

Review article

Characterized allergens causing bakers' asthma

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Although airborne allergens in bakeries and confectioneries cause one of the most common forms of occupational asthma, namely, bakers' asthma, only a few of them are known in detail so far. Here we summarize current knowledge of bakery allergens and describe our own two-dimensional (2-D) immunoelectrophoresis studies of wheat-flour allergens as well as the allergenic baking enzyme Asp o 2. Out of approximately 700 soluble wheat polypeptides, 70 show IgE binding; the following wheat-flour allergens could be identified and characterized: members of the α -amylase inhibitor family (14–18 kDa), acyl-CoA oxidase (26 kDa), peroxidase (36 kDa), and fructose-bisphosphate aldolase (37 kDa). However, the great majority of the soluble wheat-flour allergens, mainly located in the 27-, 55-, and 70-kDa areas of the 2-D immunoblots with pI values of 5.8–6.8, 5.9–6.5, and 5.5–6.1, respectively, are not known at present. Asp o 2, to which approximately 25% of all bakers with respiratory symptoms are sensitized, is a well-characterized starch-cleaving enzyme. We conclude that great effort is still needed to describe all major wheat-flour allergens. As shown by Asp o 2, knowledge of the causative allergens and their characteristics enables us to initiate very effective preventive measures such as the introduction of granulated allergenic products.

Key words: allergy; α -amylase; bakers' asthma; IgE; wheat flour.

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Bakers' asthma is the main form of occupational asthma in many countries. In Germany, approximately 1800 bakers annually claim compensation because of this disorder. Although the symptoms had already been described by Ramazzini approximately 300 years ago, the causative components and the underlying, IgE-mediated pathomechanism were unknown until two decades ago. Several studies have shown that the responsible allergens come from wheat and rye flour, soybean, baking additives, and, in a few cases, storage mites (1–7). Here we present an overview of current knowledge in this field with special reference to individual wheat allergens and fungal α -amylase Asp o 2.

Allergens causing bakers' asthma

Our recent screening showed that 60% of 405 examined bakers complaining of work-related asthma and/or rhinitis were sensitized to wheat flour, and 57% of this group were sensitized to rye

flour when commercially available extracts were used (Fig. 1). Some 22% had IgE antibodies to the baking enzyme α -amylase from *Aspergillus oryzae* (Asp o 2) (Fig. 1) (8).

Wheat-flour allergens

For the identification and characterization of wheat-flour allergens, electrophoretic methods in combination with IgE immunoblotting have been mainly applied; e.g., by Theobald et al. (9) and Pfeil et al. (10). Electrophoretic techniques are superior to other methods such as chromatography since they provide the highest resolution and can resolve the complex protein composition of wheat flour in order to characterize the responsible allergens.

Weiss et al. (11) investigated the IgE-binding capacity of three solubility fractions (albumin/globulin, gliadin, and glutenin) by immunoblotting. They found that all three fractions contain proteins which bind the IgE of allergic bakers. But

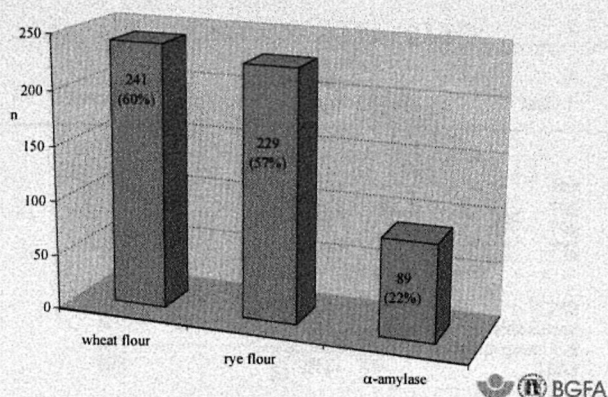


Fig. 1. Prevalence of IgE antibodies to wheat flour, rye flour, and α -amylase in 405 bakers with workplace-related asthma and/or rhinitis.

the highest percentage of IgE binding (75%) was observed with the buffer-soluble albumin/globulin fraction. Sandiford et al. (12) identified proteins of 12, 21, 26, 33, and 69 kDa as major wheat allergens by electrophoresis followed by immunoblotting. Similarly, Varjonen et al. (13) classified 26-, 38-, and 69-kDa bands, and Houba et al. (7) found wheat proteins of 14 and 30–32 kDa to be major IgE-binding proteins. However, the latter studies were purely descriptive with no attempt to identify the immunoreactive wheat proteins in detail. Posch et al. (14) and Weiss et al. (15) applied high-resolution two-dimensional (2-D) electrophoresis in combination with protein-analysis tools to identify wheat allergens (Fig. 2). They detected about 700 individual soluble wheat proteins, of which 70–80 protein spots showed IgE reactivity. IgE binding was predominantly localized in the 14–18-, 27-, 37-, 55-, and 70-kDa areas of the immunoblots with pI values mainly at 5.5–6.8. By protein sequencing and analysis of amino acid composition, they found homologies to amylase/protease inhibitors (14–18 kDa), acyl-CoA oxidase (26 kDa), and fructose-bisphosphate aldolase (37 kDa) from wheat, barley, maize, and rice, respectively. Recently, Sanchez-Monge et al. (16) identified a wheat-flour peroxidase (36 kDa) as another important allergen, since sera of 6/10 patients hypersensitive to wheat flour exhibited positive reactions in dot blotting with the isolated allergen.

