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REVIEW

Allergens in wheat and related cereals

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Summary

Wheat is one of the major crops grown, processed and consumed by humankind and is associated with both intolerances (notably coeliac disease) and allergies.

Two types of allergy are particularly well characterized. The first is bakers' asthma, which results from the inhalation of flour and dust during grain processing. Although a number of wheat proteins have been shown to bind IgE from patients with bakers' asthma, there is no doubt a well-characterized group of inhibitors of α-amylase (also called chloroform methanol soluble, or CM, proteins) are the major components responsible for this syndrome. The second well-characterized form of allergy to wheat proteins is wheat-dependent exercise-induced anaphylaxis (WDEIA), with the ω_5-gliadins (part of the gluten protein fraction) being the major group of proteins which are responsible. Other forms of food allergy have also been reported, with the proteins responsible including gluten proteins, CM proteins and non-specific lipid transfer proteins.

Processing of wheat and of related cereals (barley and rye, which may contain related allergens) may lead to decreased allergenicity while genetic engineering technology offers opportunities to eliminate allergens by suppressing gene expression.

Keywords allergy, cereals, wheat
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Introduction

World agriculture is dominated by three cereal crops – wheat, rice and maize – which each yield about 600 million tonnes per year. Of these, wheat is the most widely grown, from Scandinavia and Russia in the north to Argentina in the south, including higher areas in the tropics. It is immensely diverse, with over 25 000 different cultivars having been produced by plant breeders worldwide [1]. Much of the world production of wheat is consumed by humans, after processing to bread and other baked goods, pasta and noodles and, in the Middle East and North Africa, bulgar and couscous. In addition, the wide availability of wheat flour and the functional properties of wheat starch and gluten proteins mean that it is widely used as an ingredient in food processing. In fact, a brief inspection of processed foods currently on sale in Western Europe and North America shows that wheat flour, proteins or starch are present in a surprisingly high percentage, highlighting the problem for those suffering from dietary allergy or intolerance to wheat. In addition, many workers in the milling, baking and food processing industries are exposed to wheat flour in the atmosphere, raising the risk of respiratory allergy.

Much of the research on adverse reactions to wheat has focused on respiratory allergy (bakers' asthma) which is one of the most important occupational allergies in many countries including UK (see below) and on coeliac disease, a form of gluten intolerance which is estimated to affect about 1% of the population in Europe, North and South America, North Africa and the Indian subcontinent [2]. Dietary allergy to wheat is probably less widespread in the general population but may affect 1% of children [3] and in its extreme form may lead to anaphylaxis and death.

The proteins which are responsible for dietary allergy in wheat are also less clearly defined than those contributing to bakers' asthma but recent studies indicate that there are intriguing similarities and differences between the two conditions. The present article therefore reviews our current knowledge of the protein responsible for dietary

and respiratory allergy to wheat in relation to their structures and biological properties.

Cereals related to wheat

The taxonomic relationships of the cereals reflect, to a large extent, the chemical structures of their seed storage protein components. The cereals form part of the Gramineae (grasses) family and are divided into four subfamilies: the Bambusoideae (rice), the Chloridoideae (including ragi and tef), the Panicoideae (most millets, maize and sorghum) and the Pooideae, which are further divided into the Triticeae (wheat, barley and rye) and the Aveneae (oats). The prolamins of rice are not closely related to the other cereals, whereas the prolamins of the Panicoideae and Chloridoideae appear to be closely related to each other. The prolamins of the Triticeae are closely related with homologous groups of proteins present in all three species. They are also related to the prolamins (aveains) of oats, but less closely.

Types of cereal grain protein

The wheat grain comprises three major components: starch, protein and fibre (cell wall polysaccharides), with proteins accounting for about 10–15% of the dry weight. Although this variation is influenced by genetics, the major impact comes from nutrition with the grain protein content being directly related to the availability of nitrogen.

The analysis of the proteins of wheat and other cereals has a long and distinguished history, dating back over 250 years (the isolation of wheat gluten being first described in scientific literature in 1745). This early work established a classification based on extraction in a series of solvents, which was formalized by the American protein chemist T. B. Osborne working in the late 19th/early 20th century [4]. He defined four wheat protein fractions (often called 'Osborne fractions'), which are extracted sequentially in water (albumins), dilute saline (globulins), alcohol/water mixtures (prolamins) and dilute acid (glutenins). The terms 'albumin' and 'globulin' are also more widely used for proteins with similar solubility properties from other organisms while the prolamins are given specific names in different cereals: gliadins in wheat, hordeins in barley, secalins in rye and zeins in maize.

Gluten proteins

Although the Osborne classification is still widely used by cereal chemists, it is now more usual to classify wheat proteins based on their functions as well as other properties. Approximately 80% of the total grain protein is accounted for by the major storage protein fraction. These proteins are deposited in discrete protein bodies in the cells of the developing grain and digested during germination to provide nutrients for the growing seedling. They also form a continuous network when wheat flour is mixed with water to form dough; washing to remove most of the soluble and particulate material (notably starch) allows them to be isolated as a substantially pure cohesive mass. This mass is termed 'gluten' and comprises approximately equal amounts of the gliadin and glutenin fractions as defined by Osborne, although each comprises a complex mixture of components and all of these are structurally related to a greater or lesser extent. The gliadins are monomeric proteins which interact by non-covalent forces (notably hydrogen bonds) and are classified into three groups on the basis of their electrophoretic mobility at low pH: these are α/β-gliadins (fast), γ-gliadins (intermediate) and ω-gliadins (slow). The glutenins are polymers of individual proteins linked by interchain disulphide bonds. On reduction, the component subunits are classified into high molecular weight (HMW) and low molecular weight (LMW) groups after separation by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE). These groups are summarized in Fig. 1 and reviewed in reference [5].

Wheat gluten proteins are characterized by high contents of glutamine and other amino acids (proline, glycine, phenylalanine) in different groups. These amino acids derive from the presence of domains comprising repeated sequences, tandem or interspersed blocks based on short (up to 10 amino acids) peptide motifs. In fact, the name prolamin was coined to reflect their high contents of proline and amide nitrogen (now known to be derived from glutamine).

