

Baker's Asthma due to Xylanase and Cellulase without Sensitization to Alpha-Amylase and Only Weak Sensitization to Flour

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Abstract

Background: The baking additives xylanase and cellulase were described as baking additives causing baker's asthma. It is not known whether monosensitization to these enzymes may occur. **Methods:** We present a case report of a baker with work-related asthma evaluated by skin prick test (SPT), enzyme-linked immunosorbent assay (EAST), immunoblot, EAST and immunoblot inhibition, and specific bronchial challenge. Fungal xylanase and α -amylase were measured by two-site enzyme immunoassays in products used by the patient at work. **Results:** Allergy to xylanase and cellulase was demonstrated by SPT, EAST, immunoblot and specific bronchial challenge (for xylanase only). No sensitization to α -amylase could be demonstrated, but there was a weak flour allergy as documented by EAST and immunoblot and a positive occupational-type challenge with high concentrations of rye flour. Four baking additives contained measurable amounts of fungal α -amylase and xylanase, without a correlation between these enzymes. **Conclusions:** We conclude that occupational asthma due

to the baking additives xylanase and cellulase may occur without concomitant sensitization to α -amylase and only weak sensitization to flour.

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Introduction

Sensitization of bakers to enzymes used as baking additives have been known for about 20 years [1]. The most frequently reported allergenic enzyme in baker's asthma is α -amylase (EC 3.2.1.1) from *Aspergillus oryzae*. Additionally, glucoamylase (EC 3.2.1.3) [2, 3], cellulase (EC 3.2.1.4) [3, 4] and xylanase (EC 3.2.1.8) [3, 5] were described as occupational allergens in bakers. Recently, we reported a 20% prevalence of α -amylase-specific IgE antibodies in sera of German bakers with work-related respiratory symptoms (while the sensitization rate for rye flour was 72%) [6]. Specific IgE antibodies to glucoamylase, cellulase and xylanase were found in 4, 9 and 14% of these subjects, with strong crossreactivity between cellulase and xylanase. Only one of 93 bakers was sensitized to enzymes (xylanase) but not to flour. In practice, immunologic evaluation of bakers is often restricted to flour and α -amylase. As about one third of the bakers with work-related respiratory symptoms but without sensitization to

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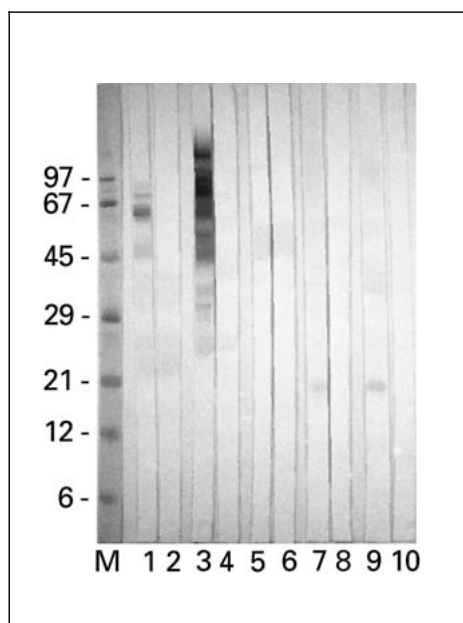


Fig. 1. Xylanase (lanes 1, 2), cellulase (lanes 3, 4), α -amylase (lanes 5, 6), rye flour (lanes 7, 8) and wheat flour proteins (lanes 9, 10) were separated electrophoretically and blotted. Membrane strips were incubated with the patient's serum (lanes 1, 3, 5, 7, 9) or with a negative pool serum as a control (lanes 2, 4, 6, 8, 10) and IgE-binding proteins were stained.

flour or α -amylase was reported [7], it may be speculated that this group is sensitized to hitherto unknown allergens. To our knowledge, epidemiological data on the prevalence of monosensitizations to enzymes other than α -amylase among bakers are not available.

Case Report

A 35-year-old baker was referred to our institute for a medical expert opinion. He has worked as a baker for 17 years and has owned a bakery with 15 employees for 13 years. No previous history of allergic symptoms to his work has been reported. Work-related nasal congestion, sneezing, running nose, watering of the eyes and shortness of breath started 3 years ago. On holiday, he was without symptoms. The patient used various nasal sprays but no bronchial medication.

