

Gastrointestinal stability of baker's yeast allergens: an *in vitro* study

O. KORTEKANGAS-SAVOLAINEN*†, J. SAVOLAINEN†‡ and R. EINARSSON§

Departments of *Dermatology, †Medical Microbiology and ‡Clinical Allergology, University of Turku, Finland and §University of Linköping and Pharmacia Diagnostics AB, Uppsala, Sweden

Summary

An *in vitro* model was established to study the stability of baker's yeast (*Saccharomyces cerevisiae*) allergens in conditions simulating the gastrointestinal tract. The protocol consisted of 2 hr incubation under gastric conditions (pH 1.2, +37°C and gastric enzymes) and 2 hr incubation under duodenal conditions (pH 6.8, +37°C and duodenal enzymes). These were studied together and separately, as well as under pure acidic conditions without gastric enzymes. The yeast extracts contained equal amounts of allergen and were analysed by IgE-immunoblotting. The acidic conditions had partly an enhancing and slightly degrading effect on the yeast allergens, whereas the gastric enzymes destroyed several allergens, including the important intermediate allergens of 31 and 45 kD. After treatment under both gastric and duodenal conditions most of the yeast allergens were destroyed, except mannan and a 10 kD protein component. The findings suggest that the allergen exposure caused by baker's yeast takes place mainly on the mucosal surfaces orally and oesophageally and through viable baker's yeast organisms that manage to pass the stomach and duodenum and possibly lead to intestinal growth of the organism. Patients with IgE production against the 10 kD allergen and mannan are, however, moderately exposed to allergens consisting of soluble antigenic material only.

Clinical and Experimental Allergy, Vol. 23, pp. 587–590. Submitted 11 September 1992; revised 20 January 1993; accepted 10 February 1993.

Introduction

Saccharomyces cerevisiae (baker's yeast) is a ubiquitous yeast in our environment—it is used in bakery, brewery and winery products. Occasionally, it also grows in the gastrointestinal (GI) tract [1] where it is subject to digestion which may change the structure of the yeast or destroy it.

Several approaches have been made to study the influence of the GI tract on the antigenicity and allergenicity of food components [2–9]. The aim of this study was to investigate the degradation of baker's yeast allergens with an *in vitro* model that has been validated in earlier studies [4,7,8]. Commercially available gastric and duodenal enzyme solutions were used as well as appropriate pH values. The effect on the allergenicity of degraded *S.*

cerevisiae was assessed by immunoblotting, a technique that allows monitoring of the IgE-binding to important individual allergens.

Materials and methods

Preparation of baker's yeast extracts

A crude cytoplasmic extract of *S. cerevisiae* was prepared as previously reported [10].

Gastrointestinal in vitro model

The GI *in vitro* model was performed as shown in Fig. 1. Briefly, gastric conditions were simulated by dissolving 100 mg of antigen in 1.3 ml PBS, after which 1.3 ml 'gastric solution' was added and the pH was adjusted to 1.25 with 0.1 N HCl. The gastric solution consisted of 1 Enzynorm® tablet, a mixture of pepsin, cathepsin and mucin (Nordmark-Werke, Hamburg, Germany), dis-

Correspondence: Dr O. Kortekangas-Savolainen, Department of Dermatology, University of Turku, Finland.

solved in 175 ml physiological saline. The extract was kept at 37°C for 2 hr in a slow shaker and the first sample was then taken, 2.6 ml of 'duodenal solution' was added and the pH was adjusted to 6.8 with NaOH. The duodenal solution consisted of 1 Västäl® tablet (pancreas lipase, pancreas amylase, pancreas protease and cholic acid; Hoechst, Frankfurt am Main, Germany) in 150 ml of physiological saline. The extract was kept at 37°C in a slow shaker for 45 min, after which the second sample was taken. As a control an equal concentration of antigen was studied in a solution with the pH adjusted to 1.25; this sample was kept at 37°C for 2 hr.