The gluten proteins are the major determinant of the processing properties of wheat flour, conferring the cohesiveness and viscoelasticity that allows dough to be processed into bread, noodles and other foods. They are also responsible for triggering coeliac disease and other intolerances in susceptible individuals. Consequently,

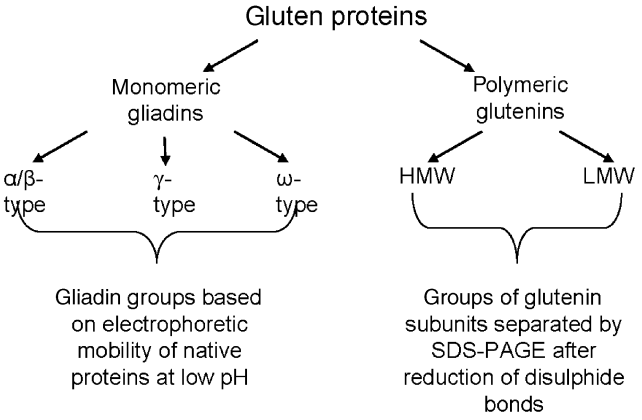

Fig. 1. Classification of wheat gluten proteins.

there is a massive literature on the structures and properties of wheat gluten and its component proteins.

Other wheat grain proteins

Proteomic analyses of developing and mature grain reveal over 1000 individual components, many of which correspond to 'house keeping' proteins (i.e. structural and metabolic proteins which are present throughout the plant) [6, 7]. However, several groups of proteins are specific to the grain and may be present in significant quantities. These include β-amylase, which is stored in a latent form in preparation for germination, inhibitors of hydrolytic enzymes (notably α-amylase and proteinases) and surface-active proteins such as non-specific lipid transfer proteins (LTPs) and puroindolines. Many of these proteins are presumed to be protective in function, contributing to a broad-spectrum resistance to fungal pathogens and invertebrate pests (as discussed in reference [8]). The degree of complexity is illustrated in Fig. 2 that shows a 2D separation of endosperm proteins at a late stage of grain development.

Wheat contains two types of α-amylase inhibitor. One of these is a bifunctional protein of M_r about 20 500, which is capable of simultaneously inhibiting endogenous wheat α-amylase and the proteinase subtilisin. This protein is called wheat amylase/subtilisin inhibitor or WASI [9] and does not appear to be an allergen. The second and quantitatively major group of α-amylase inhibitors is of particular interest as they are probably the most important wheat proteins contributing to bakers' asthma. They are often called chloroform methanol proteins or 'CM' proteins as they were initially defined based on their selective extraction in chloroform : methanol mixtures. They are now known to comprise a mixture of monomeric, dimeric and tetrameric forms based on at least 11 subunits (see Table 1 and Fig. 2) with masses ranging from about 12 000 to 16 000 [10]. These forms differ in their spectrum of activity but all inhibit mammalian and insect enzymes but not the endogenous α-amylases of wheat, implying a protective role. They are encoded by genes on the group 3, 4, 6 and 7 chromosomes of the B and D genomes of hexaploid bread wheat (which comprises three genomes called A, B and D) (see Table 1 and footnote). As the D genome is not present in tetraploid pasta wheat (which has only the A and B genomes), the inhibitory spectrum in pasta wheat will differ. Several forms are indicated as 1–6 in Fig. 2.

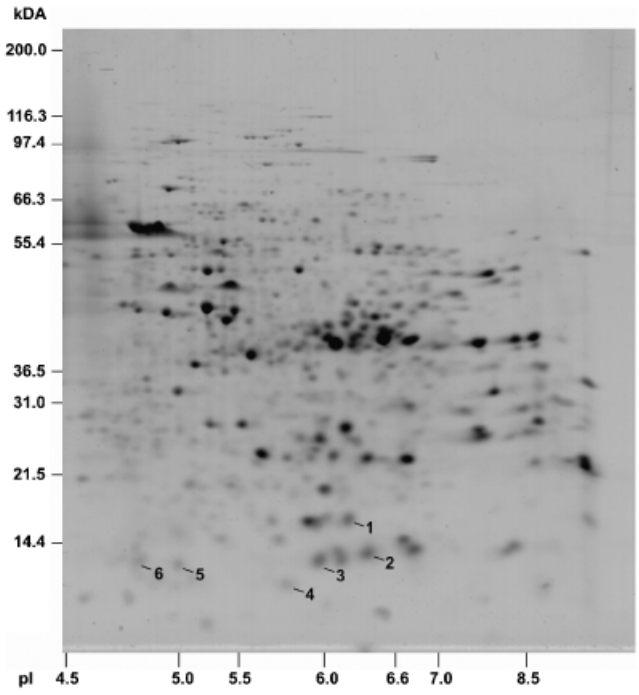

Fig. 2. Proteomic map of wheat starchy endosperm tissue at 36 days after flowering. This tissue corresponds to the white flour fraction that is the most widely consumed part of the grain. Proteins are separated by 2D isoelectric focusing/sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE). Protein identities were determined by mass spectrometry, showing that 1–6 correspond to α-amylase inhibitors that have been implicated in bakers' asthma and food allergy. Taken from Vensel et al. [7] with permission.

Three of these inhibitor forms have also been designated as 0.19, 0.53 (both dimeric) and 0.28 (monomeric) based on their relative electrophoretic mobility, while at least one subunit (WTAI-CM16) occurs in non-glycosylated and glycosylated forms (Table 1).

It is probable that the CM proteins are deposited in protein bodies in the developing grain, together with the gluten proteins. However, these deposits become disrupted as the grain matures to form a continuous proteinaceous matrix, and at least part of the inhibitor fraction becomes associated with the surface of starch granules released by dry milling [11].

