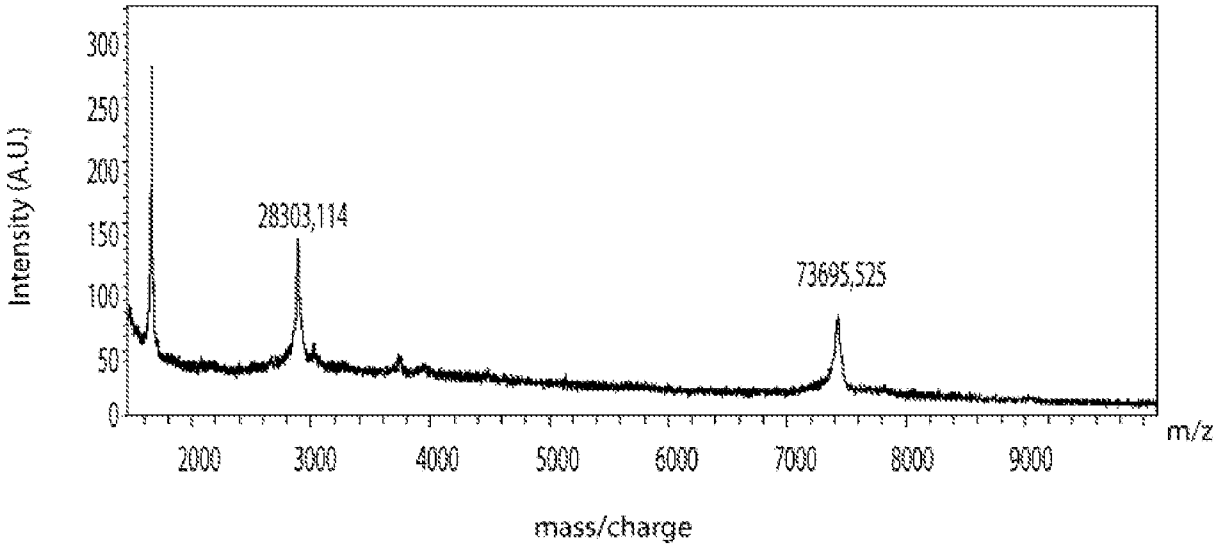
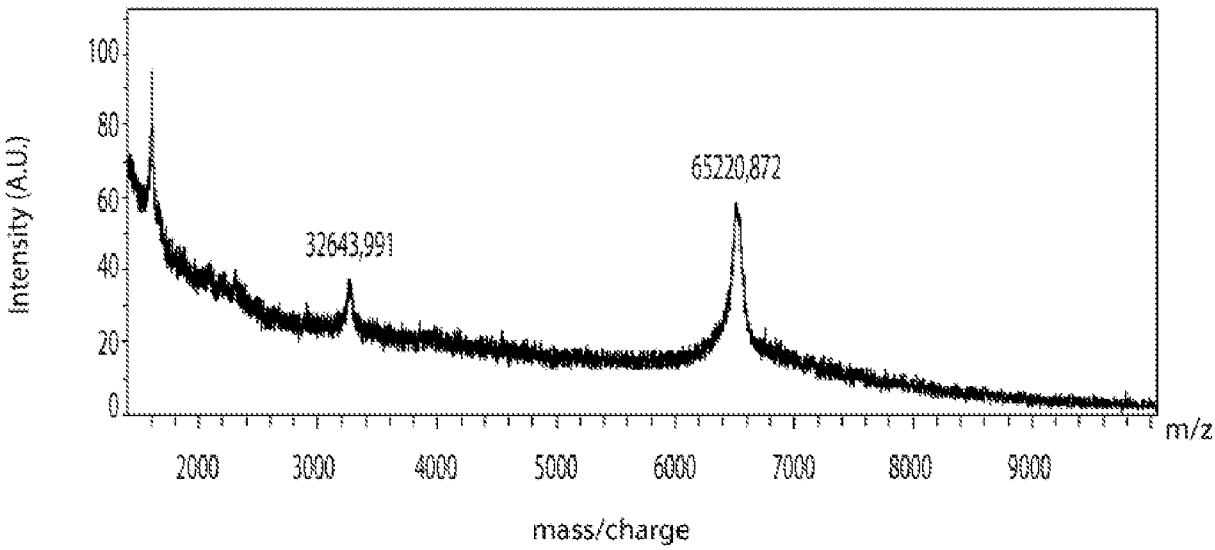

Intensity (A.U.)
300
250
200
150
100
50
0
28303,114
73695,525
m/z
2000
3000
4000
5000
6000
7000
8000
9000
mass/charge

Fig. 8c


Intensity (A.U.)
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60
40
20
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32643,991
m/z
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6000
7000
8000
9000
mass/charge

