REVIEW

# Wheat Allergens Associated With Baker's Asthma

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■ Abstract

Baker's asthma is a frequent occupational allergic disease caused mainly by inhalation of cereal flour, particularly wheat flour. This review deals with the current diagnosis and immunomodulatory treatments, as well as the role of wheat allergens as molecular tools to enhance management and knowledge of this disease. The review also discusses the current status of several salt-soluble proteins (albumins and globulins)—cereal $\alpha$-amylase/trypsin inhibitors, peroxidase, thioredoxin, nonspecific lipid transfer protein, serine proteinase inhibitor, and thaumatin-like protein—as well as salt-insoluble storage proteins (prolamins, namely, gliadins and glutenins) as allergens associated with baker's asthma . Finally, current limitations to using these proteins as molecular tools for diagnosis and immunotherapy are highlighted.

Key words: Baker's asthma. Wheat allergens. Component-resolved diagnosis. $\alpha$-amylase/trypsin inhibitors. Nonspecific lipid transfer protein. Prolamin.

■ Resumen

El asma del panadero es una enfermedad alérgica ocupacional frecuente, causada principalmente por la inhalación de harinas de cereales, en particular de trigo. Esta revisión se ocupa de la situación actual del diagnóstico, tratamientos inmunomoduladores, y papel de los alérgenos de trigo como herramientas moleculares para mejorar el conocimiento y manejo de esta enfermedad. El estatus real de varias proteínas solubles (albúminas y globulinas) –inhibidores de $\alpha$-amilasa/tripsina, peroxidasa, tiorredoxina, proteínas de transferencia de lípidos, inhibidor de serin-proteasas y proteínas tipo taumatina- , así como de las proteínas insolubles de reserva (prolaminas: gliadinas y gluteninas), como alérgenos asociados al asma del panadero es discutido. Finalmente, se enfatizan las importantes limitaciones actuales para utilizar estos alérgenos como herramientas moleculares en diagnóstico e inmunoterapia.

Palabras clave: Asma del panadero. Alérgenos de trigo. Diagnóstico por componentes. Inhibidor de $\alpha$-amilasa/tripsina. Proteína de transferencia de lípidos. Prolamina.

## Introduction

Immunoglobulin (Ig) E–mediated allergic reactions to cereal proteins are relatively frequent, can be elicited by exposure through different routes, and affect several populations and age groups worldwide. Although wheat is the most commonly involved cereal, others (eg, rye, barley, rice, maize, and oats) also play a role in allergy. Respiratory allergy to wheat proteins (baker's asthma) is one of the most common types of occupational asthma and its prevalence does not seem to be declining [1]. Baker's asthma is the most frequent type of occupational asthma in France [2]. Exposure to grain and flour dust is the second commonest reported cause of occupational

asthma in the UK [3] and Norway [4]. The estimated annual incidence of cereal-induced asthma in the UK was 811 cases per million people employed over the period 1989-1997 [3], whereas in Norway, the incidence of occupational asthma among male and female bakers was 2.4 and 1 case per 1000 person-years, respectively [4]. The incidence of baker's asthma among young bakers has been reported to range from 0.3 to 2.4 cases per 1000 person-years [5], and an increasing number of asthma cases are being reported among supermarket bakery workers [6].

Immediate hypersensitivity reactions to the ingestion of wheat are not very common and can be divided in 2 types: typical IgE-mediated food allergy, in which patients display

a variety of clinical symptoms ranging from urticaria/ angioedema to vomiting and anaphylaxis, and, sometimes, atopic dermatitis; and wheat-dependent exercise-induced anaphylaxis (WDEIA), which is a well-defined clinical picture commonly associated with sensitization to a major grain allergen, $\omega_5$-gliadin (Tri a 19) [7], whose IgE-binding epitopes have been identified [8]. Measurement of serum IgE antibodies to recombinant $\omega_5$-gliadin has been proposed as a marker of WDEIA [9].

Wheat proteins have also been shown to induce contact urticaria [10] and protein contact dermatitis [11].

Cereals form part of the Poaceae family, and there is wide allergenic cross-reactivity between wheat flour and grass pollen [12]. Recently, microarray assays with recombinant wheat seed and grass pollen allergens have been used to distinguish between baker's asthma, wheat-induced food allergy, and grass pollen allergy [13].

This review focuses on the main wheat allergens associated with baker's asthma. Wheat-induced food allergy and celiac disease, a gluten-sensitive, non–IgE-mediated enteropathy that develops in individuals with a specific genetic background, are not addressed in this article.

# Current Diagnostic Tests and Immunomodulatory Treatments for Patients With Baker's Asthma

## Skin Prick Tests

Skin prick tests (SPT) play an important role in the diagnosis of baker's asthma and in the study of sensitization to cereal flour in field studies. The frequency of sensitization to wheat flour by SPT among bakers in epidemiological studies varies from 5% to 15% [14]. Skin reactivity is related to the quality, potency, and standardization of allergen extracts, which are often poorly defined for cereal and other occupational allergens. Sander et al [15] compared different wheat and rye flour extracts used for skin testing and defined the results of specific inhalation challenge (SIC) as the gold standard. Wheat and rye flour extracts for SPT from 3 companies differed in protein concentrations and composition, resulting in a wide difference in SPT results. Sensitivity of SPTs was between 40% and 67%, specificity was between 86% and 100%, the positive predictive value (PPV) ranged from 81% to 100% and the negative predictive value (NPV) from 44% to 70% [15].