Bakers' asthma

Bakers' asthma and rhinitis are well characterized allergic responses to the inhalation of wheat and cereal flours and dusts. They have been recognized since Roman times when slaves who handled flour and dough were required to wear masks, and described by Ramazzini in about 1700 [12]. Bakers' asthma is now recognized as one of the most common types of occupational asthma, for example, it is recognized as the second commonest type by the UK Health and Safety Executive (www.hse.gov.uk/asthma/bakers.htm). A number of studies have documented the incidence of bakers' asthma in different countries (reviewed in references [13, 14]) while a recent study of apprentice bakers in Poland reported that chest symptoms ascribed to occupational asthma were observed in 4.2% of the apprentices after only 1 year and 8.6% after 2 years [15]. The corresponding values for allergic rhinitis were 8.4% and 12.5%, respectively. Diagnosis is usually

Table 1. Characteristics of the wheat α-amylase inhibitors and their identification as allergens in bakers' asthma or food allergy

				Bakers' asthma			Food allergy		
Aggregation	Subunit	Synonym	Chromosomal location	Test	Positive patients	Reference	Test	Positive patients	Reference
Monomeric	WMAI-1	0.28	*6D*	Skin prick	9/31	[25]			
				Immunoblot	Pooled sera	[31, 32, 34]			
				RAST	11/11	[22]			
	WMAI-2	–	*6B*	Not reported	Not reported				
Homodimeric	WDAI-1	0.53	*3B*	Skin prick, immunoblot	5/31	[25]	Immunoblot	5/5	[85]
	WDAI-2	0.19	*3D*	Immunoblot	Pooled sera	[34]	Expression library	*	[46]
							Immunoblot	*	[40]
	WDAI-1 or WDAI-2			Immunoblot	4/8	[33]			
	WDAI-3		*3B*	Not reported	Not reported				
Tetrameric									
First subunit	WTAI-CM1		*7D*	Not reported			Immunoblot	*	[40]
	WTAI-CM2		*7B*	Skin prick	11/31	[25]	Immunoblot	*	[40]
Second subunit	WTAI-CM16		*4B*	Skin prick	7/31	[25]	Skin prick	1/1	[84]
							Immunoblot	*	[40]
	WTAI-CM16†		*4B*	Skin prick	14/31	[25]			
	WTAI-CM17		*4D*	Not reported			Expression library		[46]
	WTAI-CM16/16† and/or WTAI-CM17						Immunoblot	7/30	[38]
Third subunit (two copies)	WTAI-CM3B		*4B*	Skin prick	11/31	[25]			
	WTAI-CM3D		*4D*	Not reported					
	WTAI-CM3A or WTAI-CM3D						ELISA, skin	8/11	[83]
							Prick	1/1	[84]
							Immunoblot	*	[40]

*Details not provided.

†Glycosylated form.

Note: The chromosomal locations of the genes encoding the individual subunits are given in italics. Bread wheat has three related genomes, A, B and D, each comprising seven pairs of chromosomes while durum (pasta) wheat (*Triticum durum*) is tetraploid, with only the A and B genomes. Hence, the subunits present in these species also differ. See also references [138, 139] for descriptions of the distributions of the major subunits in these species.

based on skin prick tests and the demonstration of specific IgE in serum.

Little was known about the proteins responsible for bakers' asthma until the application of electrophoresis combined with immunochemistry in the 1970s. Such early studies showed that multiple allergens were present, with the water-soluble albumins being particularly reactive with the IgE fractions from patients' sera [16–19]) (see also the excellent review in reference [20]).

More recent studies have identified individual proteins which are recognized by IgE from patients' sera. It is clear that one group of wheat proteins are the most important allergens, the α-amylase inhibitors. However, a number of other proteins present in wheat, enzyme improvers and other baking ingredients have also been shown to react with IgE, although in many cases these have only been identified in single studies and their clinical relevance is unclear. This apparent diversity could reflect differences in populations or in the different approaches used to identify the IgE reactive proteins.

Cereal α-amylase inhibitors

The cereal grain α-amylase inhibitors (CM proteins) appear to be the major group of wheat proteins responsible for bakers' asthma, with an extensive literature from a number of independent studies (reviewed in reference [21]). These date from 1989, when Walsh and Howden

[22] and Barber et al. [23] identified allergenic components from wheat and barley, respectively. These were followed by a series of detailed studies, notably from the group of Salcedo and co-workers in Madrid [24–28]. Their studies showed that the proteins differ in their reactivity with IgE fractions, with glycosylated forms of the wheat tetrameric subunit WTAI-CM16 (termed WTAI-CM16*) and of the related barley subunit BTAI-CMb (BTAI-CMb*) being the most active and the non-glycosylated forms showing less activity. The barley monomeric inhibitor BMAI-1 also showed strong activity. However, Armentia et al. [25] showed that four other wheat subunits (WTAI-CM2, WTAI-CM3B, WDAI-1, WMAI-1) and two other barley subunits (BDAI-1 and the related monomeric trypsin inhibitor BTI-CMe) also reacted with IgE, as did homologous inhibitors from rye [29, 30]. The reactivities of the wheat inhibitors are summarized in Table 1.

Studies from other laboratories have largely confirmed these observations. Gorg and co-workers identified WMAI-1 as part of the detailed proteomic studies discussed below [31, 32]. Fränken et al. [33] demonstrated that a mixture of dimeric wheat inhibitors WDAI-1 (0.53) and WDAI-2 (0.19) bound to IgE from German patients while Amano et al. [34] showed that WMAI-1 (0.28) and WDAI-2 (0.19) were the major immunoreactive components using sera from Japanese patients. The latter group also showed reactions with the barley monomeric trypsin inhibitor BTI-CMe.

It is probable that only WTAI-CM16* and BTAI-CMb* are glycosylated. Certainly, Amano et al. [34] showed that their WMAI-1 (0.28) and WDAI-2 (0.19) fractions contained less than one sugar residue per mole of protein, and that recombinant WDAI-2 (0.19) expressed in *Escherichia coli* (which does not carry out protein glycosylation) had the same reactivity as the native protein purified from wheat.

It is also possible that the differences in reactivity of the individual CM proteins relate to their relative abundance as well as to intrinsic differences in their structures (notably the presence or absence of glycosylation).

Other wheat proteins

A number of other wheat grain proteins have been reported to bind to IgE from patients with bakers' asthma, as summarized in Table 2.