It has already been clarified by Gomez et al. (17) that the 14–18-kDa allergens mainly belong to the α -amylase inhibitor family. Armentia et al. (18) described skin prick test responses to subunits of heterotetramer α -amylase inhibitors. Like Fränken et al. (19), they found that subunits of homodimer α -amylase inhibitors bind human IgE antibodies in immunoblot and the enzyme aller-

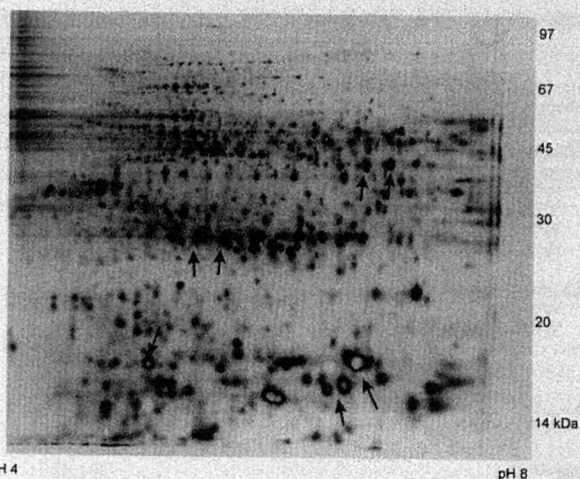


Fig. 2. Two-dimensional electrophoresis of soluble wheat-flour proteins. Polypeptides were visualized by silver staining. Arrows indicate wheat allergens α -amylase inhibitor (14–18 kDa), acyl-CoA oxidase (26 kDa), and fructose-bisphosphate aldolase (37 kDa).

gosorbent test (EAST) or induce immediate-type skin prick test reactions in symptomatic bakers. Recently, James et al. (20) demonstrated that dimeric wheat α -amylase inhibitor is also a relevant allergen in patients experiencing hypersensitivity reactions after the ingestion of wheat proteins. The allergenicity of the monomeric α -amylase inhibitors was demonstrated by Armentia et al. (18), as well as by Sutton et al. (21), with skin prick testing or IgE binding (RAST). Moreover, the IgE-binding components were shown to be the 17-kDa subunits of the homodimeric agglutinin (21).

Table 1 summarizes the properties of the already known wheat-flour allergens. While peroxidase and the members of the α -amylase inhibitor family are well characterized, and the prevalence of respective sensitization has been investigated, the respective data on acyl-CoA oxidase and fructose-bisphosphate aldolase are missing. Additionally, these allergens, including agglutinin, represent only a small subset of the approximately 70 soluble IgE-binding wheat proteins described by the different immunoblotting studies mentioned above. For example, the 26–28-kDa allergens consist of at least 11 IgE-binding polypeptides, and only two of them have been identified as acyl-CoA oxidase. Most research aims to identify soluble wheat allergens, but it has been shown that gliadins and glutenins can trigger bakers' asthma as well. By Western blotting, Sandiford et al. (22) demonstrated that water/salt-insoluble wheat gliadins and glutenins are important allergens. The most allergenic polypeptides of this protein family seem to be α - and ω -gliadins.

Table 1. Characterized wheat-flour allergens

Substance/abbreviations	MW kDa	Purity	Test	Patients with positive test results	References
α-Amylase inhibitors					
Subunits of heterotetramers					
WTAI-CM2	14	e.p. ¹	SPT	11/31	(18)
WTAI-CM3B	14	e.p. ¹	SPT	11/31	(18)
WTAI-CM16	16	e.p. ¹	SPT	7/31	(18)
WTAI-CM16*	16	e.p. ¹	SPT	14/31	(18)
Subunits of homodimers					
WDAI-1	15	e.p. ¹	SPT	5/31	(18)
WDAI-1 and/or WDAI-2		e.p. ¹	Immunoblot	4/8	(19)
		e.p. ¹	2-D immunoblot	Pool serum	(14)
		e.p. ²	EAST	13/25	(33)
Monomer					
WMAI-1	14	e.p. ¹	SPT	9/31	(18)
Agglutinin					
Subunits of homodimers					
WGA	17	e.p. ²	RAST	5/9	(21)
Acyl-CoA oxidase					
(rice homolog)	27	e.p. ¹	2-D immunoblot	Pool serum	(14)
Fructose-bisphosphate aldolase					
(rice homolog)	37	e.p. ¹	2-D immunoblot	Pool serum	(15)
Peroxidase	36	e.p. ¹	Dot blot	6/10	(16)

*Glycosylated subunit.

e.p.: electrophoretically pure; SPT: skin prick test.

¹One spot in 2-D electrophoresis.²One band in SDS-PAGE.