In their multicenter study, van Kampen et al [16] performed SPT with wheat and rye flour extracts from 4 producers in 125 symptomatic bakers. Comparisons between the SPT results of different extracts were made with flour-specific IgE. The optimal cut-off level for all SPT solutions was a wheal size ≥1.5 mm. Again, wide variability was observed between the results of SPT with wheat and rye flour extracts from different producers. These findings indicate that SPT solutions used for the diagnosis of baker's asthma must be improved and standardized.

## Specific IgE Measurements

Sander et al [15] reported that the sensitivity of specific IgE measurements (by either IgE-Enzyme Allergosorbent test or Phadia CAP-system) was higher than SPT with commercial cereal (wheat and rye) extracts. The sensitivity of specific IgE to wheat and rye flour was 83% and 72%, respectively, whereas the specificity was 59% and 81%.

## Specific Inhalation Challenge

SIC is still considered the gold standard for the diagnosis of baker's asthma [17]. Despite the broad allergenic cross-reactivity between wheat and rye flour, some patients may have a negative SIC result with wheat flour and a positive reaction to SIC with rye flour [18], indicating that SIC should be performed with flour from different cereals.

van Kampen et al [19] evaluated the relevance of flour-specific serum IgE and SPT in the diagnosis of baker's asthma and for the purpose of defining flour-specific IgE concentrations and wheal sizes that make it possible to predict the outcome of SIC. The results of the challenge with wheat flour were positive for 37 bakers, while 63 had positive results with rye flour. Depending on the flour-specific IgE concentrations (wheal size), PPV was 74%-100% for wheat and 82%-100% for rye flour, respectively. The minimal cut-off values with a PPV of 100% were 2.32 $kU_A/L$ (wheal size 5.0 mm) for wheat flour and 9.64 $kU_A/L$ (wheal size 4.5 mm) for rye flour. Thus, high concentrations of flour-specific IgE and clear SPT result in symptomatic bakers are good predictors of a positive challenge test result.

These observations show that SIC with cereal flours can be avoided in strongly sensitized bakers. In fact, a systematic literature review showed that in workers with suspected occupational asthma caused by high-molecular-weight (HMW) agents, a positive SPT result and bronchial hyperresponsiveness to methacholine correlates with SIC (high specificity, moderate sensitivity) [20]. The main determinant of a positive SIC to an allergen in patients with baker's asthma is the degree of sensitization to the allergen (as determined by skin reactivity); the SIC is modulated to a lesser extent by nonspecific bronchial hyperresponsiveness [21].

On the other hand, in bakers with persistent cough and a negative asthmatic response to the SIC, a diagnosis of nonasthmatic eosinophilic bronchitis should be considered. Monitoring of airway inflammation by noninvasive methods (induced sputum and/or exhaled nitric oxide) is necessary to confirm the diagnosis [22,23].

## Immunomodulatory Treatments

Removal from exposure to the offending agents is the cornerstone of the management of baker's asthma [24]. Allergen-specific immunotherapy (SIT) and other immunomodulatory treatments, such as anti-IgE monoclonal antibodies (omalizumab), also play a key role in management [24]. Armentia et al [25] published the results of the first double-blind placebo-controlled study of SIT with cereal flour in baker's asthma in 1990. Twenty patients were treated with an aqueous wheat flour extract (Abelló, Madrid, Spain) and 10 with placebo for 10 and 20 months. After SIT, the active group showed a significant decrease in skin sensitivity and also in bronchial hyperresponsiveness to methacholine. Specific IgE

to wheat flour decreased only in patients who were treated with SIT for 20 months. Patients in the active group also reported a significant subjective improvement, whereas patients in the placebo group showed no changes in skin sensitivity or bronchial hyperresponsiveness to methacholine.

Case reports [26] and retrospective studies [27] have also shown the efficacy of wheat flour SIT in baker's asthma. Cirla et al [27] performed an observational cross-sectional retrospective study on 41 sensitized bakers who underwent subcutaneous SIT with wheat flour extract (Lofarma Allergeni, Milan, Italy) for 4 or more years, without having to stop work. The outcome was investigated after 5 and 10 years. Thirty-four subjects out of 41 had an acceptable quality of life and were able to work normally, mainly in small businesses. In the subgroup of 19 previously treated patients, several bakers still at work had stopped SIT, in some cases for as long as 4-10 years. In the subgroup of 15 patients still in treatment, symptoms and drug use during work activity improved or were absent in most cases. The authors suggested that SIT with wheat flour may make it possible to reallocate workers with baker's asthma and may be used in combination with other environmental interventions in the workplace [27].

Omalizumab has a clinical benefit in patients with uncontrolled severe baker's asthma [28,29].