Studies with purified proteins have shown IgE reactions with wheat germ agglutinin [35], peroxidase [36] and LTP [37]. It is of interest that both peroxidase [38, 39] and LTP [40] have also been reported to be active in food allergy to wheat while LTPs are the major allergens in many other species [41–44]. Weichel et al. [45] have also identified wheat thioredoxin *h* b (Tri a 25) as a potential allergen in bakers' asthma, initially using IgE to screen an expression library constructed with endosperm cDNA and then producing recombinant protein for ELISA and immunoblotting. Cross-reactivity was also observed with maize thioredoxin (Zea m 25) and two out of the eight positive sera also reacted with the human form of the enzyme. A further study from the same group also reported IgE binding to thioredoxins in patients with food allergy to wheat and maize [46].

Table 2. Wheat grain proteins that have been shown to react with IgE from patients with bakers' asthma

Protein	$\approx M_r$	Test	Positive patients	Reference
Wheat germ agglutinin	17	RAST	5/9	[35]
Peroxidase	36	Dot blot	6/10	[36]
Thioredoxin (Tri a 25)	13 400	ELISA/immunoblot	8/17	[45]
Gliadins				
Fast ω	45–65 000	Immunoblot/RAST	21/24	[49]
Slow ω		Immunoblot/RAST	15/24	[49]
α	30–45 000	Immunoblot/RAST	24/24	[49]
β			18/24	[49]
γ			12/24	[49]
Total		Immunoblot	2/15	[50]
Total glutenins	45–90 000	Immunoblot/RAST	23/24	[49]
		Immunoblot	7/15	[50]
α-Amylase (barley)	54 000	RAST/immunoblot	29/30	[47]
β-Amylase (barley)	64 000	RAST/immunoblot	30/30	[47]
Acyl CoA oxidase (two forms)	27 000	Immunoblot	Pooled sera	[31, 32]
Glycerinaldehyde-3-phosphate dehydrogenase (five forms)	40–42 000	Immunoblot	8/10	[52]
Triosephosphate isomerase cytosolic (two forms)	26 000	Immunoblot	5/10	[52]
Serpin (two forms)	40 000	Immunoblot	4/10	[52]

See also Table 1 for details of the wheat amylase inhibitors.

Sandiford et al. [47–49] used immunoblotting to identify IgE against a range of wheat gluten proteins. All of the patients' sera tested showed IgE binding to α-gliadins, 21 out of 24 to fast ω-gliadin, 18 out of 24 to β-gliadin, 12 out of 24 to γ-gliadin and 15 out of 24 to slow ω-gliadin. Competitive binding studies with salt-soluble proteins also showed reduced binding to purified α- and γ-gliadins (but not to β- or ω-gliadins) indicating the presence of common epitopes.

Reactions with gluten proteins have also been shown by a more recent study [50], which used western blotting of fractions corresponding to total gliadins, glutenins and albumins+globulins with IgE from 15 patients with bakers' asthma. Nine of the sera reacted with albumins+globulins, two with gliadins and seven with glutenins. In the same study, sera from patients with food allergy to wheat (children and adults) and wheat-dependent

exercise-induced anaphylaxis (WDEIA) (adults) also showed reactions with the same protein fractions on immunoblotting, although the spectrum varied among individuals (children generally showing the widest spectrum).

Sandiford et al. [47] also showed that 29 out of 30 sera from patients with bakers' asthma contained specific IgE to barley α-amylase, compared with only 16 which reacted with fungal α-amylase (see above). All the sera also contained IgE to barley β-amylase and competitive RAST showed antigenic identity among the three amylase preparations. The authors used commercial enzyme preparations and SDS–PAGE showed that neither of the barley enzymes was pure. Nevertheless, western blotting with 11 sera confirmed that most of these reacted with the major proteins present which were presumed to correspond to the amylases.

Several studies have adopted a more detailed proteomic approach, separating either total flour proteins or albumin+globulin fractions by 2D electrophoresis, identifying potential allergens by immunoblotting and determining their identities by amino acid sequencing.

Weiss et al. [51] extracted wheat grain sequentially to prepare salt-soluble proteins (albumin+globulin), gliadins and glutenins. The individual fractions and total grain extracts were then separated by 2D electrophoresis and 1D SDS–PAGE and probed by western blotting to identify components which bound to IgE from serum pooled from four asthmatic bakers. This showed binding to a range of albumins, globulins and gliadins, with the highest percentage of the IgE being bound to a M_r 27 000 albumin protein. Similar results were obtained with fractions from seven different cultivars, although the relative levels of binding between components varied. The same workers subsequently carried out more detailed studies using 2D gel analysis of total proteins and albumins+globulins and *N*-terminal amino acid sequencing to determine the identities of reactive proteins excised from the gels. Posch et al. [31] separated approximately 700 proteins and probed their separations with IgE from four pooled sera. Seventeen major reactive proteins were identified of which nine were subjected to *N*-terminal sequencing. Two of these corresponded to the M_r 27 000 allergen identified by Weiss et al. [32] and comparisons with databases showed that they corresponded to forms of the enzyme acyl CoA oxidase. Similarly, two low M_r (14 000–18 000) allergens corresponded to the monomeric α-amylase inhibitor WMAI-1 (see above). The sequences of the five other proteins did not show any significant similarity to those in databases and they could not be identified.

Weiss et al. [32] extended this work using albumin+globulin fractions from 30 cultivars and pooled sera from 20 bakers suffering from asthma or rhinitis. Nine immunoreactive proteins corresponding to those studied by Posch et al. [31] were *N*-terminally sequenced, confirming that two components were related to acyl CoA oxidase and two to wheat α-amylase inhibitors. No other positive identifications were made.

A similar proteomic study was reported by Sander et al. [52], who separated about 300 albumin+globulin components from whole grain. Ten separate sera from allergic patients were used, showing between about 10 and 50 immunoreactive spots in each case. Many of these spots were unique to a single serum, but about 40 were recognized by two or more sera. Nine of the most abundant spots were selected for identification using mass spectrometry. This identified five as corresponding to forms of glyceraldehyde-3-phosphate dehydrogenase, two to forms of triose phosphate isomerase and two to serpin proteinase inhibitors. A related barley serpin, called protein Z, is also one of the major grain-derived allergens in beer [53].

