

J. Fränken^aU. Stephan^aH. E. Meyer^bW. König^a^a Institut für Medizinische Mikrobiologie und Immunologie, AG Infektabwehr, und^b Institut für Physiologische Chemie – Proteinstrukturlabor, Ruhr-Universität Bochum, Deutschland

Identification of Alpha-Amylase Inhibitor as a Major Allergen of Wheat Flour

Key Words

Baker's asthma

Allergen

Wheat flour

Abstract

We isolated a major wheat flour allergen with a molecular weight of 15-kD. The 15-kD allergen was purified from an extract of wheat flour by ammonium sulfate precipitation followed by gel filtration. Using the immunoblot technique, IgE, specifically directed towards the single isolated allergen, was detectable in 50% of the applied sera. We determined the N-terminal amino acid sequence of the allergen and identified it as an α -amylase inhibitor protein. Our data emphasize that low molecular weight wheat flour allergens play an important role in the pathogenesis of baker's asthma disease.

Hypersensitivity to foodstuffs is well recognized, and distinct components induce reactions of hypersensitivity when ingested. In some specific cases food ingredients provoke immune reactions when they are inhaled as particles. Thus, hypersensitivity to inhaled flour allergens provides an excellent model of occupational-induced type-I allergic asthma. Of main interest is the relationship between the clinical symptoms of the disease and the agents which trigger the allergic reactions. One major group of allergenic factors are flours to which bakers are exposed at their working place. The sensitization of bakers towards wheat and rye flour, especially towards the water-soluble protein constituents, has been described [1, 2]. In addition to the different kinds of common flours, baking ingredients, especially the enzyme α -amylase, may contribute to these allergic reactions as has been described in other studies [3].

Three major allergens of wheat flour with molecular weights of 47, 17 and 15 kD were identified by their ability to bind IgE from the sera of sensitized bakers and with regard to the IUIS allergen nomenclature called (*Triticum vulga-*

ris) (*Tri v*) band (Bd) 47, *Tri v* Bd 15 and *Tri v* Bd 17. The 15-kD component was detected by IgE in nearly 50% of the applied sera, and the release of histamine from the corresponding basophil leukocytes correlated with the presence of IgE antibodies against the low molecular weight component [4]. These results may indicate an important role of the 15-kD component as an initiating allergen in the etiology of baker's asthma. It was the purpose of our studies to purify the allergen and to identify its primary structure.

Commercially available wheat flour (type 405) was used to produce a water-soluble extract as described previously [4]. The extract was fractionated by stepwise addition of crystalline ammonium sulfate (ammonium sulfate precipitation) and resulting fractions were collected by centrifugation.

The fractions were then applied for further purification by gel filtration on AcA 44 columns (LKB, Gräefeling, FRG; 2.4×90 cm). Phosphate-buffered saline (PBS; pH 7.3) was used as elution buffer. Filtration fractions of 5 ml were collected and analyzed by measuring their optical density (OD)

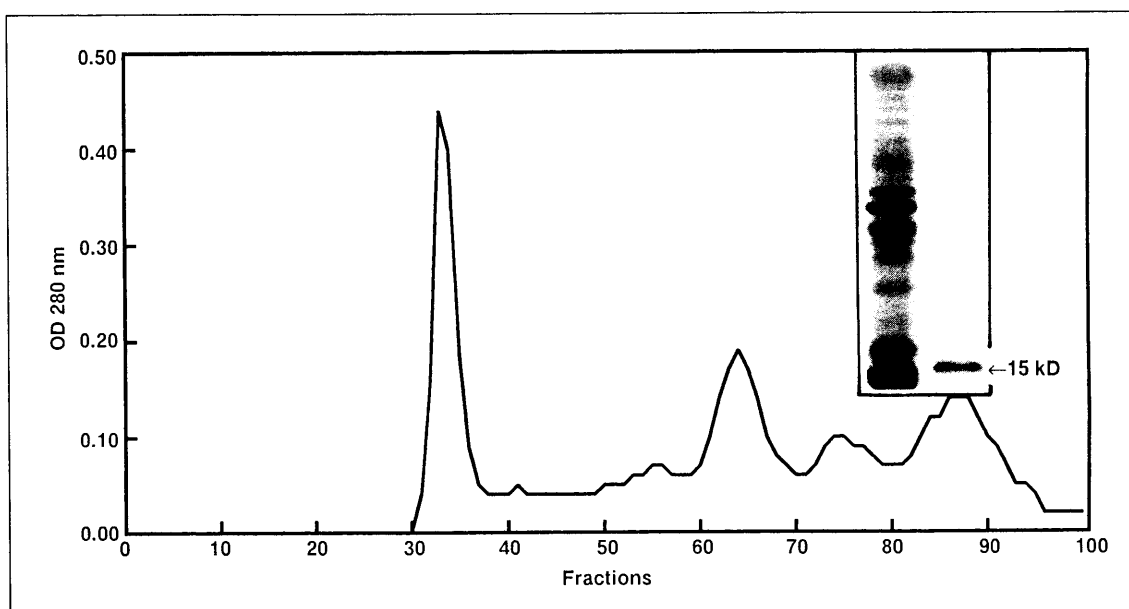


Fig. 1. Purification of the 15-kD allergen from wheat flour by the gel filtration technique. One typical representative diagram out of several runs is shown. Results are expressed as optical density (y axis) vs. sampled fractions of the run (x axis). The inset shows the purified 15-kD allergen and complete wheat flour extract by SDS-PAGE and subsequent staining with Coomassie brilliant blue R 250.

