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CROSS REACTIVITY BETWEEN ENZYMES

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Enzymes behave as potent inhalational allergens in the occupational environment and are able to sensitize a large proportion of the workers that manipulate them. Whether this great sensitization capacity is related to their enzymatic activity or simply to the fact that they are glycoprotein macromolecules able to trigger an IgE-mediated immunological response has long been a subject of debate.

A number of additives are used in the manufacture of bread, which are added to the dough with the aim to improve and accelerate the panification process.

Table I. Characteristics of the enzyme additives used in bakery processes

- Mixtures of a number of products
- Scarcely purified industrial enzymes
- Contain impurities
- Unknown composition
- Similar fungal origin
 - Aspergillus oryzae*
 - Aspergillus niger*
- Allergenic potential

Table II. Main allergens responsible for asthma in bakers

- Cereals (wheat and rye): albumins, globulins, gliadins, glutenins, prolamines
- Soybean: trypsin inhibitor, lipo-oxidase
- Fungal enzymes:
 - α -amylase (*Aspergillus oryzae*)
 - Hemicellulase and cellulase (*Aspergillus niger*)
 - Xylanase (*A. niger*)
 - Glycoamylase (*A. niger*)
- Storage mites
- Bread yeast (*Sacharomyces cerevisiae*)
- Moulds (alternaria, aspergillus)
- Grain weevil (*Sitophilus granarius*)

Table III. α -amylase: characteristics of allergologic interest

- Glycolytic enzyme usually derived from *Aspergillus oryzae* (Asp o 2).
- Sensitizes 20-32% of symptomatic bakers.
- Partially thermolabile.
- May induce symptoms upon ingestion.
- Glycoprotein with 478 aminoacid residues.
- Continuous sequential epitopes.
- Exposure/sensitization ratio: 0,25 ng/m³.

Among these additives, glycolytic fungal enzymes are one of the major groups. And yet, the enzymatic additives used are scarcely purified industrial products, so that a number of factors must be kept in mind when a possible sensitization is studied. These factors are summarised in Table I.

Bakers' and confectioners' asthma may be caused not only by cereal flour, but also by any of the additives or contaminants present in those flour and summarised in Table II. The sensitization to a number of

enzymes with glycolytic activity derived from *Aspergillus* poses questions about the possible cross reactivity between them, but also between these enzymes and the fungi producing them and, last but not least, between the fungal enzymes and the natural ones to be found in cereal grains.

Cross reactivity between *Aspergillus*-Derived enzymes

The glycolytic enzymes α -amylase and cellulase are additives that are commonly used in the panification process for accelerating the fermentation of the dough. By hydrolysing the starch in the grain flour to fermentable sugars, they continuously generate a substrate that may be used by the yeast. The usual origin of such amylases has been the flour of malted barley, but they are currently derived from microorganisms such as fungi and bacteria. The main characteristics of the fungal α -amylase enzyme are shown in Table III.

In a study of five bakers with α -amylase sensitization, four of whom were also sensitized to cellulase, we investigated the possible cross reactivity between these two fungal enzymes and between the enzymes and the fungi producing them, respectively *A. oryzae* and *A. niger*¹. We found that there was no cross reactivity between α -amylase and cellulase. However, a degree of antigenic community existed between α -amylase and *A. oryzae*, as a 60% inhibition of the α -amylase ELISA (REIA) was achieved with high concentrations of the *A. oryzae* extract. This suggested that α -amylase might represent a relevant allergen in this fungus. A discrete level of cross reactivity was also observed between cellulase and *A. niger*, although in this case the degree of inhibition of the cellulase ELISA with this fungus did not exceed 30%. In a previous study in two patients with occupational asthma who were sensitized to cellulase we had observed moderate cross reactivity between the enzyme and *A. niger*, while there was no cross reactivity between α -amylase and cellulase².

However, the ingestion of bakery products containing α -amylase and hemicellulase did not cause any problem in 17 patients sensitized to *Aspergillus* spp.³.

A very recent study investigating enzyme sensitization in a group of 171 bakers with respiratory allergic symptoms⁴ revealed a 22.8% frequency of sensitization to α -amylase, followed by cellulase (12.9%),

xylanase (10.5%) and glycoamylase (7.6%). This study confirmed that there was no cross reactivity between α -amylase and cellulase or xylanase. However, the cellulase and xylanase extracts, which contained at least six different proteins each, did show cross reactivity in the 80% level⁴. In this same study it was observed that glycoamylase, another glycolytic enzyme from *A. niger* used in bakery, inhibited the xylanase and cellulase RAST by about 50%, suggesting that common epitopes are shared by these enzymes. The authors showed that the main allergenic component in xylanase was a 105 kDa protein with a 5 isoelectric point, which was identified as the β -xylosidase enzyme from *A. niger*; this enzyme was given the designation Asp n 14⁴.