Other ingredients

Soya flour is widely used as an additive in bread, at a level of about 0.7%. Its activity is mainly due to the enzyme lipoxygenase which oxidizes flour carotenoids to increase the whiteness of the loaf and also improves mixing, loaf volume and crumb properties [54]. Soybean is a major source of food allergy and respiratory allergy in those handling the grain. Hence, it is perhaps not surprising that soybean allergens have also been implicated in bakers' asthma. Thus, Baur et al. [55] showed that the soybean trypsin inhibitor and lipoxygenase (also called lipoxidase) reacted with IgE from bakers with respiratory symptoms, while Quirce et al. [56] showed that the reactive proteins differed from those responsible for respiratory allergy in those handling soybean seeds, with only one out of four patients' sera showing a reaction with Gly m 2 and none to Gly m 1 (the two major hull allergens) [57, 58].

Asthmatic reactions in bakers to soybean lecithin [59], which is commonly used as an emulsifier [54] and to egg albumin [60, 61] have also been reported.

Fungal enzymes

Enzymes are frequently used as aids in cereal processing, the most important being α-amylase to digest starch and provide sugar for the yeast during bread proofing, hemicellulases (xylanases) to partially digest the cell wall arabinoxylans and increase their solubility, and proteinases to weaken the gluten network where doughs are too strong [62]. However, the amounts and types of enzymes used as processing aids are increasing and hence the exposure of bakers to potential allergens is also increasing [63].

The most widely used form of α-amylase is derived from the fungus *Aspergillus oryzae* and a number of studies have identified this enzyme as a cause of bakers' asthma [64–67].

Allergic responses to a number of other enzymes have also been reported: cellulase [66, 68], xylanase [68, 69], β-xylosidase [70] and glucoamylase [71].

The same enzymes may also sensitize via the skin, leading to urticaria and dermatitis [68, 72, 73].

Food allergy

Allergic responses to the ingestion of wheat can be divided into two types. WDEIA is a clearly defined syndrome that is associated with one major type of grain protein, ω_5-gliadins and is discussed in detail below. Other allergic responses that include atopic dermatitis, urticaria and anaphylaxis and appear to be related to a range of wheat proteins. These may vary between populations and be related to age and symptoms. Although less well understood than WDEIA, it is clear that most of the proteins that have been identified as related to food allergy of wheat are abundant with storage or protective functions, and some also contribute to respiratory allergy (see below).

Gluten proteins

The gluten proteins account for a high proportion of the total grain proteins so it is perhaps not surprising that they have been implicated in wheat allergy.

Mittag et al. [50] showed that IgE fractions from children and adults reacted with gliadin and glutenin fractions on immunoblots, although only eight and five sera, respectively, were used and not all of these showed reactions. Battais et al. [74] carried out more detailed studies with purified proteins using RAST assays with sera from 28 patients. They showed that 60% had IgE to α-gliadins, β-gliadins and LMW subunits, 55% to γ-gliadins, 48% to ω-gliadins and 26% to HMW subunits. Immunoblotting was used to confirm these results and to show that 67% also had IgE to albumins+globulins. Further studies from the same group [75] showed differences between sera of 60 patients that were related to age and symptoms. Patients with atopic dermatitis (AD) showed strong reactions with albumins+globulins (80% and 92%, respectively, of those with or without associated asthma) while children frequently showed antibodies to α-, β- and γ-gliadins. All patients with anaphylaxis or WDEIA and 55% of those with urticaria had IgE to ω_5-gliadins.

Similar wide ranges of reactions with gluten proteins were reported by Maruyama et al. [76] who used recombinant gliadins (α, γ, ω) and gluten subunits (LMW, HMW) for blotting with 10 sera, and Pastorello et al. [40] who used western blotting of wheat protein fractions with 22 antisera and mass spectrometry to identify α-gliadin and LMW subunits among a number of immunoreactive bands. Gliadins (α, γ) were also identified by screening a wheat endosperm cDNA expression library with sera from patients with anaphylaxis after ingestion of wheat or with positive double-blind placebo-controlled food challenge to wheat [46]. Similarly, Mesa-del-Castillo et al. [77] showed the presence of a major immunoreactive band of M_r 35 000 which was extractable in 1% acetic acid and sensitive to pepsin digestion: this probably corresponded to a mixture of gliadins. Akagawa et al. [78] also showed IgE binding to γ-gliadin and LMW subunits in a proteomic study using pooled sera from seven patients.

Watanabe et al. [79] isolated a peptide from a chymotryptic digest of gluten, using serum from a single allergic patient. The peptide comprised 30 residues and corresponded to part of the repetitive domain of an LMW subunit of glutenin. Further studies using synthetic peptides and sera from four patients with atopic dermatitis allowed the IgE-binding epitope to be defined as QQQPP [80].

Battais et al. [81] also used synthetic peptides to scan for gliadin epitopes in patients presenting different symptoms to ingested wheat. Patients with WDEIA (three adults), anaphylaxis (one child) and urticaria (two adults) reacted with the same epitopes corresponding to sequences in the repetitive domains of gliadins. These included two immunodominant epitopes on ω_5-gliadin that were related to those identified by Matsuo et al. [82] (Table 3). In contrast, sera from four children with atopic eczema/dermatitis (one of whom also had asthma) did not react with any linear gliadin epitopes while serum from a third child with eczema/dermatitis and asthma reacted with non-repetitive sequences present in α-, β- and γ-gliadins. The authors also concluded that the B epitopes in wheat allergy were not related to those established for coeliac disease.

Table 3. Protein epitopes identified as associated with wheat-dependent exercise-induced anaphylaxis (WDEIA)

	Epitopes	
Protein	Dominant	References
ω_5-Gliadin	QQIPQQQ	[82]
	QQFPQQQ	
	QQSPEQQ	
	QQSPQQQ	
	Others	
	QQLPQQQ	
	QQYPQQQ	
	PYPP	
	QQFHQQQ	[100]
	QSPEQQQ	
	YQQYPQQ	
	QQPPQQ	
	QQQLPQQQ	[81]
	QQQFPQQQ	
HMW subunits	QQPGQ	[105]
	QQPGQGQQ	
	QQSGQGQ	

HMW, high molecular weight.