### Wheat Allergens as Molecular Tools to Enhance Diagnosis and Management of Baker's Asthma

The preceding sections point out the strong limitations of the diagnosis and treatment of baker's asthma at present. Isolation and characterization of cereal allergens associated with baker's asthma, particularly from wheat flour, enable us to better define major and minor allergens, thus helping to produce an adequate diagnostic panel of molecular markers. This approach is useful in several ways: *1)* To establish potential links between sensitization profiles and clinical symptoms, geographical areas, or age; *2)* To compare molecules involved in different routes of sensitization (inhalation versus ingestion); *3)* To predict potential cross-reactions with allergens from plant foods or pollens; *4)* To investigate changes in allergenic capacity in cereal (wheat)-derived foodstuffs; *5)* To engineer allergen variants with modified allergenic properties (ie, lower IgE-binding potency).

## Wheat Grain Proteins

Protein represents about 10%-15% (dry weight) of wheat grain. It can be classified in 4 different fractions based on sequential extraction in a series of solvents [30,31] (Figure 1). Interestingly, salt-soluble fractions—albumins and globulins—include only 15%-20% of total protein, whereas most protein components—prolamins (gliadins plus glutenins)—are not extracted by salt solutions. Gliadins are monomeric proteins that are grouped into 3 types—α/ß-, γ- and ω-gliadins—according to their biochemical characteristics and electrophoretic mobility at low pH [32]. Glutenins form polymers maintained by interchain disulphide bridges and are classified into HMW and low-molecular-weight (LMW)

glutenin subunits after reduction and separation using sodium dodecyl sulfate-polyacrylamide gel electrophoresis [32]. Wheat gluten comprises about half amount of gliadin and glutenin fractions.
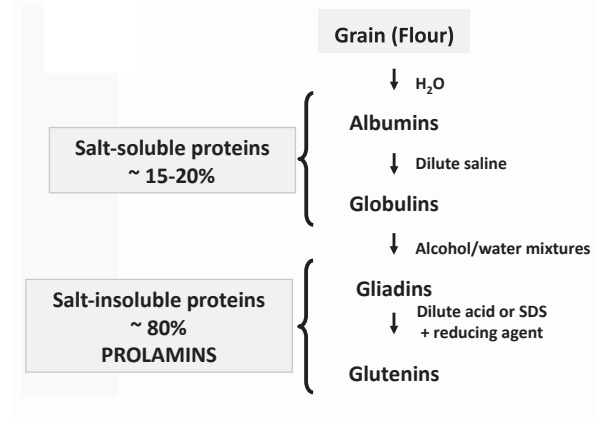


Figure 1. Protein fractions from wheat grain obtained by sequential extraction in different solvents.

Sequential extraction does not render clear-cut preparations, as expected. Thus, cross-contamination among protein fractions may occur; for example, salt-soluble proteins such as α-amylase inhibitor subunits can appear residually in the glutenin fraction [33]. Nevertheless, the peculiar extractability properties of wheat grain proteins constrain commercial diagnostic products; for example, wheat Immuno CAP contains mainly salt-soluble proteins and has to be complemented with glutenin Immuno CAP and/or ω-5 gliadin Immuno CAP in order to ensure a correct diagnosis.

Testing of IgE reactivity in patients with different clinical profiles of wheat allergy (food, WDEIA, baker's asthma) to salt-soluble and salt-insoluble protein fractions from wheat flour [34] revealed a high degree of heterogeneity among recognized allergens in groups with different clinical profiles, as well as within each group. However, mainly salt-soluble proteins seem to be associated with baker's asthma, and prolamins with WDEIA, whereas both protein fractions reacted to IgE from food-allergic patients.

## Wheat Allergens Associated With Baker's Asthma

In the present article we review our current knowledge only of those allergens that have been linked to baker's asthma and assayed as purified proteins in either their natural or recombinant forms. Both criteria have been fulfilled by the α-amylase/trypsin inhibitor family, lipid transfer protein (LTP), peroxidase, thioredoxin, serine proteinase inhibitor, thaumatin-like protein, and some prolamins. Several studies on these allergens show 2 main limitations: no evidence of immunological equivalence between recombinant and natural forms [35] and lack of assays to determine in vivo activity (skin

prick test, SPT) or biological activity (eg, basophil activation test, histamine release tests). Wheat allergens associated with food allergy and/or WDEIA, but not with baker's asthma, are not specifically considered.

Besides the allergens mentioned above, additional IgE-binding proteins (eg, acyl-CoA oxidase, fructose-bisphosphate aldolase, triosephosphate isomerase, glycerinaldehyde-3-phosphate dehydrogenase, and serpin), have been located using a proteomic approach based on 2D-electrophoresis to separate total flour protein or protein fractions and IgE-immunodetection to detect putative allergens [36-38]. Interestingly, considerable interindividual variation of the 2D IgE-binding profile of protein maps has been found in patients with baker's asthma [38]. Furthermore, ß-amylase (from barley flour) [39] and Tri a Bd 27K, a member of the γ-interferon-inducible thiol reductases [40], have been reported as putative allergens, although neither protein purity [39] nor the number and type of sera tested have been clarified [40]. Finally, recent data on a potential association between baker's asthma and allergy to kiwifruit [41] suggest that wheat thiol-proteases homologous to kiwi Act d 1 may be responsible for wheat–kiwi cross-reactivity.