at 280 nm. The corresponding filtration fractions of the runs were pooled, lyophilized and resuspended in PBS for further analyses by SDS-PAGE. After electrophoresis the gels were stained with Coomassie brilliant blue R 250 (Serva, Heidelberg, FRG).

The subsequent identification of the primary structure of an isolated allergen was achieved by N-terminal protein sequence analysis. Edman degradation was performed with an Applied Biosystem 470A gas-phase sequencer using the 03RPTH standard program. The resulting PTH amino acid derivatives were analyzed using an Applied Biosystems Model 120A on-line PTH analyzer.

In addition we analyzed the IgE-binding pattern towards the isolated allergen in individuals exposed to wheat flour dust. The protein was transferred to nitrocellulose by the Western blot technique [5] followed by probing with sera from allergic bakers.

The bound human IgE was detected with a secondary radiolabelled (^{125}I) anti-human IgE (40,000–50,000 cpm/4 ml = 1.48–2.22 kBq; Tago, distributed by Medac, Hamburg, FRG) as described previously [6].

Wheat flour extract was fractionated by stepwise addition of crystalline ammonium sulfate. Four fractions precipitating at 20, 40, 60 and 80% ammonium sulfate saturation were collected by centrifugation. SDS-PAGE was used to analyze the protein pattern of the fractions. Most of the low

molecular weight components were precipitated in the presence of 20% ammonium sulfate (data not shown). Therefore, we took this fraction (20% precipitation) for gel filtration. As an example, figure 1 is a diagram of such a separation by gel filtration.

Four UV-positive (OD) fractions (1–4) from each single gel filtration were collected: fraction 1 (sample 30–37); fraction 2 (sample 60–68); fraction 3 (sample 73–78), and fraction 4 (sample 81–92). The highest protein concentration was determined in fraction 1 (OD up to 0.36), containing proteins of higher molecular weight. Lower protein concentrations were obtained in fractions 2, 3 and 4. Fraction 4 only contained the 15-kD component as revealed by SDS-PAGE analysis (fig. 1).

We then analyzed the IgE-binding pattern towards the 15-kD component in individuals exposed to wheat flour dust. Therefore a sample from peak 4 (purified 15-kD component) was separated on SDS-PAGE and electroblotted on to nitrocellulose sheets.

The sera used for these experiments were not the same as in the previous studies [4] in which IgE binding towards complete wheat flour extract was demonstrated. The selection principles for atopic bakers were in line with previous studies with regard to the RAST criteria (wheat >2), prick test (wheat $\geq$ ++) and a personal history of atopy. As is apparent the control (buffer instead of serum = nonspecific

Fig. 2. Identification of IgE binding towards the purified 15-kD wheat allergen by autoradiography (Western blot): 1 = wheat flour extract; 2 = nonspecific binding; 3–10 = sera from atopic bakers.

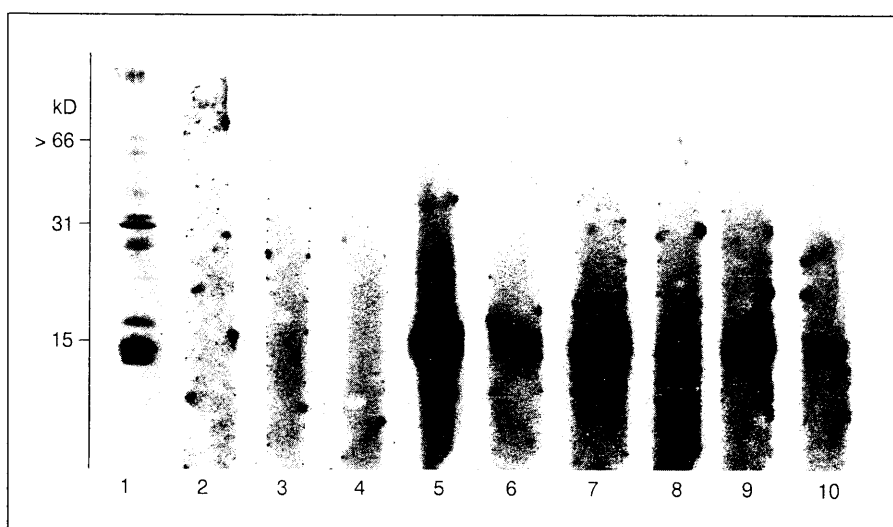
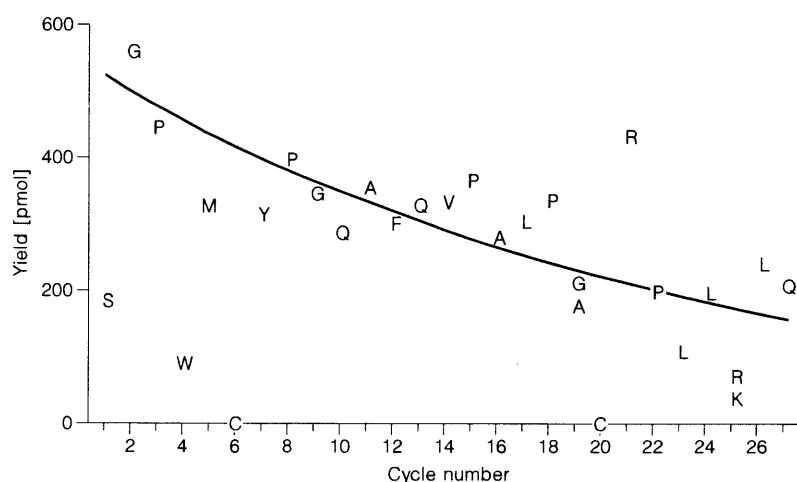