These results confirm the report from the same group that reactions with gliadins vary depending on age and symptoms [75].

Other proteins

Pastorello et al. [40] identified a number of other proteins that bound to IgE, present in albumin+globulin, gliadin and glutenin fractions, using immunoblotting combined with mass spectrometric analysis to determine the identities of the reactive proteins. Furthermore, a number of these proteins had been identified previously in other studies and/or have been shown to be active in bakers' asthma. Several of the IgE-reactive proteins appeared to be α-amylase inhibitor subunits, notably WTAI-CM3 and WTAI-CM16 but also WTAI-CM1, WDAI-2 (0.19) and WTAI-CM2. Similarly, Akagawa et al. [78] identified wheat dimeric inhibitors by proteomic analysis using 2D gels. A wide screen used IgE to probe a cDNA expression library and also identified α-amylase inhibitors; in this case WDAI-2 (0.19) and WTAI-CM7 [46]. Kusaba-Nakayama et al. [83] also showed the binding of IgE from 8 out of 11 patients with atopic dermatitis to purified WTAI-CM3 but not to WTAI-CM2 or WTAI-CM16, while Zapatero et al. [84] reported positive skin prick tests to WTAI-CM3 and WTAI-CM16 but not WTAI-CM2 in a 9-month-old child with anaphylaxis to wheat flour. Similarly, James et al. [85] identified WDAI-1 (0.53) as binding to IgE from five children with wheat allergy while Kimoto et al. [38] showed that 7/30 patients with atopic dermatitis showed IgE binding on immunoblotting with WTAI-CM16 and/or WTAI-CM17. However, the latter authors also reported that their WDAI-CM16 and WDAI-CM17 preparations were *N*-glycosylated. Hence, the former may have corresponded to WDAI-CM16* but the glycosylation of WDAI-CM17 has not been reported by other workers. Simonato et al. [86] also reported that 12/20 patients with atopic symptoms showed IgE binding to a protein of M_r about 16 000 on immunoblotting, but did not identify the precise subunit. It is clear therefore that α-amylase inhibitor subunits are food allergens in wheat but the precise subunits which have been identified vary between studies (Table 1). This may reflect differences between the populations studied or between the analytical approaches.

It is of interest that the α-amylase inhibitor subunits are also active in bakers' asthma, although the precise spectrum of activity may differ between the two syndromes (see Table 1). However, it is notable that so far only two of the 11 α-amylase inhibitor subunits listed in Table 1 have not been described as allergens in bakers' asthma or food allergy. Furthermore, related inhibitors in other cereals are also allergens. Most notably, M_r 14 000–16 000 inhibitors are important dietary allergens in rice, particularly in the Far East [87]. Cristaudo et al. [88] also suggested that a related protein was responsible for contact urticaria and dermatitis in a patient exposed to cornflour, although the immunoreactive protein (M_r 14 000) was not positively identified.

The nsLTP of wheat and other cereals have also been identified as food allergens in several studies. Pastorello et al. [40] used immunoblotting followed by mass spectrometry to identify IgE reactions with wheat LTP in 9 out of 22 patients with food allergy to wheat while Battais et al. [75] showed that 28% of 60 patients with food allergy to wheat showed IgE reactions with purified wheat LTP. The wheat nsLTP is also an allergen in bakers' asthma (Table 2) while the homologues of barley and maize have been reported to be allergens in beer [53, 89] and in maize products [40], respectively. Weichel et al. [45] also identified the LTP of maize as a dietary allergen by expression screening of a cDNA library, but a similar expression screen of wheat failed to identify the wheat LTP.

The expression screening reported by Weichel et al. [45] and immunoblotting study of Pastorello et al. [40] also identified a number of other proteins that bound to IgE from patients with food allergy to wheat. Several of these (wheat germ agglutinin, peroxidase, serpin, β-amylase, thioredoxin *h* B) have also been reported as allergens in bakers' asthma while others (globulin, β-purothionin, puroindolines a and b, tritin, granule-bound starch synthase) have not so far been identified in other studies. Akagawa et al. [78] also identified serpins using pooled sera from seven patients to probe 2D immunoblots.

Wheat-dependent exercise-induced anaphylaxis

Food-dependent exercise-induced anaphylaxis (FDEIA) is a form of allergic reaction induced by the ingestion of a causative food and subsequent physical exercise. Neither the food nor the exercise alone will trigger the reaction; the combination of food and exercise is required. The first cases of exercise-induced anaphylaxis (EIA) were reported by Sheffer and Austen [90], where increased physical activity resulted in patients experiencing the symptoms of anaphylaxis. However, Sheffer and Austin reported no specific allergen exposure before the symptoms developing. Maulitz et al. [91] reported the first case of EIA that was food-related, in a patient who had ingested shellfish before exercise. Since then, a number of different foods have been reported to have caused FDEIA; however, wheat is the most widely reported food to be associated with FDEIA [92–96].

Patients with WDEIA display a range of clinical symptoms from generalized urticaria to severe allergic reactions including anaphylaxis. Challenge tests have been used to determine the causative foods followed by exercise; however, this is not always safe as in some cases the test induces anaphylactic shock. The use of skin prick testing and CAP-RAST has been used to detect those patients with a predisposition to WDEIA.

The major allergens in wheat associated with WDEIA have been identified in Japanese patients by Morita et al. [97–99] and Matsuo et al. [82, 100, 101] and in Finnish patients by Varjonen et al. [102], Palosuo and co-workers [96, 103] and Lehto et al. [104]. Initially a γ-gliadin-like protein was identified by immunoblotting as the major allergen [96–98] but subsequently Palosuo et al. [103] and Morita et al. [99] identified ω_5-gliadins (Tri a 19) as the major allergen in WDEIA. Matsuo et al. [82] used synthetic peptides to identify seven epitopes (QQIPQQQ, QQLPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, QQYPQQQ and PYPP) within the primary sequence of a ω_5-gliadin, pooling the sera of 15 patients. Four of these epitopes were found to be dominant: QQIPQQQ, QQFPQQQ, QQSPEQQ and QQSPQQQ. No full-length sequences were then available for the ω-type gliadins, so Matsuo et al. [82] searched an EST (expressed sequence tag) wheat DNA library for ω_5-gliadin-like sequences. This identified four sequences with GenBank accession numbers BE590637, BQ608902, BQ895830 and BQ245835. Matsuo et al. [105] subsequently identified four further IgE-binding epitope sequences, QQFHQQQ, QSPEQQQ, YQQYPQQ and QQPPQQ, in three patients with WDEIA using a recombinant ω-gliadin. Mutational analysis of the QQIPQQQ and QQFPQQQ peptides indicated that the amino acids at positions Gln 1, Pro 4, Gln 5, Gln 6 and Gln 7 were critical for IgE binding.