Fig. 9a

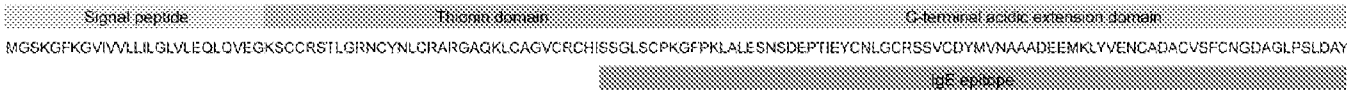


Fig. 9b

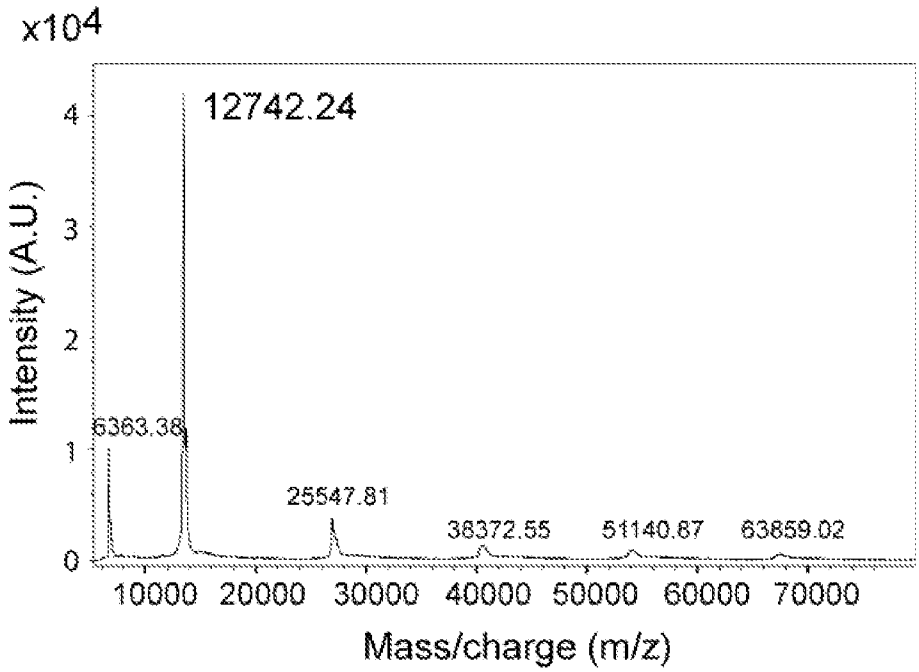


Fig. 10

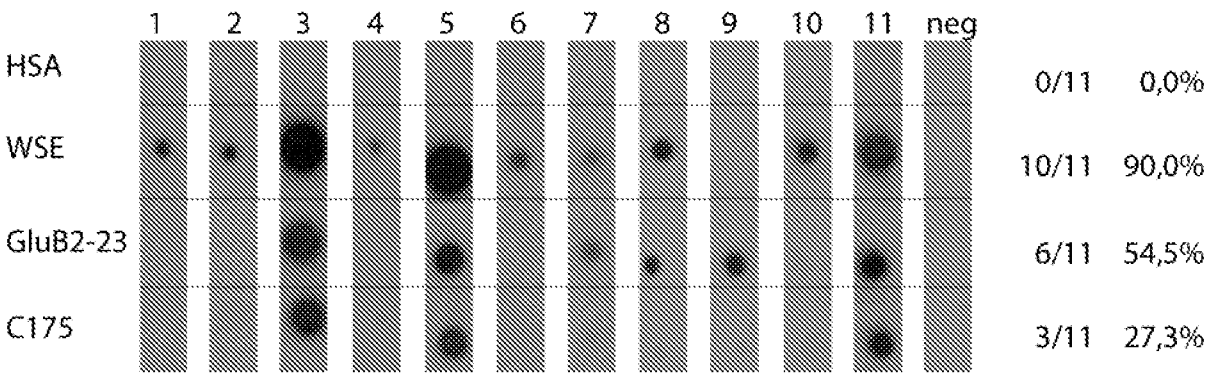


Fig. 11

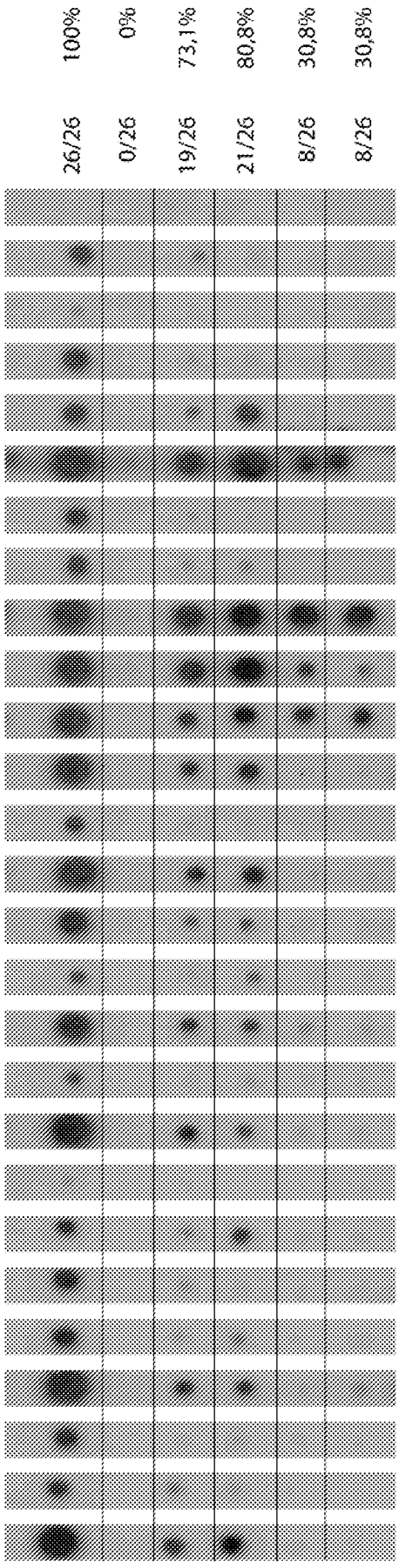

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 neg
WSE 26/26 100%
HSA 0/26 0%
C175 19/26 73,1%
GluB3-23 21/26 80,8%
mal43 8/26 30,8%
mal82 8/26 30,8%

Fig. 12

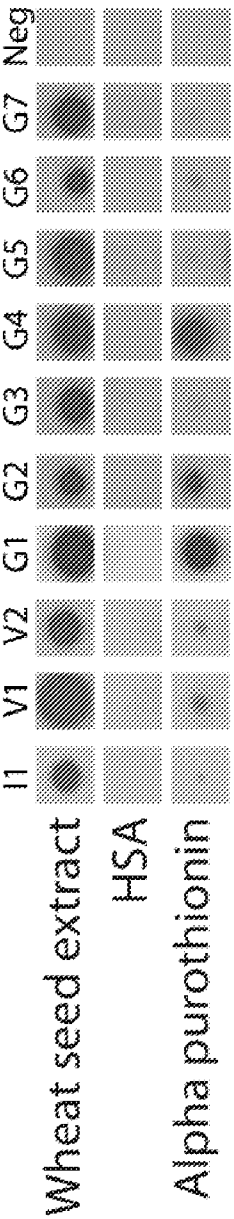
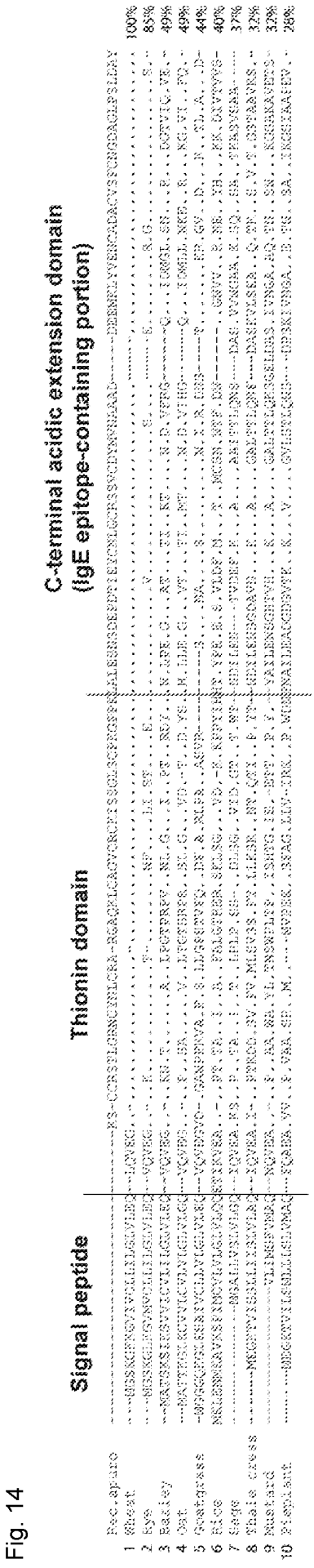

