Detection of a Novel Allergen in Raw Tomato

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Abstract

The study reports a case of "pure" tomato allergy in an adult female. The responsible allergen was partially characterized by immunoblot analysis, pepsin digestion, and heating. It had a molecular weight of about 9 kDa and was heat-labile and pepsin-resistant, thus confirming the clinical history. Unfortunately, due to the failure of both 2-dimensional electrophoresis analysis and N-terminal sequencing experiments, it was not possible to characterize the protein further. Based on a comparison with currently known tomato allergens, this seems to be a novel allergen protein.

Key words: Food allergy. Tomato. Allergens. Processing.

Resumen

Este estudio informa de un caso de alergia al tomate "puro" en una mujer adulta. Se caracterizó de manera parcial al alérgeno responsable mediante análisis de inmunotransferencia, digestión con pepsina y calentamiento. Tenía un peso molecular aproximado de 9 kDa y era termolábil y resistente a la pepsina, confirmando de este modo los antecedentes clínicos. Desgraciadamente, debido al error tanto de la electroforesis bidimensional, como de los experimentos de la secuencia N-terminal, no fue posible caracterizar de un modo más exhaustivo dicha proteína. Basándonos en la comparación con otros alérgenos del tomate que se conocen en la actualidad, esta parece ser una nueva proteína alérgena.

Palabras clave: Alergia a los alimentos. Tomate. Alérgenos. Procesamiento.

Introduction

Tomato is known mainly as a cause of oral allergy syndrome in patients who are allergic to pollen and food due to sensitization to the cross-reacting allergen protein, profilin. We report a case of "pure" tomato allergy caused by a novel, heat-labile allergen.

Case Description

A 53-year-old woman recently attended our allergy department because of a 4-year history of perennial rhinitis and a 3-year history of severe abdominal pain, cramps, and diarrhea following the ingestion of raw tomatoes. Pain developed about 30-60 minutes after ingestion and lasted about 2 hours. The patient reported good tolerance of cooked tomato and all other foods.

Skin prick testing (SPT) with commercial extracts (Allergopharma, Reinbeck, Germany) of both seasonal and perennial airborne allergens including grass, mugwort, ragweed, Parietaria, plantain, birch, olive, cypress, molds (*Alternaria, Aspergillus, Cladosporium, Candida, Penicillium,* and *Trichophyton*), house dust mites, and cat and dog dander revealed hypersensitivity to *Candida albicans* only. SPT with a large panel of commercial food extracts (Alk-Abelló, Lainate, Italy; 5% w/v) showed skin reactivity to tomato extract (mean wheal diameter, 6.5 mm), whereas the remaining food extracts (egg, cow's milk, shrimp, peanut, wheat, maize, soybean, walnut, hazelnut, sunflower seed, cod, carrot, orange, potato,

peach, celery, almond, and banana) did not induce any skin reaction. SPT with latex extract was negative, as was SPT with a purified natural palm profilin extract (Pho d 2, 50 µg/mL; Alk Abelló, Madrid, Spain).

The pulp and peel of 100 g of fresh tomato were homogenized. The homogenate was mixed with 300 mL of precooled acetone and equilibrated at –20°C overnight. The precipitate was washed twice with acetone and once with acetone/ether (1:1, v/v) and dried. The resulting powder was extracted [1]; the protein concentration of the extract was 1 mg/mL [2] (BioRad, Milan, Italy).

In direct enzyme-linked immunosorbent assay (ELISA), 2 µg of raw tomato extract or 0.1 µg of recombinant peach lipid transfer protein (LTP), both diluted in 100 µL of coating buffer (15 mM Na_2CO_3, and 35 mM $NaHCO_3$)/well, was used to coat 96-microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) [3]. After washings with 0.1 M phosphate-buffered saline (PBS), pH 7.4, and 0.05% Tween 20 (Sigma, Milano, Italy), the wells were saturated with 2% bovine serum albumin in PBS for 2 h at room temperature. After further washing, 100 µL of undiluted serum was added to each well and incubated for 2 h at room temperature. After washing, solid phase–bound specific immunoglobulin (Ig) E was detected by adding peroxidase-conjugated anti-human IgE from goat (1:1500; BiosPacific, Emeryville, California, USA). The enzyme reaction, induced using tetramethyl-benzidine/H_2O_2 as substrate, was stopped after 20 minutes by 1 mol/L HCl. Absorbance, read at 450 nm, confirmed the patient's IgE reactivity to tomato: 0.2 OD vs 0.05 OD using a normal control serum. No IgE reactivity to recombinant peach LTP was detected.