Battais et al. [81] also reported two immunodominant epitopes on ω_5-gliadin: QQQLPQQQ and QQQFPQQQ, which agree well with those of Matsuo and co-workers [82, 100, 105].

Palosuo et al. [106] studied cross-reacting allergens from barley, rye and oats and identified γ-70 and γ-35 secalins in rye and γ-3 hordeins in barley as the major allergens; there was no cross-reactivity with oat proteins. Of 23 patients with WDEIA, 21 (91%) showed IgE binding to γ-70 secalin, 19 (83%) to γ-35 secalin and 21 (91%) to γ-3 hordein.

The HMW subunits of wheat glutenin also reacted with IgE from patients with WDEIA [99]. Matsuo et al. [105] determined the IgE-binding epitopes of wheat HMW glutenin subunits using synthetic peptides and identified three epitopes from the repetitive domain, QQPGQ, QQPGQGQQ and QQSGQGQ. Twenty-nine out of thirty patients with WDEIA had specific IgE to these epitopes, and to those of ω_5-gliadin. Twenty-five patients with atopic dermatitis with specific IgE to wheat showed little or no cross-reactivity with these epitopes and the authors suggested that these peptides might be useful for diagnosis of WDEIA in patients.

Palosuo et al. [106] also identified ω_5-gliadin as an allergen in children with an immediate-type allergy to wheat. Sixteen out of nineteen children (84%) had IgE antibodies to ω_5-gliadin but no IgE to ω-5 was found in children with delayed challenge symptoms or in the negative controls. Palosuo et al. [107] also studied the effects of transglutaminase-mediated cross-linking of a pepsin–trypsin digest of ω_5-gliadin to determine whether cross-linking could be involved in modulating IgE-binding activity. Transglutaminase cross-linked peptides from the pepsin or pepsin–trypsin digested ω_5-gliadin caused marked increases in IgE binding both *in vitro* and *in vivo* as determined by ELISA and skin prick testing. It was suggested that activation during exercise of transglutaminase in the intestinal mucosa of patients with WDEIA could lead to the formation of large allergen complexes capable of eliciting anaphylactic reactions.

Aspirin has been shown to increase the levels of circulating gliadin peptides in patients with WDEIA. Harada et al. [108] examined the effects of aspirin as a substitute for exercise in three patients with FDEIA, two of whom had WDEIA, and reported that aspirin up-regulated the allergic response to specific foods. Aihara et al. [109] also reported that aspirin pre-treatment enhanced the effects of skin prick testing in five of eight WDEIA patients and five of seven when administered in oral testing; aspirin and wheat alone without exercise provoked reactions in two out of five patients. Matsuo et al. [101] studied six patients with anaphylaxis associated with wheat ingestion and found increased serum levels of gliadin peptides indicating that exercise and aspirin facilitated gliadin peptide absorption from the intestine. Serum gliadin levels also increased in healthy individuals but did not elicit an allergic reaction. Morita et al. [110] found that the levels of gliadin peptides in the serum of patients increased during provocation tests with both wheat–exercise and wheat–aspirin challenges, and that the increases in the level of serum gliadin peptides occurred in parallel with the development of allergic symptoms. This indicates that both exercise and aspirin facilitate allergen absorption from the intestine.

Effects of processing on allergenicity

Wheat and related cereals are only consumed by humans after processing, which varies from simple cooking (e.g. pearl barley in stews) to complex fermentation processes (beer, spirits). These processes clearly have impacts on the food components, resulting in both chemical and physical modifications, which may affect the allergenicity of protein components. Processing can result in a reduction, no change or an increase in the number of epitopes in a protein. Thermal treatment of proteins in processed foods may result in a number of modifications including denaturation, hydrolysis of peptide bonds, aggregation by non-covalent and disulphide bonding, and reaction with other food molecules, such as sugars in Maillard reactions, lipids and carbohydrates. Biochemical processes can also occur including a range of enzyme-mediated reactions including proteolysis, oxidation or reactions with transglutaminases. The potential degree of complexity of the

reactions in a food material is, therefore, enormous. The most well-defined covalent reactions of proteins in foods are Maillard reactions between reducing sugars (and sugar breakdown products) and the protein amino groups. The chemical processes are complex but the resultant protein adducts have brown colouration which is often desirable in cooked foods. These reactions can occur at room temperature on storage, but at a much slower rate. Maillard products of peanut processing have been implicated in enhancing the allergenicity of peanut proteins through the formation of higher molecular weight aggregates and increasing resistance to digestion in the gut [111]. Furthermore, the nature of the food matrix may affect the allergenic properties of proteins: in one study the reaction to peanut proteins was modified by the fat content of the protein mixture [112]. Enzymic treatment to target the allergenic epitopes while maintaining the functional/quality/processing properties of the proteins, has so far yielded mixed results although hypoallergenic milk formulations have been produced with reduced allergenicity. However, there is scant literature of the effects of processing on cereal allergens.

Heating

Varjonen et al. [113] extracted proteins from wheat, barley and rye flours with buffered saline (albumins+globulins) and with sodium acetate buffer at pH 3.8 (gliadins, hordeins and secalins), after heating at 80 °C, 100 °C or 120 °C for 10, 20 or 60 min. Similar fractions were also extracted from doughs containing 50% water that had been subjected to the same heating regime. The IgE-binding capacity of the extracted proteins was reduced after heating, particularly after heating of doughs, but was not abolished even after heating at 120 °C for 60 min. However, the authors were unable to rule out the effects of heating on protein extractability which could be expected as denaturation is known to reduce protein solubility. Sutton et al. [19] also showed that heating decreased the allergenicity of extracted gluten.