Clinical Investigations

The clinical examination showed an almost total nasal obstruction. No other pathological findings were detected. Auscultation of the lungs and spirometry [8] were normal (FEV_1 97% predicted). A methacholine challenge [9] demonstrated bronchial hyperresponsiveness with a $PD_{20}FEV_1$ of 0.2 mg methacholine. Skin prick tests (SPT) with environmental allergens and commercial wheat and rye flour extracts of three manufacturers (Allergopharma, Reinbek; Ben-card, Neuss; ALK-Scherax, Hamburg, Germany) were completely

negative. SPT with enzymes [3] yielded positive reactions to cellulase (6 mm wheal diameter) and xylanase (16 mm), both from *Aspergillus niger* and an equivocal reaction to α -amylase from *Aspergillus oryzae* (2 mm).

Rye Flour Challenge

Occupational-type challenge with rye flour dust (type 1150) from the patient's bakery was performed in a chamber with total dust concentrations as assessed by personal and area sampling of about 25, 50, 100 and 200 mg/m³. Exposure lasted about 15 min for each flour concentration. After a total exposure time of 1 h, the patient complained of nasal congestion, running nose, sneezing and shortness of breath. Specific airway resistance increased from 0.8 to 3.2 kPa·s, FEV_1 fell by 44% and PaO_2 from 92 to 68 mm Hg. Anterior rhinomanometry showed complete obstruction of both noses. The time course was an immediate-type reaction, peak expiratory flow measurements performed for 8 h did not show a delayed reaction.

Xylanase Challenge

Bronchial challenge with xylanase [3] was performed (1 day after the challenge with rye flour) with a dosimeter (APS, Jaeger, Würzburg, Germany) and a MedicAid nebulizer (Sussex, UK) with an output of 160 ml/min. The nebulizer was activated for 0.6 s during each of 10 deep breaths. Concentrations were increased by 5-fold steps. After application of a xylanase concentration of 50 µg/ml (cumulative dose 1.056 µg), the patient reported shortness of breath, specific airway resistance increased from 1.0 to 2.3 kPa·s, FEV_1 fell by 16% and PaO_2 from 76 to 68 mm Hg. The time course was an immediate-type reaction, peak expiratory flow measurements performed for 5 h did not show a delayed reaction.

EAST and EAST Inhibition

Total IgE was 164 kU/l (Phadezym PRIST, Pharmacia, Freiburg, Germany). Specific IgE for xylanase, cellulase and α -amylase were determined by EAST (Phadezym RAST, Pharmacia) using self-coupled allergen disks. Specific IgE antibodies to rye flour (0.61 kU/l; CAP, Pharmacia), wheat flour (1.62 kU/l; CAP), cellulase (>17.5 kU/l) and xylanase (8.82 kU/l) but not to α -amylase were demonstrated in the patient's serum.

The binding of the patient's IgE to xylanase allergen disks could be inhibited by xylanase and cellulase (10 µg) by more than 80%. With wheat flour, rye flour and α -amylase from *A. oryzae* (10 µg protein each), no inhibition was obtained. When wheat flour was used as solid phase, preincubation with cellulase and xylanase resulted in increased IgE binding, probably due to the binding of the enzymes to cellulose [3].

SDS-PAGE and Immunoblot

Briefly, 15 µg protein per lane (xylanase and cellulase from *A. niger*, α -amylase from *A. oryzae*, wheat flour and rye flour) were separated electrophoretically in a pre-cast NuPAGE® Bis-Tris gel (Invitrogen, Groningen, The Netherlands) with 10% acrylamide and electroblotted in 50 mM Tris base/50 mM boric acid, pH 8.5 to polyvinylidene difluoride membrane. Blocking and immunostaining of membrane strips were performed as described previously [3] with the patient's serum or a pooled serum from patients without sensitization. For inhibition experiments, the sera were pre-incubated with 20 µg xylanase or cellulase.