I1 V1 V2 G1 G2 G3 G4 G5 G6 G7 Neg
Wheat seed extract
HSA
Alpha purothionin

Fig. 13


GluB3-2 MKTFLIFALLAIVATSAIAQMENSHIPGLERPSQQQ-PLPPQQTLSHHQQQQPIQQQPQPFSQQQPCSQQQQQPLSQQQQPPFSQQQPPFSQQQQQPLSQQQQ 102
Rye .....V......A.........T.RV....K.W...-.....---------..P.......--------- 51
Barley .....V.....V....S.....I.P.S...G.WP..-.F.Q.---PYP..P..YP.....-YP...YP..P.--------- 67
Oat ......L....MA..M.T.--QYDPS----------EQY--------- 27
Spelt ...L..LTI..MAI.IGT.N.QVDPSSQVQW.Q..PV.Q.H.PFSQQP..TF.QP..TF.HQP..QFP.P..P----------..QFL.P.. 82
Rice ..I.V.LS...LA.S..S..FDACTYG---------- 27
GluB3-2 PPFSQQQPPFSQQQQPPFSQQQPPFSQQQQPVLPQQPSFSQQQLPPFSQQQSPFSQQQQIVLQQQPPFLQQQQPSLPQQPPFSQQQQQLVLPQQQIPFVHPSI 205
Rye --------..P.....---------.IIL..S..S......S-.....P..H..--F......V.Q..V 100
Barley --------.YP..P..YP..---------QPFP..S..S...-.CPQ..T.LQ.VY..--.L....S..Q..V 120
Oat -------Q.YPE..----------..MQ.LL 41
Spelt .FPQ.P.Q.YP..P.Q..P.T.Q.----------QQ.FP.SQQP...FSQPQ..F.QP..P..-SF....P..IQ..L 149
Rice --------QCQ..----------..MQ.-- 37
GluB3-2 LQQLNPCKVFLQQQCSPVAMPQSLARSQMLQQSSCHVMQQQCCQQLPQIPQQSRYEAIRAIIYSIILQEQQQVQGSIQTPQQQPQQLGQCVSQPQQ--QSQQQ 306
Rye .....H...S.R......W.....E...S......V.....--..FV.PQ......S..G...H..--..... 199
Barley .....V..RIT.....L.....RE.....E...N......V.....EQ.RD-EDFV.-Q...L..SVRG...L..AI.GVS. 221
Oat Q..MI..QM..M......E.VP-FL...I.R..T....RR...R..A...R.L.CP..HSM.HA..M----------...Q..----LV.A..------- 119
Spelt Q..V....N..L...K..SLVS-SLW.MIWP..D.Q..R.......A.....LQCA..HTVIH...M----------..EQ..GMHILLPLY.------- 231
Rice --IM...NE.VR.....MSL.W--EQ.RR..L...Q..R......MRLMA..YHCQ..CTM.Q..MQ---------- 100
GluB3-2 ----------LGQQPQQQQLAQGTFLQPHQIAQLEVMTSIALRTLPTMCRVNVPLYRTTTSVPFG-VGTGVGSY 369
Rye QQLGQCSFQQPQQLQQ.....IP..I.....S.....G.....SS..IM..S-I.....G. 278 76%
Barley PQIGQCSFQQPQ-LQQ.....VPL.A.....H.....N.....I....A...-..I...G. 299 64%
Oat ----------M.LVQP.T.M.GQV.I..Q.LS.YQT.KVV.MQ...A..N.Q..P.CS--TGQV.GMAA.I.GC 181 48%
Spelt ----------QQ.VG.GTLVQGQGII..Q.P....AIR.LV.Q.....N.Y..PECSIIKA..SS.VA.I.GQ 295 46%
Rice ----------.V.FDA.FVGEP----.AQAQAQV..N-..S..G.YPRYCS.PCK.AT.----HC..W 149 40%

Fig. 14


Signal peptide
Thionin domain
C-terminal acidic extension domain
(IgE epitope-containing portion)
Rec.ap-ure
1 Wheat
2 Rye
3 Barley
4 Oat
5 Goatgrass
6 Rice
7 Sage
8 Thale cress
9 Mustard
10 Ryegrass
100%
83%
49%
49%
44%
40%
37%
32%
32%
28%