Patient sera

A serum pool with different broad spectrum IgE specificities from seven *S. cerevisiae* radio allergosorbent test (RAST)-positive patients was used for the IgE blotting experiments. By immunoblotting the IgE-binding pattern of these sera identified all important allergenic bands, including the 85, 75, 48, 45, 39, 32, 31, 25 and 10 kD bands and mannan. Thus, most of the important allergens previously reported were represented [10]. As a control pooled sera from five non-atopic subjects were used.

SDS-PAGE and electrophoretic blotting

The SDS-PAGE was run with 30 µl of all processed baker's yeast extracts, diluted with 15 µl of Tris buffer, adjusted to a pH of 8.6, containing 10% (w/v) SDS and 10% (w/v) glycerol. All samples had equal final antigen concentrations. The samples were incubated for 5 min at 100°C and then separated with SDS-PAGE in a 5–17.5% gradient gel as described previously [11]. Molecular weight markers (Rainbow, Amersham, Buckinghamshire, U.K.) were run in parallel with SDS-PAGE. Separated antigens were electroblotted onto nitrocellulose sheets (Schleicher & Schuell, Kassel, Germany) in a Transphor apparatus (LKB, Bromma, Sweden) as previously described [12].

IgE antibody detection

The specific IgE-binding of the allergens on nitrocellulose was identified as described [13]. The serum pools were not diluted. ¹²⁵I-labelled anti-IgE antibodies (Phadebas-RAST, Pharmacia Diagnostics, Uppsala, Sweden) were used diluted to 20 000 c.p.m./ml in PBS-Tween. The ¹²⁵I-labelled bands were recorded on a Trimax X-ray film in a cassette with intensifying screens (Trimax) after storage at –70°C prior to developing.

Results

The *in vitro* model presented in Fig. 1 was easy to carry out and repeatable. A correct pH in both simulated

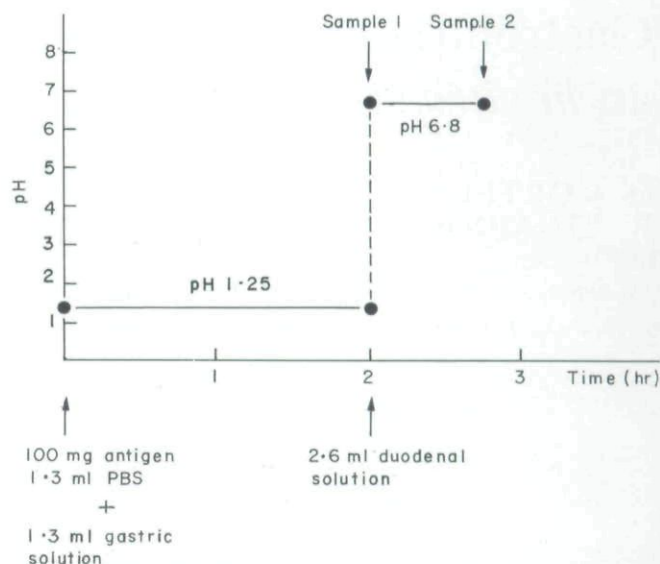


Fig. 1. Gastrointestinal degradation of baker's yeast allergens *in vitro*. Natural enzymes are included in the gastric and duodenal solutions.