Immunoblots (fig. 1) of the patient's serum showed strong reactions to several protein bands of xylanase and cellulase preparations

Table 1. Concentrations of fungal α -amylase and xylanase in products used in the patient's bakery

	Product labelled 'enzymes'	Percentage of additives in flour ^a	α -Amylase $\mu\text{g/g}^b$	Xylanase $\mu\text{g/g}^c$
<i>Wheat flour</i>				
Type 550	no	–	0.16	BD
Type 1050	no	–	BD	0.18
<i>Rye flour</i>				
Type 1150 ^d	no	–	BD	BD
Type 1370	no	–	BD	BD
<i>Additives not containing flour</i>				
A	yes	3	80.16	0.46
B	yes	5	33.34	4.34
C	yes	1–3	0.43	52.57
D	no	2	BD	BD
F	no	1–3	BD	BD
<i>Additives containing flour</i>				
G	no	20	BD	BD
H	yes	20	98.64	0.34

^a Information was provided by the manufacturers.

^{b/c} Detection limits for fungal α -amylase and xylanase were ~ 0.13 and $0.1 \mu\text{g/g}$, respectively.

^d Rye flour type 1150 used for the provocation test.
BD = Below the detection limit.

(lanes 1 and 3). The molecular weights of the IgE-binding proteins of the xylanase preparation are below 100 kD with a main reaction at about 67 kD indicating that the previously identified 105-kD allergen β -xylosidase (Asp n 14) [3] does not play an important role in this patient. The same applies for α -amylase (Asp o 2); in lane 5 no reaction was observed. A weak reaction to rye (lane 7) and wheat flour (lane 9) proteins was observed. This binding to wheat and rye flour proteins could not be inhibited by xylanase or cellulase (results not shown).

Measurements of Fungal Xylanase and α -Amylase in Products Used by the Patient at Work

The patient provided ten samples of flour and baking additives from his workplace. We extracted the samples (45 mg in 10 ml phosphate-buffered saline with 0.5% Tween 20) by repeated vortexing and sonication [10]. The xylanase content was determined by a two-site ELISA with rabbit antibodies directed to the xylanase preparation used for provocation and EAST. Serial dilutions of a fungal xylanase preparation (Röhm, Darmstadt, Germany) with protein concentrations between 180 ng/ml and 80 pg/ml were used as standard. The capture antibodies in this assay were purified with a protein A column, and the detection antibodies were purified with the antigen coupled to N-hydroxysuccinimide-ester-activated Sepharose and then biotinylated with biotin-N-hydroxysuccinimide ester. The α -amylase content was measured with an assay based on monoclonal

antibodies described recently [10]. Four baking additives contained measurable amounts of fungal α -amylase and xylanase, without a correlation between these enzymes. Interestingly, in wheat flour samples, contamination with fungal α -amylase or xylanase was observed (table 1).

Discussion

This case demonstrates that allergy to xylanase and cellulase may occur in subjects without cutaneous sensitization to α -amylase or flour as assessed by SPT. Although SPTs with flour were completely negative, in vitro tests (CAP and immunoblot) yielded weak sensitizations to wheat and rye flour indicating higher sensitivity compared to SPT with commercial flour extracts. Immunoblot and inhibition results clearly indicate that the minor reactions to flour were not due to contamination of the enzyme preparations by flour. Challenge with rye flour dust produced a significant bronchial and nasal reaction at high dust concentrations (thus indicating that the in vitro results were not false positive), but challenge with a very small dose of xylanase induced a significant bronchial reaction.

Although the patient was not monosensitized to the enzymes but also showed low concentrations of specific IgE antibodies to flour, it is evident that the most relevant allergens were enzymes. They were detected in baking additives used in the patient's bakery. According to the manufacturer's instructions, the dosage of the additive varied somewhat between products but was usually between 1 and 5% (some additives were premixed with flour and used in dosages of about 20%). The enzyme quality was not specified. Our results prove that both xylanase and α -amylase may be found in baking additives. As one enzyme always predominated in the preparations, labeling of baking additives with respect to the quality (but also the quantity) of enzymes would be helpful for allergic patients.

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