In addition to wheat (cereal) proteins, a wide array of components, mostly additives used to improve wheat flour quality for baking, such as fungal enzymes (mainly *Aspergillus*-derived α-amylase) [39,42,43], have also been associated with baker's asthma.

All the wheat allergens described below, except prolamins, are salt-soluble proteins. Consequently, their presence in commercial diagnostic tools would be warranted.

## The Cereal α-Amylase/Trypsin Inhibitor Family

Current immunological and clinical data point to the α-amylase/trypsin inhibitor family as the main culprit of baker's asthma [31,44]. The family comprises a large proportion of the salt-soluble proteins from wheat flour, including the so-called CM proteins, which can be extracted with chloroform/methanol mixtures, as well as with salt solutions [45].

The cereal α-amylase/trypsin inhibitor subunits are 12-16–kDa polypeptides with 4-5 intrachain disulphide bridges that are essential for their inhibitory activity [45]. Members of the inhibitor family are restricted to the seed storage tissue (endosperm), and seem to have a common fold (4-5 α-helices and a short antiparallel ß-sheet) [45,46].

The inhibitor subunits are encoded by a multigene family in wheat, rye, and barley. Thus, up to 12 different subunits have been characterized in a single bread wheat (*Triticum aestivum*) cultivar, and most of them had IgE-binding capacity (see below) [44,45]. Amino acid sequence identity between members of the family ranges from around 30% to 95%.

Based on their degree of aggregation, 3 types of α-amylase inhibitors have been identified in wheat flour, namely, monomeric (1 subunit), homodimeric (2 identical subunits), and heterotetrameric (3 different subunits, one of them in two copies) [44,45,47] (Figure 2). Trypsin inhibitors belong to the monomeric type [45].
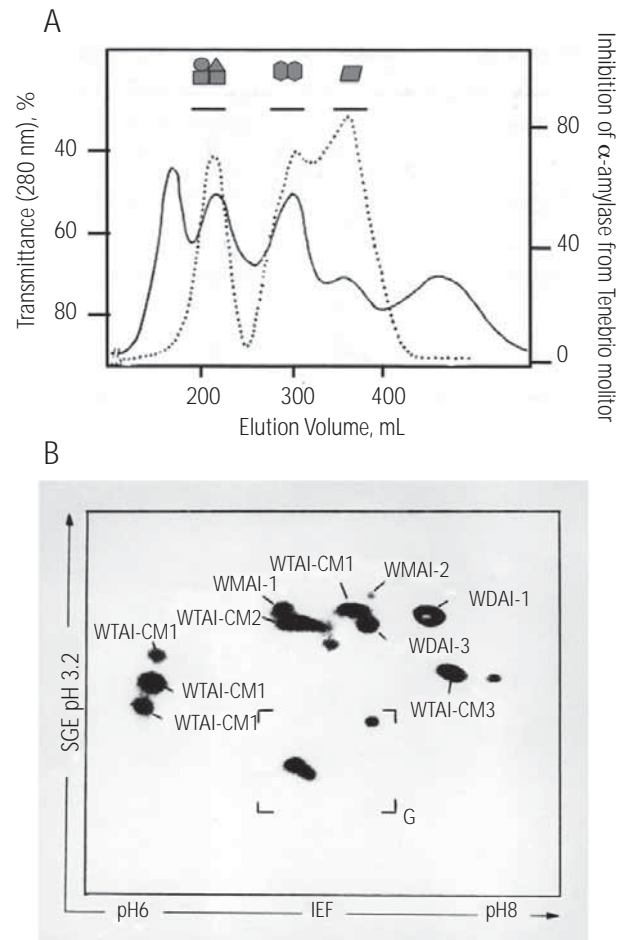


Figure 2. A, Gel filtration profile under nondissociating conditions (protein and inhibition of α-amylase activity from the insect pest *Tenebrio molitor*) of a protein preparation enriched in inhibitors from bread wheat flour showing the 3 types of α-amylase inhibitors: tetrameric, dimeric, and monomeric. B, Two-dimensional electrophoretic map of α-amylase inhibitor subunits from bread wheat. A indicates α-amylase; CM, CM protein; D, dimeric; G, globulins not belonging to the inhibitor family; I, inhibitor; IEF, isoelectrofocusing; M, monomeric; SGE, starch-gel electrophoresis; T, tetrameric; W, wheat.

Activity against heterologous α-amylase from insects, mites, mammals and/or bacteria, but not against the endogenous α-amylases present in the cereal kernel, has been described for wheat inhibitors [44,45]. Their role in plant defense is supported by the negative effects on the amylase activity of coleoptera and lepidoptera, which feed on stored cereal grains [48,49]. Additionally, the interaction between wheat inhibitors and the α-amylase from *Dermatophagoides pteronyssinus* (Der p 4 allergen) suggests that wheat/mite allergen complexes might be present in house-dust mite–infected flours [50]. The members of the family with antitrypsin activity usually affect bovine trypsin and trypsin-like proteases from lepidoptera [44,45].