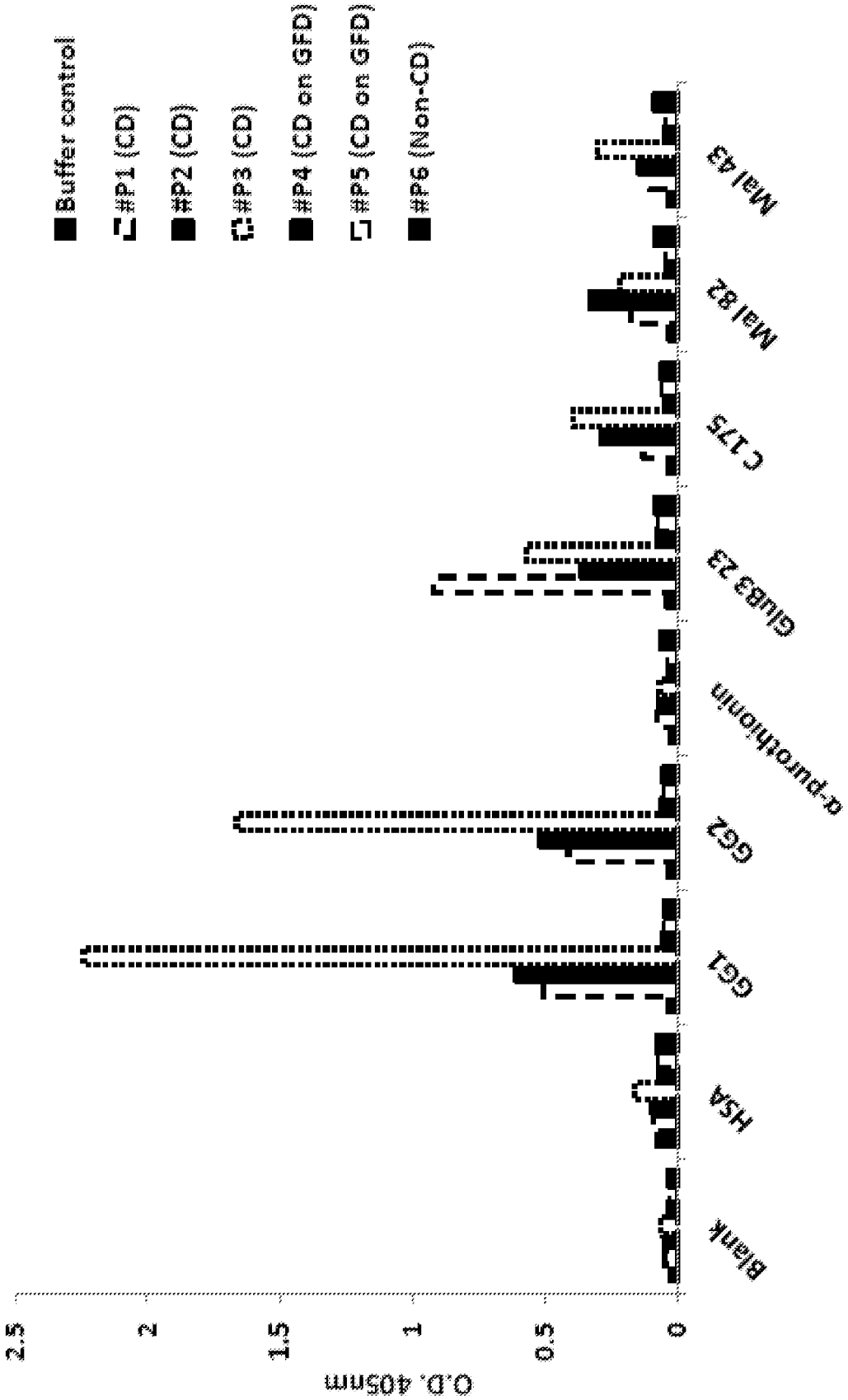

Buffer control
#P1 (CD)
#P2 (CD)
#P3 (CD)
#P4 (CD on GFD)
#P5 (CD on GFD)
#P6 (Non-CD)
O.D. 405nm
2.5
2
1.5
1
0.5
0
Blank
HSA
GG1
GG2
α-purothionin
Glu83 23
C175
Mal 82
Mal 43

Fig. 15

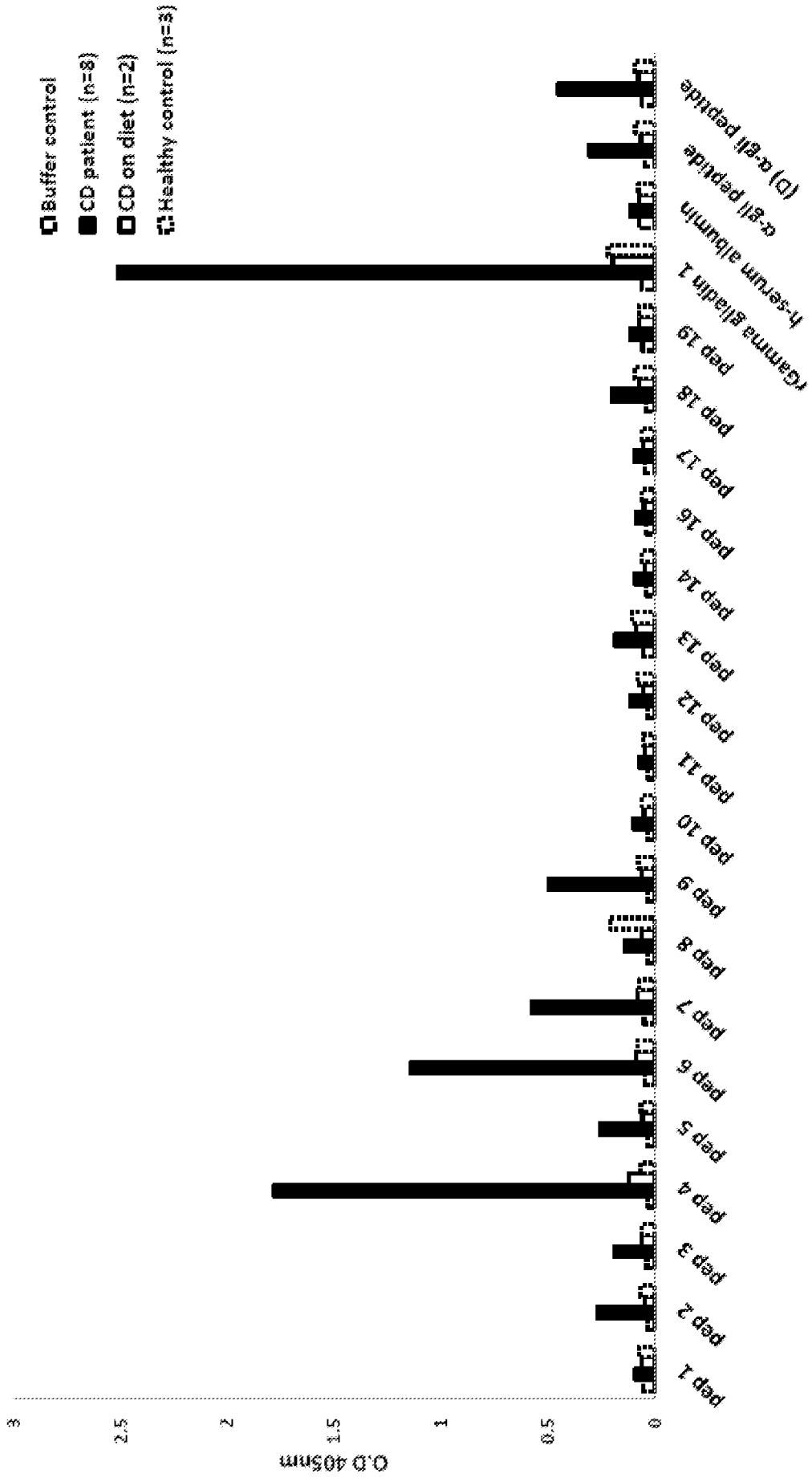

Buffer control
CD patient (n=8)
CD on diet (n=2)
Healthy control (n=3)
O.D 405nm
3
2.5
2
1.5
1
0.5
0
pep 1
pep 2
pep 3
pep 4
pep 5
pep 6
pep 7
pep 8
pep 9
pep 10
pep 11
pep 12
pep 13
pep 14
pep 16
pep 17
pep 18
pep 19
(Gamma gliadin 1
h-serum albumin
α-gli peptide
(D) α-gli peptide