*α -Amylase of *A. oryzae* as an important bakery allergen*

α -Amylase is a starch-cleaving enzyme often used in the baking industry as a flour additive. It is usually of fungal origin, produced by *A. oryzae*. Recent studies have shown that 20–32% of bakers suffering from workplace-related rhinitis and/or asthma are sensitized to fungal α -amylase. Corresponding results have been reported from Switzerland, Germany, Italy, Spain, and The Netherlands (3, 6, 23–26). We examined a group of 89 symptomatic bakers by skin prick test and EAST. Some 48% ($n=43$) of bakers reported work-related symptoms such as asthma, rhinitis, conjunctivitis, and/or eczema. Some 16% ($n=14$) of all bakers, representing 32% of symptomatic bakers, had positive results in EAST; 18% of the total (16 subjects, one of whom had no symptoms) and 35% of the symptomatic bakers showed immediate-type reactions to α -amylase in skin prick tests. The results of skin prick tests and EAST correlated in 96% ($n=85$) of all cases. In the control group of 43 subjects without occupational contact with baking materials, including 23% atopics, only one subject (2%) had a borderline EAST result, and another one (2%) had a positive prick test.

Our recent studies demonstrate that heating results in a decrease, but not a total loss, of IgE binding to α -amylase (4).

Furthermore, there is evidence that the remaining α -amylase in baking products can cause allergic reactions in consumers (8, 27).

The primary and 3-D structures of this enzyme are already known. According to Toda et al. (28), α -amylase is a 478-amino acid glycoprotein. Radio-graph crystallography of the α -amylase demonstrates that the molecule consists of three structural domains (Fig. 3) (29). The conformation is stabilized by four disulfide bonds. Calcium binding is obviously necessary for enzyme activity; two calcium-binding sites are near the enzymatically active site cleft of the enzyme.

α -Amylase mainly contains continuous B-cell epitopes, since its complete digestion by trypsin and endoprotease Arg-C did not destroy its IgE-binding capacity (30).

Concluding remarks

The main allergens in bakeries and confectioneries are members of the α -amylase inhibitor family (heterotetramers, homodimers, and a monomer of α -amylase inhibitors) as well as peroxidase, agglutinin homodimer, acyl-CoA oxidase, and fructose-bisphosphate aldolase of wheat flour. Various soluble flour allergens not identified and not further characterized also exist, since about 70 out of 700 soluble wheat-flour allergens bind the IgE of affected workers. While the 14–18-kDa allergens

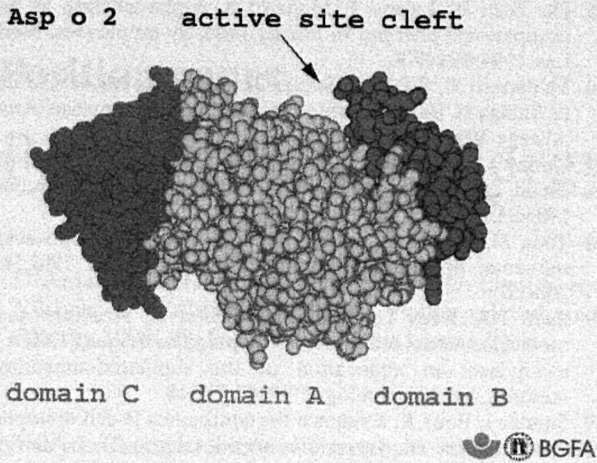


Fig. 3. Three-dimensional model of bakers' allergen α -amylase from *Aspergillus oryzae* (Asp o 2), consisting of three domains.

mainly belong to the α -amylase inhibitor family, little information is available on the 27-, 55-, and 70-kDa allergens.

An important nonflour allergen is α -amylase from *A. oryzae*, causing type I sensitization of approximately 23% of symptomatic bakers (2, 6, 25).

Recent studies by Houba et al. (6) demonstrated exposure-response relationships between the workplace load of wheat-flour allergens or α -amylase on the one hand and type I sensitization on the other hand; threshold values were 1–2.4 mg/m³ and 0.25 ng/m³, respectively.

Our findings prompted the development of new granulated enzyme products, which have been shown to prevent airborne exposure. However, bakers have not accepted them on a large scale so far.

It should be mentioned that additional allergenic components may be important in individual cases; e.g., soybean allergens (4) and allergens from storage mites, insects, pollen, and molds contaminating flour. Furthermore, irritative gases have been shown to occur in bakeries and confectioneries; i.e., NO, NO₂, acetaldehyde, formaldehyde, and acrylaldehyde (31). However, the clinical relevance of these gases in the respective workplaces has not been evaluated yet.

Another current question is whether flour components retain their allergenic potency during the baking process and cause atopic diseases in the general population. There is evidence that this is the case in some individuals. Recently, a 15-kDa subunit of dimeric α -amylase inhibitor (20), as well as 20- and 47-kDa wheat components (32), was shown to bind IgE antibodies from patients suffering from hypersensitivity to ingested wheat products.

In conclusion, many questions remain on individual putative pathogenetic factors in bakers'

rhinitis and asthma. Moreover, it must be clarified whether conserved or newly developed allergenic flour and enzyme epitopes in baking products are of major importance. These questions should be answered by intensified research to enable the development of more effective primary and secondary preventive measures.

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