### In Vitro IgE Binding and In Vivo Reactivity

Extensive data reported by different groups during the period 1989-1998, based mainly on 1- and 2-dimensional immunoblotting of wheat flour salt-soluble proteins, made it possible to identify several 12-16–kDa members of the α-amylase/trypsin inhibitor family as major IgE-binding proteins in sera from wheat-induced asthmatic patients [36-38,51-56]. In vitro analysis of purified inhibitor subunits fully confirmed their IgE-binding capacity [52,54,56,57], particularly in the case of monomeric members (WMAI-1; synonymous [syn] 0.28) and homodimeric members (WDAI-1 and -2; syn 0.53 and 0.19) [56,57]. However, our group [52,54,58] has reported very different types of reactivity among wheat members of the inhibitor family, with glycosylated members (see below) showing the strongest responses.

The biological activity of allergenic wheat α-amylase inhibitors was indicated by the induction of histamine release from peripheral basophils by wheat flour fractions enriched in salt-soluble 14-kDa electrophoretic bands [59] and by the stimulation of leukotriene release from peripheral leukocytes by both purified WMAI-1 (syn 0.28) and WDAI-2 (syn 0.19) allergens [56].

Data on IgE (B) epitopes of members of the wheat inhibitor family are very scarce. Only a sequential IgE-binding region spanning residues 9-26 has been located in the WMAI-1 subunit [60]. Interestingly, unidentified IgE epitopes shared by

Table. Positive Skin Prick Test Responses Induced by Purified Wheat α-Amylase Inhibitor Subunits in a Group of Patients With Baker's Asthma (n=31) Sensitized to Wheat[a]

| Allergenic sample | Positive SPT Responses, No. (%) |
|---|---|
| Crude inhibitor preparation | |
| Wheat | 27 (87) |
| Purified subunits | |
| WMAI-1 (syn 0.28) | 9 (29) |
| WDAI-1 (syn 0.53) | 5 (16) |
| WTAI-CM2 | 11 (35) |
| WTAI-CM16 | 7 (22) |
| gWTAI-CM16[b] | 14 (45) |
| WTAI-CM3 | 11 (35) |

Abbreviation: A indicates α-amylase; CM, CM protein; D, dimeric; I, inhibitor; M, monomeric; SPT, skin prick test; T, tetrameric; W, wheat.
[a]Data summarized from Armentia et al [62].
[b]Glycosylated form.

the inhibitor WDAI-2 and wheat α-gliadin has been suggested by RAST-inhibition assays [61].

SPTs performed by Armentia et al [62] with 6 purified inhibitor subunits from wheat flour made it possible to evaluate the in vivo reactivity of representative members of this allergen family (Table). Most asthmatic patients (27/31; 87%) sensitized to wheat flour reacted to a protein preparation enriched in inhibitor subunits, as well as to at least 1 isolated wheat subunit (25/31; 80%). However, positive responses to the purified inhibitor allergens assayed to date have varied from 16% to 45%, with a glycosylated tetrameric inhibitor subunit (gWTAI-CM16) being the highest reactive protein.

### An Unknown Role of N-Complex Asparagine-Linked Glycans

A glycosylated (g) form of the wheat tetrameric inhibitor subunit WTAI-CM16 and of its allergenic barley counterpart BTAI-CMb has been identified [54,58]. These modified variants, which are around 10-fold less abundant than their nonglycosylated forms in the corresponding flour, harbor complex N-glycans with ß1→2 xylosyl and α1→3 fucosyl residues, which are typical of the so called cross-reactive carbohydrate determinants (CCDs) of plant glycoproteins [58,63]. BMAI-1, a glycosylated barley monomeric inhibitor with allergenic reactivity in vitro and in vivo, seems to bind similar N-glycans [58,62,64].

The strongest in vitro and in vivo responses of the wheat and barley inhibitor subunits assayed to date were observed with the 3 glycosylated allergenic components mentioned above, and their reactivity was significantly stronger than those of their natural nonglycosylated forms [54,58,62]. Furthermore, chemical or enzymatic deglycosylation in vitro led to a drastic loss of their IgE-binding capacity [58]. Taken together, these data indicate a prominent role for the attached N-complex glycans in the allergenic potency of the glycosylated subunits. However, further research is necessary to ascertain the actual enhancement in IgE-binding potency and SPT response of the glycosylated inhibitor subunits compared with their nonglycosylated counterparts.

### Cross-Reactivity Between Cereal Flours: Rye and Barley Inhibitors

Homology among inhibitor subunits from wheat, rye, and barley partially accounts for cross-reactivity between flours from these cereals [44,53,55]. Allergens belonging to the α-amylase inhibitor family and associated with baker's asthma have been isolated from rye flour [65-67] and barley flour [62,64,68], and their reactivity has been confirmed by in vivo SPTs [62,67]. Interestingly, 3 of the rye inhibitors tested, namely Sec c 1, RDAI-1, and RDAI-3, provoked positive SPT responses in more than 50% of 21 patients with baker's asthma induced by rye flour. Moreover, these 3 rye allergens have also been associated with occupational allergy in the wood industry, as have other purified inhibitor subunits from wheat and barley [69,70]. Cereal flour, particularly from rye, is added to increase the viscosity of the urea-formaldehyde glue used in veneer panels, and its inhalation by wood-derivative factory workers induced sensitization to cereal α-amylase inhibitor subunits.