Fig. 16

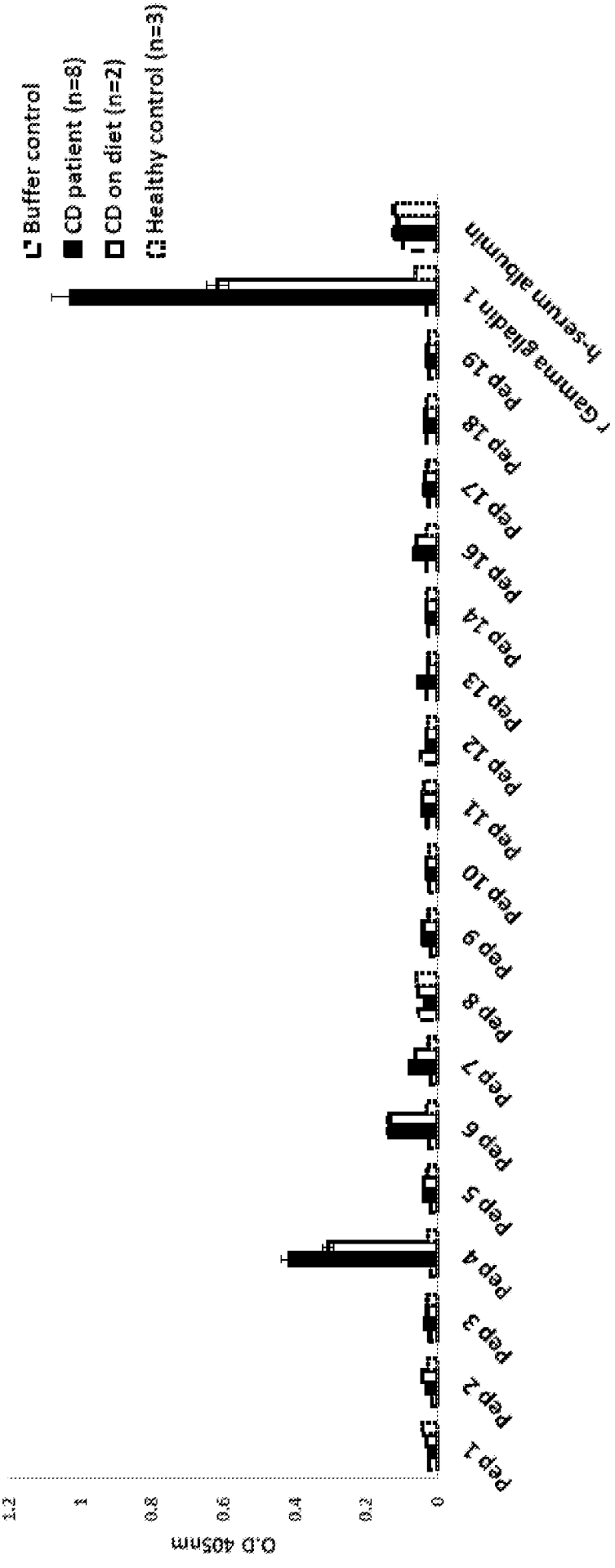

Buffer control
CD patient (n=8)
CD on diet (n=2)
Healthy control (n=3)
O.D 405nm
1.2
1
0.8
0.6
0.4
0.2
0
h-serum albumin
r Gamma gliadin 1
pep 19
pep 18
pep 17
pep 16
pep 14
pep 13
pep 12
pep 11
pep 10
pep 9
pep 8
pep 7
pep 6
pep 5
pep 4
pep 3
pep 2
pep 1

Fig. 17

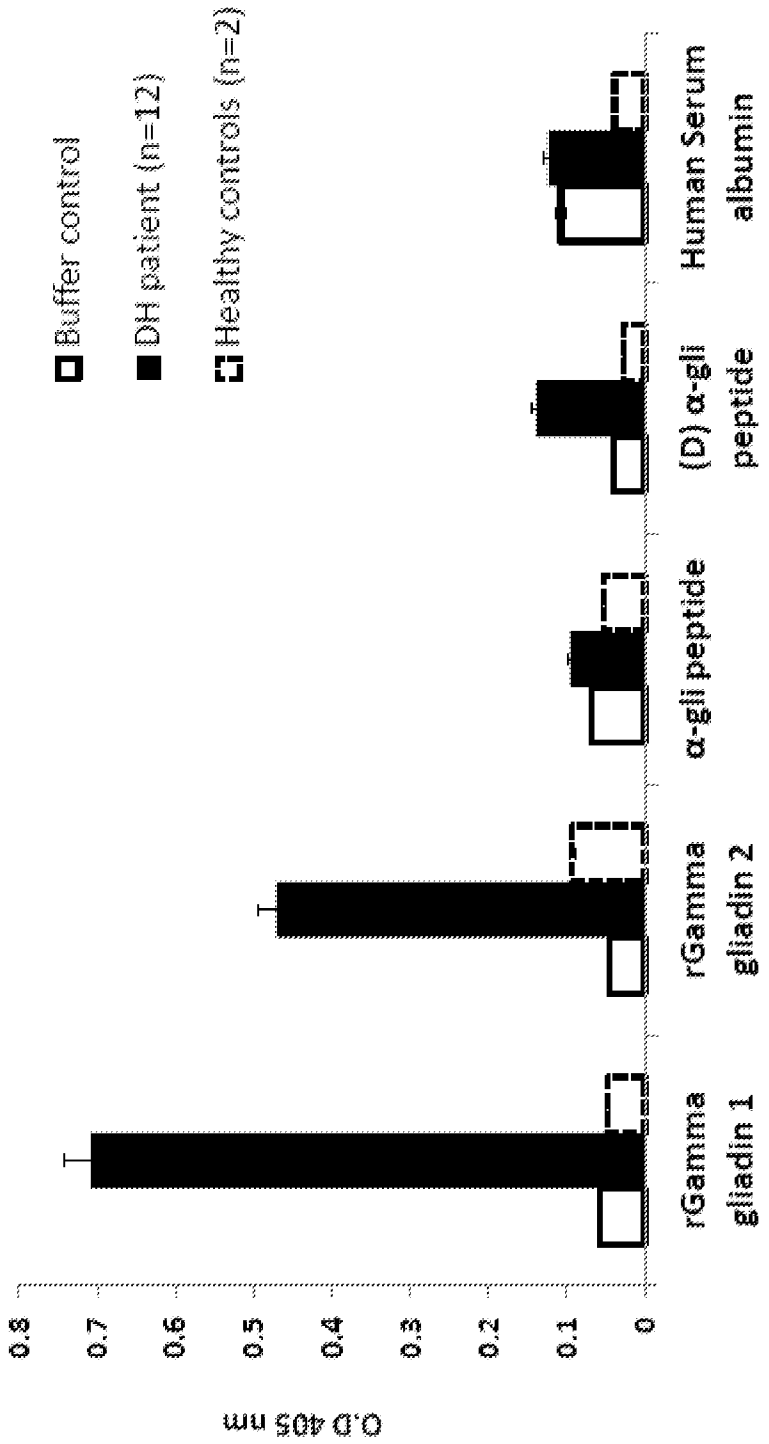

Buffer control
DH patient (n=12)
Healthy controls (n=2)
O.D 405 nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
rGamma gliadin 1
rGamma gliadin 2
α-gli peptide
(D) α-gli peptide
Human Serum albumin

Fig. 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K, A61P, C07K, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, WPI data, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE, EBI;Registry