In contrast, Simonato et al. [114] suggested that the allergenicity of wheat gluten proteins might actually be increased by cooking. They showed that the ability of enzymes to digest allergenic epitopes *in vitro* was reduced in bread compared with dough and suggested that baking increases the resistance of potential allergens to digestion *in vivo*, allowing them to reach the small intestine and elicit allergenic responses.

Pastorello et al. [40] also reported that the *in vitro* reactions of extracted wheat protein fractions with IgE from patients with dietary allergy to wheat were similar whether they had been extracted from flour or from a suspension of flour and water that had been heated to boiling and then cooled. Similar observations were also made with double-blind placebo-controlled food challenges and some patients reacted with a lower dose of cooked flour than raw flour: this is consistent with the hypothesis of Simonato et al. [114].

Enzymic digestion

Watanabe and co-workers have developed enzymic methods for the production of hypoallergenic wheat, focussing on the digestion of known epitopes and particularly the QQQPP epitope identified for LMW subunits of glutenin [79, 115–117]. However, it has also been suggested that processing of gluten to give functional ingredients may introduce epitopes, possibly by deamination [118]. Lauriére et al. [119] also showed that severe allergic reactions (urticaria or anaphylaxis) can occur to hydrolysed wheat proteins used in cosmetics, despite the patients showing tolerance to unmodified wheat proteins in skin tests. However, the same patients did show IgE reactions with unmodified proteins, in all cases with albumins+globulins and in some cases with gliadins.

Malting and brewing

Allergic reactions have been reported to beers brewed from barley and wheat, with anaphylaxis occurring in extreme cases [120, 121]. The major allergens derived from barley have been identified as the non-specific lipid transfer protein LTP1 and the serpin proteinase inhibitor Z_4 [53]. Furthermore, the LTP1 retained its allergenicity despite being unfolded and highly modified [122]. It is possible that related proteins are responsible in wheat beers as both LTP1 and serpins have been identified as food allergens (as discussed above), but this has not been investigated.

Application of genetic engineering technology

The use of genetic engineering to down-regulate gene expression is now routine in many crop plants, including all the major cereals, and is hence an attractive option for reducing allergens in cereals and other plants (including pollen allergens). The first documented use of this approach was in rice, where Tada et al. [123] used anti-sense technology to down-regulate the expression of the gene family encoding the allergenic α-amylase inhibitors (CM proteins). These are major food allergens in Japan and are related to the wheat α-amylase inhibitors listed in Table 1 (for a review see reference [21]). The anti-sense suppression reduced the protein level to about 20% of that in the control plants, resulting in grain that is hypoallergenic rather than non-allergenic.

An alternative approach, transgene-induced gene silencing, was used by Hermann et al. [124, 125] to suppress the expression of the Gly m Bd 30k (cysteine proteinase-related) allergen of soybean while RNAi technology was

used to silence Mal d 1 in transgenic apple [126]. Similarly, anti-sense suppression has been used to reduce pollen allergens in rye grain (*Lolium* spp.): Lol p 1, Lol p2 [127] and Lol p 5 [128, 129].

Anti-sense suppression, transgene-induced gene silencing and RNAi silencing all act at the mRNA level, preventing translation rather than transcription (reviewed in reference [130]). Although there is no evidence that they will not provide stable suppression of allergen synthesis in the long term, it is fair to say that they have not been assessed over the many generations that many crop plants are grown.

Two more sophisticated approaches may therefore be more attractive, although neither is currently routinely applicable to crop plants. Targeted gene replacement by homologous recombination is now feasible for many microbes but has only been reported at a low frequency for one crop plant, rice [131]. Chimeraplasty uses chimeric RNA/DNA oligonucleotides to induce specific mutations in DNA sequences and has been applied to maize [132] and rice cells [133].

Both these approaches could be used to reduce allergenicity by making stable heritable changes in the plant DNA.

Conclusions

Although it is probable that the full range of wheat proteins responsible for bakers' asthma and food allergy has not been identified, it is likely that the major components have been characterized. Based on this, it is possible to draw some conclusions.

Firstly, there is a clear overlap between the spectra of proteins responsible for the two syndromes but their relative importance differs. There is little doubt that the most important components contributing to bakers' asthma are α-amylase inhibitor subunits whereas these appear to be less important in food allergy. Similarly, the gluten proteins appear to be more important in food allergy, particularly ω_5-gliadin in WDEIA, and less so in bakers' asthma. Several other proteins such as wheat germ agglutin, thioredoxin, serpin and β-amylase have also been reported to contribute to both forms of allergy, although all appear to be minor contributors and their clinical relevance requires further study.

Secondly, many of the most active proteins belong to a large group of proteins called the 'prolamin superfamily'. This was initially defined as comprising proteins related to the prolamin storage proteins of cereal grain [134, 135] and also contains a range of small sulphur-rich proteins including the cereal α-amylase inhibitors, the nsLTPs and puroindolines, all of which have been described as allergens in wheat. In fact, Jenkins et al. [136] surveyed the 129 food allergens then included in the PROTALL (now InformALL, www.informall.eu.com) and FARRP (www.allergenonline.com) databases and showed that almost 40% belonged to the prolamin superfamily, compared with less than 5% of the 152 respiratory allergens in the FARRP database. Several of the groups of allergens in the prolamin superfamily are also sufficiently widely distributed to be classified as panallergens, such as the nsLTPs [43, 44] and the 2S albumins [137].

Thirdly, the relative activities of the allergens may relate to their presentation. The binding of some α-amylase inhibitors to starch granules could facilitate their absorption by inhalation while the resistance of the prolamin repetitive domains to digestion, particularly after cooking, could facilitate their presence in the small intestine in largely intact form. It is therefore of particular interest that the epitopes identified in the ω_5-gliadin responsible for WDEIA are derived from the repetitive domain where digestion is inhibited by the high content of prolyl residues.

Finally, it should be noted that the vast majority of the proteins discussed above, and all of those which can be considered to be major allergens, have storage and/or protective functions, although some metabolic proteins have been identified as minor allergens.

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