### Cereal α-Amylase Inhibitors and Food Allergy

Wheat, rice, and maize are the staple foods consumed by most of the world's population [71]. Allergic responses to the ingestion of wheat include, besides WDEIA, atopic dermatitis,

urticaria, gastrointestinal disorders, and anaphylaxis. Allergens belonging to the α-amylase inhibitor family have been found in flour proteins implicated in wheat food allergy, thus indicating their capacity to sensitize not only by inhalation (baker's asthma), but also by ingestion. The pioneering report by James et al [72] established the involvement of WDAI-1 in US pediatric patients with wheat hypersensitivity. Several subsequent works have identified inhibitor subunits as relevant in vitro IgE-binding proteins by testing sera from European patients [73,74] and Japanese patients [75,76] or by proving their in vivo reactivity in a Spanish child [77]. Pastorello et al [33] recently provided strong evidence for the role of this allergen family in food allergy. They found that over 70% of sera from 22 European patients with allergic reactions to wheat-containing foods presented specific IgE to inhibitor subunits WDAI-2, WTAI-CM1, WTAI-CM2, WTAI-CM3, and WTAI-CM16.

Finally, the major rice food allergens [78,79], as well as the maize trypsin inhibitor responsible for food-induced allergic reactions reported in a series of Italian patients [80], are also members of the cereal α-amylase/trypsin inhibitor family.

## Peroxidase

A prominent 36-kDa IgE-binding protein was isolated from diploid wheat flour (*Triticum monococcum*) by Sanchez-Monge et al [81] in 1997. The protein, which is also present in tetraploid wheat (pasta) and hexaploid wheat (bread), was identified as a seed-specific peroxidase harboring N-linked complex glycans (CCDs). Sera from 6 out of 10 patients with baker's asthma displayed an in vitro reaction (dot-blot) to the purified allergen. The biochemical characteristics of the peroxidase were later confirmed by Yamashita et al [82], who suggested that IgE from a patient's serum bound to the glycan moiety.

Wheat peroxidase has also been involved in contact dermatitis [11] and food allergy [33].

Additional clinical and biochemical data are necessary to establish the relevance of cereal peroxidases in baker's asthma. Their in vivo reactivity in particular requires further evaluation, as do the role of the N-linked glycans in their allergenic potency and potential cross-reactions among cereal flours.

## Thioredoxin

Thioredoxins are ubiquitous 12-14–kDa regulatory proteins that reduce intrachain disulphide bridges of target proteins (thioredoxin [–SH HS–] + target protein [–S-S–] →thioredoxin [–S-S–] + target protein [–SH HS–]), such as the wheat storage prolamins (gliadins and glutenins), thus enhancing mobilization of these proteins in germinating wheat seeds [83]. A putative thioredoxin-linked mitigation mechanism of allergic responses based on the aforementioned enzymatic function has been proposed in a canine model of wheat allergy [84].

Wheat thioredoxin (Tri a 25) has been identified as a novel allergen related to baker's asthma by screening of a wheat cDNA phage display library with sera from patients suffering from this occupational disease [85].

Both Tri a 25 and its homologous maize thioredoxin Zea m 25 (74% of amino acid sequence identity) have been produced as recombinant proteins in *Escherichia coli* and tested against 17 sera from patients with baker's asthma [85]. Sensitization (specific IgE) rates of 47% were found for both recombinant allergens. Interestingly, the α-amylase inhibitor rWDAI-2 (syn 0.19) reached a sensitization rate of 65% in the same group of patients. Specific IgE to the wheat and maize thioredoxin allergens was also detected in 35% and 20% of 20 sera from subjects with grass pollen allergy, but no clinical history of wheat or maize allergy, thus suggesting cross-reactivity between Tri a 25, Zea m 25, and grass pollen thioredoxins.

Tri a 25 could also be involved in wheat-dependent food allergy [74], as are other wheat allergens associated with baker's asthma (see above).

The true role of Tri a 25 in baker's asthma can only be established by determining the immunological equivalence between the natural and recombinant forms of Tri a 25, evaluating its in vivo reactivity and sensitization rates in larger populations with wheat flour–induced asthma, and analyzing the relationship between sensitization and clinical symptoms.

## Nonspecific Lipid Transfer Protein (LTP)

Plant lipid transfer proteins (LTPs) constitute a panallergen family of 9-kDa basic polypeptides (90-95 amino acid residues) that show a 3D fold characterized by a compact domain composed of 4 α-helices strongly linked by a network of 4 conserved disulphide bridges [86,87]. A main in vivo function of these proteins seems to be their involvement in plant defense against phytopathogens (bacteria and fungi), thus leading to their classification as pathogenesis-related (PR) proteins (PR-14 family) [86].