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005105129 A2 (BTG INT LTD ET AL), 10 November 2005 (2005-11-10); Comprising peptides 100% similar to parts of SEQ ID NO 26 and the whole or parts of SEQ ID NO: 90-98; see extra sheet for details --	1-19
X	EP 0905518 A1 (ACADEMISCH ZIEKENHUIS LEIDEN ET AL), 31 March 1999 (1999-03-31); The following sequences are related to SEQ ID NO: 26: SEQ ID NO 8 (15 aa) 100.0% identity in 15 aa overlap between aa 33-47; --	1-19

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25-07-2012	26-07-2012
Name and mailing address of the ISA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Patrick Andersson Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SNEGAROFF J et al., "Recombinant Proteins and Peptides as Tools for Studying IgE Reactivity with Low-Molecular-Weight Glutenin Subunits in Some Wheat Allergies", 2007, vol 55, pages 9837-9845, Journal of Agricultural Food Chemistry; whole document	1-6, 10-13, 17-19
A	--	7-9, 14-16
X	AKAGAWA et al. "Proteomic Analysis of Wheat Flour Allergens", 2007, vol 55, pages 6863-6870, Journal of Agricultural Food Chemistry; table 2 points 14-16	1-8, 12-13, 17-19
A	--	9-11, 14-16
A	UNIPROT Accession no. B2Y2Q7, 2008-07-01 [online] [2011-08-25] Retrieved from: EBI; Database UNIPROT; whole document; 100% identity in 369 aa overlap with SEQ. ID. NO. 51 and 76,6 % identity in 333 aa overlap with SEQ ID NO:26	1-6
X	--	7-19
X	WO 03104273 A2 (ISIS INNOVATION ET AL), 18 December 2003 (2003-12-18); Comprising peptides 100% similar to parts of SEQ ID NO 26 and the whole or parts of SEQ ID NO: 89-94, 96-107; see extra sheet for details	1-19
	--	
X	JP 2006126083 A (UNIV SHIMANE), 18 May 2006 (2006-05-18); figure 3; SEQ ID NO: 3 has identity with SEQ ID NO: 26 in a 7 aa overlap & Database JPOP[online]Accession number BD937799, 26 March 2007, retrieved from EBI 58.1% identity (65.4% similar) in 356 aa overlap with SEQ ID NO: 26	1-8, 12-19
A	--	9-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0125793 A2 (ISIS INNOVATION ET AL), 12 April 2001 (2001-04-12); The following sequences are related to SEQ ID NO 26: SEQ ID NO 57 (15 aa) 100.0% identity in a 15 aa overlap between aa 33-47; SEQ ID NO 660 100.0% identity in 20 aa overlap between aa 176-195; SEQ ID NO 667 (20 aa) 100.0% identity in 20 aa overlap between aa 224-243; SEQ ID NO 659 (20 aa) 100.0% identity in 20 aa overlap between aa 168-187; SEQ ID NO 656 (20 aa) 100.0% identity in 20 aa overlap between aa 144-163; SEQ ID NO 670 (20 aa) 100.0% identity in 20 aa overlap between aa 248-267; SEQ ID NO 669 (20 aa) 100.0% identity in 20 aa overlap between aa 240-259; SEQ ID NO 662 (20 aa) 100.0% identity in 20 aa overlap between aa 190-209; SEQ ID NO 671 (20 aa) 100.0% identity in 20 aa overlap between aa 256-275; SEQ ID NO 664 (20 aa) 100.0% identity in 20 aa overlap between aa 200-219; SEQ ID NO 100 (15 aa) 100.0% identity in 15 aa overlap between aa 33-47; --	1-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROCHER A et al., "Characterization of distinct alpha- and gamma-type gliadins and low molecular weight components from wheat endosperm as coeliac immunoreactive proteins.", 1995, vol 1247, pages 143-148, Biochim. Biophys. Acta; whole document; table 1,gamma2-40 100.0% identity (100.0% similar) in 18 aa overlap with SEQ ID NO: 89	5-19
A	--	1-4
X	VACCINO, P et al. " A catalogue of Triticum monococcum ge nes encoding toxic and immunogenic peptides for celiac disease patients", 2009, vol 281, pages 289-300, Molecular Genetics and Genomics; STN International File Registry Retrieved on 26 august 2011 RN:s= 1144154-61-9, 1144154-59-5, 1144154-57-3, 1144154-55-1, 1144154-51-7, 1144154-47-1, 1144154-45-9	1-7, 10-19
A	--	8-9
X	BOUCHET-MAHIOUT I et al. "Low Molecular Weight Glutenins in Wheat-Dependant, Exercise-Induced Anaphylaxis:Allergenicity and Antigenic Relationships with Omega 5-Gliadins", 2010, vol 153, sida 35-45; whole document	1-6, 10-13, 17-19
A	--	7-9
X	WO 2006112925 A2 (UNIV MARYLAND ET AL), 26 October 2006 (2006-10-26); SEQ ID NO 7 is identical with present SEQ ID NO: 87	5-13, 17-19
	--	
A	SKERRITT JH, Antigenicity of Wheat Prolanis: Detailed Epitope Analysis using a Panel of Monoclonal Antibodies", 2000, vol32, pages 259-279, Journal of Cereal Science; whole document	1-13, 17-19
	--	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009139887 A2 (UNIV LELAND STANFORD JUNIOR ET AL), 19 November 2009 (2009-11-19); The following sequences are related to SEQ ID NO 92: SEQ ID NO 10 100.0% identity (100.0% similar) in 20 aa overlap The following sequences are related to SEQ ID NO 94: SEQ ID NO 19 100.0% identity (100.0% similar) in 19 aa overlap ---	1-19
X	US 20060286601 A1 (MARTI THOMAS ET AL), 21 December 2006 (2006-12-21); The following sequences are related to SEQ ID NO 92:SEQ ID NO 48 100.0% identity (100.0% similar) in 20 aa overlap SEQ ID NO 49 100.0% identity (100.0% similar) in 20 aa overlap SEQ ID NO 50 100.0% identity (100.0% similar) in 18 aa overlap The following sequences are related to SEQ ID NO 94: SEQ ID NO 59 100.0% identity (100.0% similar) in 19 aa overlap ---	1-19
X	WO 9956698 A2 (KOEBENHAVNS UNI ET AL), 11 November 1999 (1999-11-11); The following sequences are related to SEQ ID NO 97: AAY53687 100.0% identity (100.0% similar) in 18 aa overlap, page 11;AAY53686 100.0% identity (100.0% similar) in 17 aa overlap, page 76;AAY53679 100.0% identity (100.0% similar) in 17 aa overlap, page 27 ---	1-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02083722 A2 (ACADEMISCH ZIEKENHUIS LEIDEN ET AL), 24 October 2002 (2002-10-24); The following sequences are related to SEQ ID NO 105: AAE34158 100.0% identity (100.0% similar) in 15 aa overlap, see fig 4;AAE34159 100.0% identity (100.0% similar) in 15 aa overlap, see fig 4	1-19
A	-- --------	1-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050121