Tarvainen *et al.*⁵ had previously described four workers in biotechnology laboratories who evidenced occupational asthma and contact urticaria after exposure to cellulase or xylanase (a type of hemicellulase that cleaves xylane). These four patients had positive RAST both to cellulase and to xylanase. RAST inhibition studies showed that xylanase inhibited the cellulase RAST by 92%, indicating a high level of cross reactivity between these two enzymes. A primary sensitization to cellulase or hemicellulase will therefore be very difficult to identify because of the high degree of antigenic community between these two enzymes.

Cross reactivity between the natural amylases in cereals and the fungal amylase

A further problem that arises is the possible cross reactivity between the amylases naturally present in cereal grains and in their flours and the fungal amylases used as additives. In our previously discussed study¹ we found that wheat flour caused slight inhibition of the fungal α -amylase RAST, dose-dependently and up to a maximum of 40%. This was interpreted as probably due to a low level of cross reactivity between the natural cereal amylases and the fungal α -amylase, or perhaps to the possible presence of fungal contaminants in the wheat flour.

This aspect was later studied and confirmed by Sandiford *et al.*⁴, who used the sera from 30 patients with inhalational sensitization to wheat flour and compared the IgE levels (RAST) to natural barley α -amyla-

se and β -amylase to those to the fungal α -amylase. Using RAST inhibition studies they demonstrated a low level of cross reactivity between the natural amylases and the fungal α -amylase (in the order of 30 to 40%). They concluded that the natural α -amylase and β -amylase are important allergens in patients with respiratory allergy to cereal flours, but that there is almost no cross reactivity between these enzymes and the fungal α -amylase.

Cross reactivity between bromelain and papain

Bromelain is a mixture of very similar proteolytic enzymes derived from the stalk and the fruit of tropical pineapple (*Ananas comosus*). The two first cases of occupational asthma caused by bromelain were described in 1978⁷. Baur and Fröhmann⁸ later reported a 58-year-old female who had been working in a pharmaceutical laboratory for ten years. In her work she was environmentally exposed to papain and bromelain, and she became sensitized to both enzymes. The patient had positive skin tests, RAST and specific bronchial challenge tests with both enzymes. The bromelain RAST could be inhibited with pineapple juice, but this agent did not inhibit the papain RAST. An oral challenge test with pineapple induced abdominal pain and bronchospasm within 30-40 minutes. A positive RAST to bromelain was observed in five out of six patients sensitized to papain, and also in 8 out of 60 control asthmatics not exposed to proteases. Furthermore, bronchial challenge tests with bromelain were carried out in two of the papain-sensitized patients, with positive results; however, the challenge test with bromelain was negative in three of the control patients with positive RAST.

In an enlarged follow-up of that study⁹, Baur investigated a group of four bakers sensitized to wheat and rye flour and a group of 25 pollen-sensitive asthmatics. One of the bakers and seven of the pollenosis patients had positive RAST to bromelain, and three of the pollenosis patients had positive RAST to papain. In the RAST inhibition studies using the sera of the bakers and of the pollenosis patients the authors observed homologous inhibition of the bromelain and papain RAST with the corresponding allergen, and also RAST inhibition between papain and bromelain, although in the latter case the level of the inhibitor

was highly variable from one patient to another. Using the serum of the patient who was clinically sensitized to papain and bromelain and pollen extracts as inhibitors, a certain degree of inhibition (mild to moderate) of the papain RAST was seen (grass pollens 9-63%, birch 3-63%, wheat 9-74%), and also of the bromelain RAST (grass pollens 15-86%, birch 9-69%, wheat 13-89%, rye 6-90%). In a further experiment, the RAST to grass pollens and to bromelain could be inhibited in almost all cases with bromelain, but only in a few cases and to a lesser extent with papain.

A high level of cross reactivity has also been reported between papain and chymopapain, an enzyme that is widely used for carrying out chymonucleolysis in patients with intervertebral disc hernia and which might induce sensitization and severe anaphylactic reactions in patients sensitized to papain¹⁰.

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