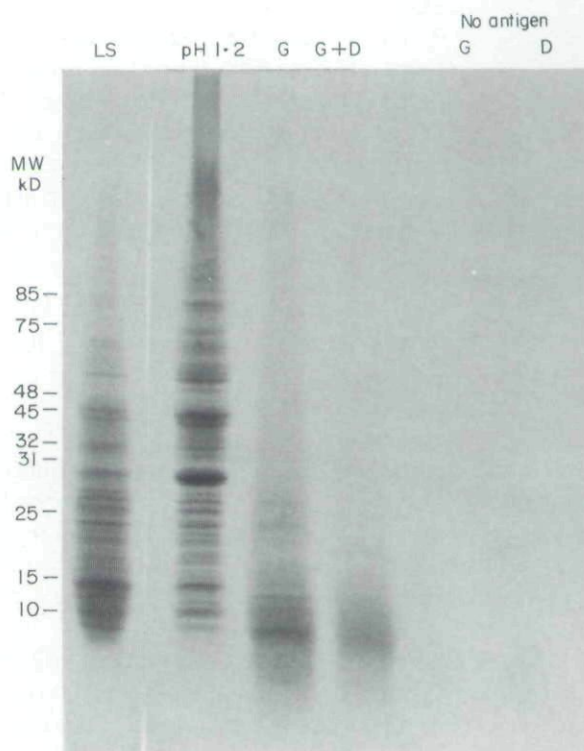


Fig. 2. SDS-PAGE analysis of the baker's yeast samples after various treatments (Coomassie brilliant blue). LS, Lyophilized control sample; pH 1-2, acidic treatment alone; G, gastric conditions (sample 1, Fig. 1) and G+D, both gastric and duodenal conditions (sample 2, Fig. 1). The gastric and duodenal solutions alone were also analysed with the absence of dissolved baker's yeast antigen.

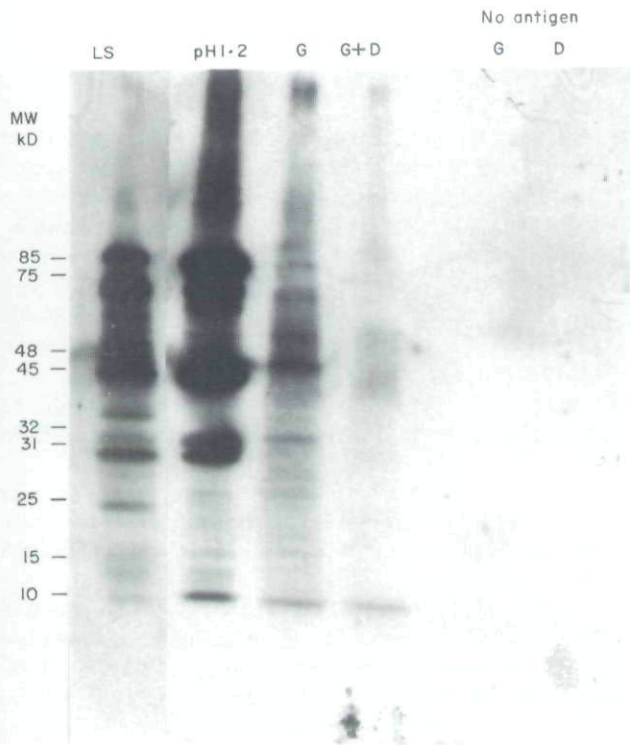


Fig. 3. IgE-immunoblots of the SDS-PAGE samples. Electrophoretic conditions as in Fig. 2.

conditions, gastric and duodenal, was essential for the solubility of the commercial preparations.

Acidic treatment had an enhancing and gastric and duodenal solutions a deleterious effect on the proteins. Figure 2 shows the Coomassie-stained gel and Fig. 3 shows the IgE immunoblot patterns of the pH treatment and acidic, gastric and duodenal conditions. Acidic conditions alone had only a slight degrading effect on the proteins, whereas the high molecular weight diffuse mannan stain was strongly enhanced. The protein bands demonstrating enhanced IgE-binding due to acidic treatment had molecular weights of 85, 45, 32 and 10 kD.

The deleterious effect of the gastric and especially of the duodenal conditions on the yeast proteins was evident. The gastric conditions degraded the 45, 31 and 25 kD bands most markedly and all detected IgE-binding bands, except the 48 kD band, showed loss of IgE-binding capacity. The high molecular weight mannan stain also showed reduced IgE binding. After duodenal treatment, all the IgE-binding activity was abolished, except for the 10 kD band and the high molecular weight diffuse mannan stain. Even these showed weakened binding, although the diffuse mannan stain was now visualized down to 40 kD, due to degraded proteins.

The pooled non-atopic sera showed no IgE binding.