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Invention 1: Parts of claims 1-13 and 17-19 directed to a polypeptide or an epitope related to respectively amino acid sequence of SEQ ID NO: 26, 51 and nucleic acid SEQ ID NO 1.
Inventions 2-72: Parts of claims 1-19 directed to a polypeptide or an epitope related to respectively amino acid sequence SEQ ID NO: 27-50, 62-86 and 89-110, as well as the corresponding proteins SEQ ID NO: 52-61 and corresponding nucleic acid SEQ ID NO 2-25.
Inventions 73-74: Parts of claims 7-19 directed to a polypeptide or an epitope related to SEQ ID NO:s 87 or 88 respectively.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: parts of 1-19, see below

Invention 1, and subject matter of claims 1-19 related to SEQ ID NO: 87, and 89-107

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of: citation of documents

Indication of relevant sequences

WO 2005105129 A2 (BTG INT LTD ET AL), 10 November 2005 (2005-11-10);

The following sequences are related to SEQ ID NO 26:
SEQ ID NO 1114 100.0% identity in 20 aa overlap between aa 176-195;
SEQ ID NO 1119 (20 aa) 100.0% identity in 20 aa overlap between aa 179-198;
SEQ ID NO 522 (20 aa) 100.0% identity in 20 aa overlap between aa 168-187;
SEQ ID NO 518 (20 aa) 100.0% identity in 20 aa overlap between aa 170-189;
SEQ ID NO 1116 (20 aa) 100.0% identity in 20 aa overlap between aa 183-202;
SEQ ID NO 1294 (20 aa) 100.0% identity in 20 aa overlap between aa 233-252;
SEQ ID NO 1292 (20 aa) 100.0% identity in 20 aa overlap between aa 232-251;
SEQ ID NO 1537 (20 aa) 100.0% identity in 19 aa overlap between aa 147-165;
SEQ ID NO 1298 (20 aa) 100.0% identity in 20 aa overlap between aa 229-248;
SEQ ID NO 1299 (20 aa) 100.0% identity in 20 aa overlap between aa 229-248;
SEQ ID NO 1122 (20 aa) 100.0% identity in 20 aa overlap between aa 186-205;
SEQ ID NO 515 (20 aa) 100.0% identity in 19 aa overlap between aa 167-185;
SEQ ID NO 1282 (20 aa) 100.0% identity in 20 aa overlap between aa 256-275;
SEQ ID NO 617 (20 aa) 100.0% identity in 20 aa overlap between aa 200-219;
SEQ ID NO 629 (20 aa) 100.0% identity in 20 aa overlap between aa 200-219;
SEQ ID NO 1847 (16 aa) 100.0% identity in 16 aa overlap between aa 34-49:

The following sequences are related to SEQ ID NO: 90:
SEQ ID NO 923 100.0% identity (100.0% similar) in 19 aa overlap;
SEQ ID NO 924 100.0% identity (100.0% similar) in 15 aa overlap
SEQ ID NO 1037 100.0% identity (100.0% similar) in 20 aa overlap
SEQ ID NO 1046 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1035 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1042 100.0% identity (100.0% similar) in 18 aa overlap

The following sequences are related to SEQ ID NO 91:
SEQ ID NO 1037 100.0% identity (100.0% similar) in 20 aa overlap
SEQ ID NO 1046 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1035 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1042 100.0% identity (100.0% similar) in 18 aa overlap

The following sequences are related to SEQ ID NO 92:
SEQ ID NO 749 100.0% identity (100.0% similar) in 20 aa overlap
SEQ ID NO 747 100.0% identity (100.0% similar) in 20 aa overlap

The following sequences are related to SEQ ID NO 93:
SEQ ID NO 672 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1231 100.0% identity (100.0% similar) in 18 aa overlap

The following sequences are related to SEQ ID NO 94:
SEQ ID NO 649 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 1255 100.0% identity (100.0% similar) in 19 aa overlap

.../...

Continuation of: extra sheet

SEQ ID NO 669 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 1726 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 634 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1229 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 670 100.0% identity (100.0% similar) in 18 aa overlap

The following sequences are related to SEQ ID NO 95:
SEQ ID NO 653 100.0% identity (100.0% similar) in 20 aa overlap
SEQ ID NO 1749 100.0% identity (100.0% similar) in 20 aa overlap
SEQ ID NO 1735 100.0% identity (100.0% similar) in 20 aa overlap

The following sequences are related to SEQ ID NO 96:
SEQ ID NO 272 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 273 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 1492 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 489 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 1494 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 458 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 487 100.0% identity (100.0% similar) in 19 aa overlap
SEQ ID NO 440 100.0% identity (100.0% similar) in 19 aa overlap

The following sequences are related to SEQ ID NO 97:
SEQ ID NO 1741 100.0% identity (100.0% similar) in 18 aa overlap
SEQ ID NO 1478 100.0% identity (100.0% similar) in 17 aa overlap

The following sequences are related to SEQ ID NO 98
SEQ ID NO 1479 100.0% identity (100.0% similar) in 16 aa overlap

WO2003104273, A2 (ISIS INNOVATION ET AL), 18 December 2003 (2003-12-18);
The following sequences are related to SEQ ID NO 26:
SEQ ID NO 57 (15 aa) 100.0% identity in a 15 aa overlap between aa 33-47;
SEQ ID NO 660 100.0% identity in 20 aa overlap between aa 176-195;
SEQ ID NO 667 (20 aa) 100.0% identity in 20 aa overlap between aa 224-243;
SEQ ID NO 659 (20 aa) 100.0% identity in 20 aa overlap between aa 168-187;
SEQ ID NO 656 (20 aa) 100.0% identity in 20 aa overlap between aa 144-163 ;
SEQ ID NO 670 (20 aa)100.0% identity in 20 aa overlap between aa 248-267;
SEQ ID NO 669 (20 aa) 100.0% identity in 20 aa overlap between aa 240-259;
SEQ ID NO 662 (20 aa) 100.0% identity in 20 aa overlap between aa 190-209;
SEQ ID NO 671 (20 aa) 100.0% identity in 20 aa overlap between aa 256-275;
SEQ ID NO 664 (20 aa) 100.0% identity in 20 aa overlap between aa 200-219;
SEQ ID NO 100 (15 aa) 100.0% identity in 15 aa overlap between aa 33-47

The following sequences are related to SEQ ID NO 89:
ADH15845 100.0% identity (100.0% similar) in 17 aa overlap, see page 110
ADH15445 100.0% identity (100.0% similar) in 17 aa overlap, see page 103

.../...