Fig. 3. Sequence analysis of the isolated and purified wheat flour protein with a molecular weight of 15 kD. The yield (pmole) of each amino acid in each degradation steps is plotted against the cycle number.



binding; fig. 2, lane 2) exhibits no binding of the radiolabelled antibody to the 15-kD component. Sera from 4 bakers showed a detectable IgE binding towards the purified component. The sera from the remaining 4 bakers (50%) did not reveal binding towards the 15-kD component (fig. 2). However, no binding of serum IgE antibodies towards any protein band besides the 15-kD component was apparent. Binding of IgE antibodies towards the 15-kD component was not detectable in the pooled sera from 5 nonallergic bakers (data not shown).

Further identification of the 15-kD component (a sample from peak 4 = purified 15-kD component) was achieved by NH₂ terminal sequence analysis. The N-terminal amino acid sequence of the isolated allergen was identical within

the first 25 positions with the published primary structure of the α -amylase inhibitor. In positions 19 (alanine and glycine) and 25 (arginin and lysin), two different amino acids in equimolar quantities were identified demonstrating the presence of an equimolar mixture of two known isoforms of the inhibitor called 0.53 and 0.19 (fig. 3).

These inhibitors form dimers and show very high sequence homology with amino substitutions at seven positions. They belong to the 24-kD family of α -amylase inhibitors acting on mammalian and insect α -amylases [7]. The in vitro allergenic capacity as well as the purity of the isolated component was proven by the immunoblot technique using sera from atopic bakers. The reactivity of the 15-kD allergen on immunoblot towards specific human IgE demon-

strates its immunological activity in vitro. 50% of the applied sera from atopic bakers possessed specific IgE antibodies towards the isolated 15-kD component which correlates with the results concerning the IgE-binding pattern of complete wheat flour extract [4].

Besides the 15-kD band, no other band was detected by IgE in sera from bakers. This proves the accuracy of the purification procedure and the purity of the 15-kD component. Normally all components of wheat flour extract between 134 and 12 kD exhibit IgE binding in wheat flour-exposed donors, as detected by Western blot analysis [4].

Recently other investigators not only described the presence of α -amylase inhibitory activity in wheat kernel but also their role as important allergens causing baker's asthma [8, 9].

It has been demonstrated by in vitro and in vivo immunological tests that the most prominent allergenic components are glycoproteins which belong to the subunits of tetrameric α -amylase inhibitors; the allergenic activity of the dimeric form of the α -amylase inhibitor 0.53 was found to be of negligible importance. Armentia et al. [10] suggested that the carbohydrate moieties of the proteins are due to the higher reactivities of the glycoproteins. In addition, allergenic activity was described for the monomeric wheat α -amylase inhibitor protein 0.28. The strong IgE-binding capacity of the 15-kD component was described for complete

wheat flour extract and was obviously not influenced by the purification methods shown by our results with the isolated component. One may assume that the allergenicity of the isolated 15-kD protein is due to the composition of the allergen as a mixture of two isoforms although each single isoform seems to be of minor allergenicity. Recently cross-reactivities between the low molecular weight allergens of wheat flour with a molecular weight of 15- and 17-kD and a main allergen of rye flour has been described [6]. One may suggest that members of the α -amylase/trypsin inhibitor family will also be shown to be allergens in rye flour as described for other cereals [8]. Our results support the important role of proteins from the α -amylase/trypsin inhibitor family as allergens for baker's asthma. In addition, for the possible consequences of a more effective diagnosis and treatment of allergy to flours, it is of great interest to determine the influence of proteins on immunological functions possessing both allergenic and enzymatic or enzyme-inhibitory activity. An association between enzymatic/enzyme inhibitory activity and allergenicity was discussed for particular allergens from the mite *Dermatophagoides pteronyssinus* [11]. The results require further investigations into the role of the main flour allergens in baker's disease especially of the enzymatic/enzyme inhibitory activity of characterized purified allergens.

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