Discussion

Methods used to assess allergenicity in GI models include skin tests [3], the Schultz-Dale test, the uterus slice technique [2,6], RAST [7-9,14], IEF-immunoprint [8] and RRIE [9]. In this study, IgE immunoblotting allowed the analysis of individual allergens separated by size. In most studies enzymatic treatment has consisted of pepsin [5,6,14,15] or pepsin and trypsin only [2-4,7,8]. Pure human duodenal juice has also been used, but it is difficult to obtain [9]. The experimental conditions created in this study simulated *in vivo* conditions very closely and yet only commercial enzyme preparations with a variety of natural enzymes were used. This kind of study design could be used to investigate all suspected food allergens.

Most experimental studies on the degradation of allergens in the GI-tract and on the role of IgE-mediated immune response in the gut in atopic dermatitis have used cow's milk allergy as a model, which has laid emphasis on paediatric conditions. In contrast to young children, adults and older children have no abnormal permeability of the gut, and physiological absorption of the ingested allergens is enough to maintain symptoms [16-18]. Allergen-specific IgE has been demonstrated in intestinal washings of children with cow's milk allergy [19]. IgE-stained cells have been recovered in intestinal biopsies in adults as well as children with atopic dermatitis and food allergy [20,21]. The IgE-mediated recognition of ingested allergens is thus likely to be of importance in atopic dermatitis. The stability of the IgE-binding structures in the GI tract may thus be an important determinant of a food allergen.

The digestion of components of cow's milk by pepsin exposes new allergenic epitopes [3,5,7,14] which are allergenic only to subjects that have been sensitized to the native allergens [14]. These neo-allergens are thus likely to be formed in the stomach. In our study, the GI conditions did not, in fact, expose new epitopes but, rather, showed that most native epitopes, except for the 10 kD band and high molecular weight diffuse mannan, are destroyed by this treatment. This is in agreement with studies on pepsin and trypsin treatment of cereal components [8] and digestion of birch and timothy allergen by natural human duodenal juice [9]. In contrast, pH treatment demonstrated enhanced IgE-binding activity, especially in the high molecular weight mannan region, while some acid-sensitive IgE-binding bands disappeared. Acid hydrolysis splits the mannan polysaccharide possibly exposing new epitopes [22].

When baker's yeast is consumed orally, the immediate allergen exposure takes place in the native form on the oral mucosa. The degraded yeast proteins that enter the duodenum still have allergenic potency and even after

1 hr, when almost all allergenic activity has disappeared, traces of the 10 kD allergen and mannan may cause important exposure to patients sensitized to these allergens. On the other hand, viable yeast cells might pass the stomach and the duodenum and continue to grow thereafter in the intestine. *Saccharomyces cerevisiae* is occasionally found within the intestinal flora of atopic subjects [1].

The GI conditions simulated by our *in vitro* study destroyed more than 90% of the allergenic activity of baker's yeast. However, large amounts of ingested baker's yeast may cause clinically important exposure, especially to individuals sensitized to the 10 kD allergen or mannan. This *in vitro* model of allergen degradation in the GI tract provides a tool for studying the sensitizing properties of food allergens.

Acknowledgments

This study was supported by grants from the Emil Aaltonen Foundation, the Finnish Allergy Research Foundation and the Finnish Dermatologists' Society. We thank Mrs Leena Kaven-Honka for excellent technical assistance. Mrs Maire Mäkimartti MSc (Pharm) gave valuable advice when the gastrointestinal conditions were constructed.