LTPs have been identified as major allergens in many plant foods, mainly Rosaceae fruits (eg, peach, apple), and in some pollens display an unexpected geographical distribution of sensitization in Europe, with a high prevalence in the Mediterranean, but much lower prevalence in northern and central regions [86-88]. Moreover, LTPs, particularly peach Pru p 3, have been proposed as a model of true food allergens based on their high resistance to both digestive proteolysis and heat treatments [86-88]. These characteristics have been associated with severe and systemic clinical symptoms and the activity in processed beverages and foodstuffs shown by several members of this allergen family [86-88]. LTPs sensitize through the oral route, but can also act as inhalant allergens, which, in some cases, are linked to plant food and pollen cross-reactions [86,87,89]. The characteristics of the LTP panallergen family described above are confirmed by identification of wheat flour LTP (Tri a 14) as an important food allergen linked to IgE-mediated disorders induced by ingestion of wheat-derived foodstuffs [33,90,91], maize LTP (Zea m 14) as a major allergen responsible for food-induced

allergic reactions [80], barley LTP as a relevant beer allergen [92], and rice LTP as an inhalant allergen in dust derived from rice grains [93].

Palacin et al [94] have characterized Tri a 14 as a major allergen associated with baker's asthma. Specific IgE to this wheat flour LTP was detected in 60% of sera from 40 Spanish patients with baker's asthma, and in vivo reactivity (positive SPT response) was found in 15 (62%) of 24 of these patients. Furthermore, recombinant Tri a 14 has been produced in *Pichia pastoris*, and its physicochemical properties, resistance to heat and proteolysis, and IgE-binding capacity have been shown to be almost equivalent to those of its natural counterpart [95]. These characteristics, together with its biological [95] and in vivo potency (SPT; A Armentia, A Palacin, and A Diaz-Perales, unpublished), make rTri a 14 a helpful tool in the diagnosis of baker's asthma.

Tri a 14 and peach fruit Pru p 3 (the model food LTP allergen) show an amino acid sequence identity of 45%, and, therefore, cross-reactivity in patients with baker's asthma
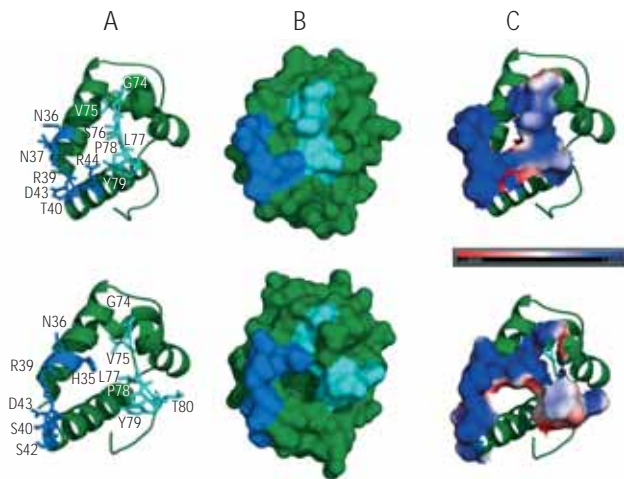


Figure 3. Mimotopes of peach Pru p 3 (upper row) and wheat Tri a 14 (lower row). A, Ribbon diagrams showing side chains of amino acid residues forming the mimotope. B, Surfaces of residues in A. C, Electrostatic potential isosurfacecs of the mimotopes (–1, red; +1, blue).

[94,95]. However, very different levels of cross-reactivity have been detected, reflecting the common and specific IgE (B) epitopes of both allergens [96] (Figure 3). Amino acid residues 31-40 and 71-80 have been mapped as common sequential IgE-binding regions of both allergenic LTPs, whereas regions Tri a $14_{51-60}$ and Pru p $3_{11-20}$ as specific to each allergen. Moreover, a conformational epitope (mimotope), L34H35N36R39S40S42D43G74V75L77P78Y79T80, which comprises the 2 common sequential epitopes (residues 31-40 and 71-80), has been located in Tri a 14. A very similar epitope has been located in Pru p 3. However, substantial differences in the surface electrostatic potential of both mimotopes have been found around residues 74-80, a region that markedly negative in Tri a 14, but neutral-positive in Pru p 3.

## Serine Proteinase Inhibitor

In 2008, Constantin et al [97] identified a serine proteinase inhibitor as a novel allergen in baker's asthma by screening a wheat seed cDNA library with serum IgE from asthmatic patients. The allergen is a 9.9-kDa protein, which usually forms 40-kDa tetramers and is a new member of the potato inhibitor I family. The inhibitor is mainly expressed in mature seeds and is accumulated in the starchy endosperm and aleuron layer. It is probably involved in plant defense and belongs to the pathogenesis-related (PR) protein-6 family.

The recombinant form of this allergen produced in *E. coli* (but not the natural one) has been assayed against sera from patients with baker's asthma [13,97]. The recombinant allergen reacted with specific IgE from 3 (14%) [97] and 6 (27%) [13] out of 22 sera from Spanish asthma patients when tested in dot-blotted or microarrayed samples, respectively. In contrast, sera from patients with wheat food or grass pollen allergy did not recognize the inhibitor [13,97]. The biological activity of the recombinant allergen was confirmed by 3 out of 3 positive results in the basophil histamine release assay [97].