Continuation of: extra sheet

The following sequences are related to SEQ ID NO 90:
ADH15860 100.0% identity (100.0% similar) in 20 aa overlap
ADH14939 100.0% identity (100.0% similar) in 20 aa overlap

The following sequences are related to SEQ ID NO 91:
ADH15877 100.0% identity (100.0% similar) in 18 aa overlap , see page 111
ADH14956 100.0% identity (100.0% similar) in 18 aa overlap, see page 99
ADH14751 100.0% identity (100.0% similar) in 18 aa overlap, see page 94

The following sequences are related to SEQ ID NO 92:
ADH15894 100.0% identity (100.0% similar) in 20 aa overlap,page 111
ADH14973 100.0% identity (100.0% similar) in 20 aa overlap

The following sequences are related to SEQ ID NO 93:
ADH14825 100.0% identity (100.0% similar) in 18 aa overlap, page 95
ADH14990 100.0% identity (100.0% similar) in 18 aa overlap, page 99
ADH14753 100.0% identity (100.0% similar) in 18 aa overlap, see page 94
ADH15911 100.0% identity (100.0% similar) in 18 aa overlap, see page 94

The following sequences are related to SEQ ID NO 94:
ADH15068 100.0% identity (100.0% similar) in 19 aa overlap, page 100
ADH14730 100.0% identity (100.0% similar) in 19 aa overlap, page 94
SEQ ID NO 34 100.0% identity (100.0% similar) in 19 aa overlap
ADH15923 100.0% identity (100.0% similar) in 19 aa overlap, page 112
ADH15066 100.0% identity (100.0% similar) in 19 aa overlap, page 100
ADH15925 100.0% identity (100.0% similar) in 19 aa overlap, page 112

The following sequences are related to SEQ ID NO 96:
ADH15083 100.0% identity (100.0% similar) in 19 aa overlap, page 100
ADH14742 100.0% identity (100.0% similar) in 19 aa overlap, page 94
ADH15940 100.0% identity (100.0% similar) in 19 aa overlap, page 112

The following sequences are related to SEQ ID NO 97:
ADH6193 100.0% identity (100.0% similar) in 17 aa overlap, see example 6, fig 12 h

The following sequences are related to SEQ ID NO 98:
ADH15955 100.0% identity (100.0% similar) in 16 aa overlap, see page 112
ADH15098 100.0% identity (100.0% similar) in 16 aa overlap, see page 100
ADH15103 100.0% identity (100.0% similar) in 16 aa overlap, see page 100
ADH15960 100.0% identity (100.0% similar) in 16 aa overlap, see page 113

The following sequences are related to SEQ ID NO 99:
ADH15108 100.0% identity (100.0% similar) in 17 aa overlap, see page 100
ADH15965 100.0% identity (100.0% similar) in 17 aa overlap, see page 113
ADH15113 100.0% identity (100.0% similar) in 15 aa overlap, see page 100
ADH15970 100.0% identity (100.0% similar) in 15 aa overlap, see page 113

.../...

Continuation of: extra sheet

The following sequences are related to SEQ ID NO 100:
ADH15975 100.0% identity (100.0% similar) in 20 aa overlap, page 113
ADH15118 100.0% identity (100.0% similar) in 20 aa overlap, page 100

The following sequences are related to SEQ ID NO 101:
ADH15986 100.0% identity (100.0% similar) in 20 aa overlap, page 113
ADH15192 100.0% identity (100.0% similar) in 20 aa overlap, page 101

The following sequences are related to SEQ ID NO 102:
ADH15205 100.0% identity (100.0% similar) in 20 aa overlap, page 101
ADH15999 100.0% identity (100.0% similar) in 20 aa overlap, page 113

The following sequences are related to SEQ ID NO 103:
ADH15219 100.0% identity (100.0% similar) in 19 aa overlap, page 101
ADH16006 100.0% identity (100.0% similar) in 13 aa overlap, page 113
ADH15212 100.0% identity (100.0% similar) in 13 aa overlap, page 101

The following sequences are related to SEQ ID NO 104:
ADH15236 100.0% identity (100.0% similar) in 17 aa overlap, see page 101
ADH16030 100.0% identity (100.0% similar) in 17 aa overlap, see page 114
ADH15228 100.0% identity (100.0% similar) in 15 aa overlap, see page 100
ADH16022 100.0% identity (100.0% similar) in 15 aa overlap, see page 114

The following sequences are related to SEQ ID NO 105:
ADH16034 100.0% identity (100.0% similar) in 16 aa overlap, see page 114
ADH15240 100.0% identity (100.0% similar) in 16 aa overlap, see page 101
ADH16035 100.0% identity (100.0% similar) in 16 aa overlap, see page 114
ADH15241 100.0% identity (100.0% similar) in 16 aa overlap, see page 101
ADH15244 100.0% identity (100.0% similar) in 17 aa overlap, see page 101
ADH16038 100.0% identity (100.0% similar) in 16 aa overlap, see page 114
AAE15242 100.0% identity (100.0% similar) in 15 aa overlap, see page 101
ADH16036 100.0% identity (100.0% similar) in 16 aa overlap, see page 114

The following sequences are related to SEQ ID NO 106:
ADH16051 100.0% identity (100.0% similar) in 18 aa overlap, page 114
ADH15321 100.0% identity (100.0% similar) in 18 aa overlap, page 102

The following sequences are related to SEQ ID NO 107:
ADH15333 100.0% identity (100.0% similar) in 18 aa overlap, see page 102
ADH16063 100.0% identity (100.0% similar) in 18 aa overlap, see page 114
ADH15327 100.0% identity (100.0% similar) in 15 aa overlap, see page 102

Continuation of: second sheet

International Patent Classification (IPC)

C07K 14/415 (2006.01)
A61K 38/16 (2006.01)
A61P 37/00 (2006.01)
G01N 33/68 (2006.01)

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Use the application number as username. The password is **YDSRZFZGRE**.

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Cited literature, if any, will be enclosed in paper form.

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				CA	2564521	A1	10/11/2005
				EP	2412380	A1	01/02/2012
				EP	1755639	A2	28/02/2007
				JP	2008508856	A	27/03/2008
				MX	PA06012322	A	31/01/2007
				NZ	550600	A	26/03/2010
				US	20080318852	A1	25/12/2008
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WO	03104273	A2	18/12/2003	AU	2003244771	A1	22/12/2003
				CA	2488218	A1	18/12/2003
				EP	2292649	A2	09/03/2011
				EP	1513873	A2	16/03/2005
				JP	2010229138	A	14/10/2010
				JP	2006512893	A	20/04/2006
				MX	PA04012117	A	19/04/2005
				NZ	537226	A	30/06/2008
				US	20060178299	A1	10/08/2006
				ZA	200409740	A	27/09/2006
JP	2006126083	A	18/05/2006	JP	4157947	B2	01/10/2008
WO	0125793	A2	12/04/2001	AT	319091	T	15/03/2006
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