References

- Savolainen J, Koivikko A, Kalimo K, Nieminen E, Viander M. IgE, IgA and IgG antibodies and delayed skin response towards *Candida albicans* antigens in atopics with and without saprophytic growth. *Clin Exp Allergy* 1990; 20:549-54.
- Urbach E, Jaggard G, Crisman DW. Experimental approach to oral treatment of food allergy. II. Immunologic properties of food propeptans. *Ann Allergy* 1945; 3:172-86.
- Bloom S, Markow H, Redner B. Studies in food sensitivity. II. The effect of protein digestion on antigenicity of foods as determined by skin tests and clinical food trials. *J Allergy* 1953; 24:64-72.
- Frazer AC, Fletcher RF, Ross CAC, Shaw B, Sammons HG, Schneider R. Gluten-induced enteropathy. The effect of partially digested gluten. *Lancet* 1959; 2:252-55.
- Spies JR, Stevan MA, Stein WJ, Coulson EJ. The chemistry of allergens. XX. New antigens generated by pepsin hydrolysis of bovine milk proteins. *J Allergy* 1970; 45:208-19.
- Spies JR, Stevan MA, Stein WJ. The chemistry of allergens. XXI. Eight new antigens generated by successive pepsin hydrolyses of bovine beta-lactoglobulin. *J Allergy Clin Immunol* 1972; 50:82-91.
- Haddad ZH, Kalra V, Verma S. IgE antibodies to peptic and peptic-tryptic digests of betalactoglobulin: significance in food hypersensitivity. *Ann Allergy* 1979; 42:368-71.
- Sutton R, Hill DJ, Baldo BA, Wrigley CW. Immunoglobulin E antibodies to ingested cereal flour components: studies with sera from subjects with asthma and eczema. *Clin Allergy* 1982; 12:63-74.
- Einarsson R, Renck B, Taudorf E. *In vitro* studies of degradation of birch and timothy pollen allergen preparations by human duodenal juice. *Allergy* 1988; 43:469-72.
- Kortekangas-Savolainen O, Kalimo K, Lammintausta K, Savolainen J. IgE-binding components of baker's yeast (*Saccharomyces cerevisiae*) recognized by immunoblotting analysis. Simultaneous binding to mannan and 48/46 kD allergens of *Saccharomyces cerevisiae* and *Candida albicans*. *Clin Exp Allergy* 1993; 23:179-84.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680-5.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76:4350-4.
- Bengtsson A, Rolfsen W, Einarsson R. Characterization of allergens and patient sera by a nitrocellulose immunoprint technique. *Int Arch Allergy Appl Immunol* 1985; 78:139-44.
- Schwarz HR, Nerurkar LS, Spies JR, Scanlon RT, Bellanti JA. Milk hypersensitivity: RAST studies using new antigens generated by pepsin hydrolysis of beta-lactoglobulin. *Ann Allergy* 1980; 45:242-5.
- Ishizaka T, Campbell DH, Ishizaka K. Internal antigenic determinants in protein molecules. *Proc Soc Exp Biol Med* 1960; 103:5-9.
- Du Mont GCL, Beach RC, Menzies IS. Gastrointestinal permeability in food-allergic eczematous children. *Clin Allergy* 1984; 14:55-9.
- Fälth-Magnusson K, Kjellman NIM, Sundqvist T, Magnusson KE. Gastrointestinal permeability in atopic and non-atopic mothers, assessed with different-sized polyethyleneglycols (PEG 400 and PEG 1000). *Clin Allergy* 1985; 15:565-70.
- Pike MG, Hedde RJ, Boulton P, Turner MW, Atherton DJ. Increased intestinal permeability in atopic eczema. *J Invest Dermatol* 1986; 86:101-4.
- Marcuzzi F, Sensi LG, Bizzarri G. Specific IgE to food and inhalant allergens in intestinal washings of children affected by atopic eczema. *Clin Allergy* 1985; 15:345-54.
- Rosekrans PCM, Meijer CJLM, Cornelisse CJ, vd Wal AM, Lindeman J. Use of morphometry and immunohistochemistry of small intestinal biopsy specimens in the diagnosis of food allergy. *J Clin Pathol* 1980; 33:125-30.
- Kalimo K, Lammintausta K, Klemi P, Leino R, Panula P, Kalimo H. Mast cells and IgE in intestinal mucosa in adult atopic dermatitis patients. *Br J Dermatol* 1988; 119:579-85.
- Faille C, Michalski JC, Strecker G, Mackenzie DWR, Camus D, Poulain D. Immunoreactivity of neoglycolipids constructed from oligomannosidic residues of the *Candida albicans* cell wall. *Infect Immun* 1990; 58:3537-44.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.