Despite around 50% amino acid sequence identity, no relevant cross-reactivity was found with homologous inhibitors from maize and rice [97].

In order to establish the clinical significance of serum proteinase inhibitor in baker's asthma, it will be necessary to compare natural and recombinant allergens, evaluate in vivo reactivity, and test against a wider spectrum of sera from affected patients.

## Thaumatin-Like Protein

Thaumatin-like proteins (TLPs) form the most recently identified wheat flour salt-soluble protein family to be associated with baker's respiratory allergy [98]. Most TLPs have molecular masses ranging from 21 kDa to 26 kDa and 16 conserved cysteine residues forming 8 disulphide bridges that are responsible for a compact 3D structure and resistance to low pH conditions, proteolysis, and heat treatment [99]. TLP antifungal activity supports the role of these proteins in plant defense against fungal pathogens and their assignment to form family 5 of the pathogenesis-related (PR) proteins [99].

Several TLPs have been identified as main allergens in pollens, as well as in plant foods [100]. Some of them are N-glycosylated, although the actual relevance of the glycan moiety in the allergenic potency of TLPs remains to be established.

Purified wheat TLP (a preparation containing different isomorphs) induced positive SPT responses in 6 (30%) and 9 (45%) out of 20 Finnish patients with baker's asthma when tested at 50 μg/mL and 500 μg/mL, respectively [98]. Surprisingly, specific IgE to TLP analyzed using enzyme-linked immunosorbent assay (ELISA) was detected in only 2 out of 20 of the corresponding sera. Interestingly, over 40% of the patients studied by Lehto et al [98] showed positive SPT responses to protein preparations enriched in other wheat allergens mentioned above, such as α-amylase inhibitor and peroxidase.

## Prolamins: Gliadins and Glutenins

Besides the salt-soluble allergens discussed above, several of the major water/salt-insoluble wheat flour proteins (prolamins) also appear to be implicated in baker's asthma. Studies by authors such as Walsh et al [101], Sandiford et al [61], and Mittag et al [34] demonstrated IgE-binding in the prolamin fraction, including binding by α-, ß-, γ-, and ω-gliadins and LMW-glutenin subunits [61]. However, to our knowledge, only 2 studies have evaluated the allergenicity of purified (recombinant) prolamins [102,103].

Snegaroff et al [102] produced 2 recombinant LMW-glutenin subunits in *E. coli*, namely, LMW-GS B16 (37.7 kDa) and LMW-GS P73 (32.4 kDa). Taken together, the results of dot-blot and Western blot experiments using the purified recombinant subunits and 7 individual sera from 7 French patients with baker's asthma indicated specific IgE-binding from 6 and 5 sera by P73 and B16, respectively. Both glutenin subunits also recognized IgE from patients with WDEIA or immediate hypersensitivity to hydrolyzed wheat proteins. Bittner et al [103] expressed a cDNA encoding a 20-kDa αß-gliadin in *E. coli* and isolated the corresponding recombinant prolamin. ELISA screening of 153 sera from bakers with occupational asthma using the recombinant αß-gliadin detected specific IgE in 12% of the sera tested. Sensitization to natural total gliadin (commercial gliadin IgA/IgG ImmunoCAP, Phadia [Uppsala, Sweden], with detection of IgE) was observed in 33% of the asthmatic bakers. Obviously, most issues related to the potential role of prolamins in baker's asthma remains to be uncovered (eg, reactivity of natural forms and other purified gliadins and glutenins, in vivo tests).

Prolamins have been also identified as relevant allergens in WDEIA, particularly ω-5 gliadin, and in wheat food allergy (review in [31]).

## Component-Resolved Diagnosis of Baker's Asthma: Are Molecular Tools Ready?

In addition to discussing currently available data on wheat allergens associated with baker's asthma, this review has also tried to highlight the substantial limitations of these data. A recently reported microarray [13] comprising 6 recombinant wheat allergens, most of which are specific for occupational asthma, reflects several of these limitations, such as lack of comparison with their natural counterparts from wheat flour and very low reaction rates (32% to 5%) against sera from patients with baker's asthma.

Our knowledge of the wheat allergens involved in occupational asthma is subject to the following limitations: *1)* The low number of patients included in most reports; *2)* The low prevalence of most purified allergens (specific IgE in <50% of sera assayed), which could be partly explained by the wide individual heterogeneity in sensitization patterns [38]; *3)* The lack of in vivo tests in most reports; *4)* The lack of clear markers of clinical symptoms and route of sensitization; *5)* The lack of comparison between natural and recombinant allergens; *6)* The unknown role of N-linked complex glycans (eg, α-amylase inhibitors, peroxidase); and *7)* The absence of conclusive results on cross-reactivity with other cereal flours (rye, barley), plant foods (eg, Rosaceae fruits), and pollens (eg, grasses). Therefore, component-resolved diagnosis and immunotherapy of baker's asthma warrant further study.

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