The patient's IgE reactivity was further investigated by immunoblotting under reducing conditions against cooked, raw, and pepsin-digested tomato extract. Electrophoresis of extracts (15 µg/lane) was carried out in a 10% polyacrylamide precast Nupage Bis-Tris gel with MES buffer according to the manufacturer's instructions (Invitrogen, Milano, Italy) at 180 mA for 1 h. The gel-resolved proteins were transferred for 1 h onto a nitrocellulose membrane [4]. The membrane was saturated with 0.1 mol/L Tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with sera. After 3 washings, bound specific IgE was detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:1000 in saturation buffer; BiosPacific) using an ECL Western blotting kit substrate (Amersham, Milano, Italy).

Pepsin digestion was carried out as previously described [5]. Briefly, tomato extract was dissolved in 10 mM HCl and pepsin (Sigma, Milano, Italy) was added to a final concentration of 0.35% (w/v) and pH 2. Digestion was performed at 37°C for 4 h, and stopped by the addition of 0.5 M K_2HPO_4.

Immunoblotting revealed clear IgE reactivity at about 9 kDa (Figure 1). This disappeared completely if the tomato extract was cooked at 100°C for 30 min. In contrast, IgE reactivity did not change if pepsin-digested tomato extract was used for immunoblotting.

In order to isolate the tomato allergen for subsequent N-terminal sequencing, 2-dimensional electrophoresis (2-DE) analysis was performed. The protein extract for 2-DE analysis was prepared as described elsewhere [6]. Briefly, 1 g of dried powder was extracted with 50 mL of Tris-base 0.5 M, KCl 0.1 M,

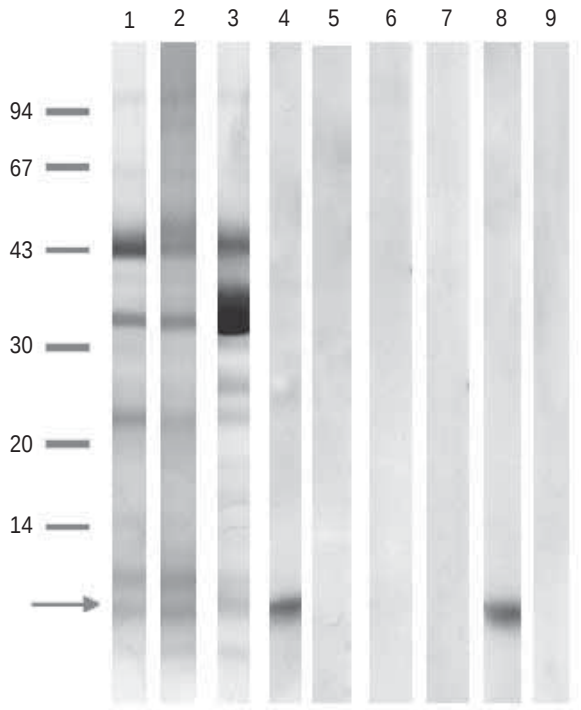

Figure 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of raw (lane 1), cooked (lane 2), and pepsin-digested (lane 3) tomato extract. The gel is stained with Coomassie colloidal blue (Invitrogen, Milan, Italy). The arrow indicates the protein(s) on which N-terminal sequencing was attempted.
Immunoblot analysis: IgE reactivity of patient's serum to raw (lane 4), cooked (lane 6), and pepsin-digested (lane 8) tomato extract; IgE reactivity to the same extracts of a pool of normal sera is shown in lanes 5, 7, and 9, respectively.

1% polyvinyl polypyrrolidone (PVPP), and complete protease inhibitor cocktail (Roche Applied Science, Milano, Italy) for 3 hours at 4°C. Thirty milliliters of extract were precipitated at –20°C overnight with 4 volumes of ice-cold acetone containing 10% TCA, 1% PVPP, and 2% ß-mercaptoethanol. The protein pellet, recovered by centrifugation at 10 000*g* for 30 min underwent multiple ice-cold acetone washes, before being dried and redissolved in isoelectric focusing (IEF) buffer containing 7 M Urea, 2 M thiourea, CHAPS 2%, Tris-Base 20 mM tributylphosphine (TBP) 5 mM (Bio-Rad, Milan, Italy), 1% w/v carrier ampholytes, pH 3-10 (Bio-Rad), and 0.002% bromophenol blue. The protein concentration, measured using the RcD5 Protein Assay (Bio-Rad) was 620 µg/mL. IPG strips (7 cm pH 3-10 Bio-Rad ReadyStrip) were rehydrated overnight with 175 µL of IEF buffer. Proteins were focused using a Protean IEF Cell (Bio-Rad) at 23°C with a current limit of 50 µA/strip, by applying the following program: a linear increase from 0 V to 250 V for 1h, 250 V to 2000 V for 4 h, held at 2000 V for a total of 20 kVh, followed by a rapid increase from 2000 V to 4000 V, where it was held for a total of 30 kVh. After focusing, proteins were reduced by incubating the strips with equilibration buffer 1 (6M urea, 20% glycerol, 2% sodium dodecyl sulphate [SDS], 0.002%

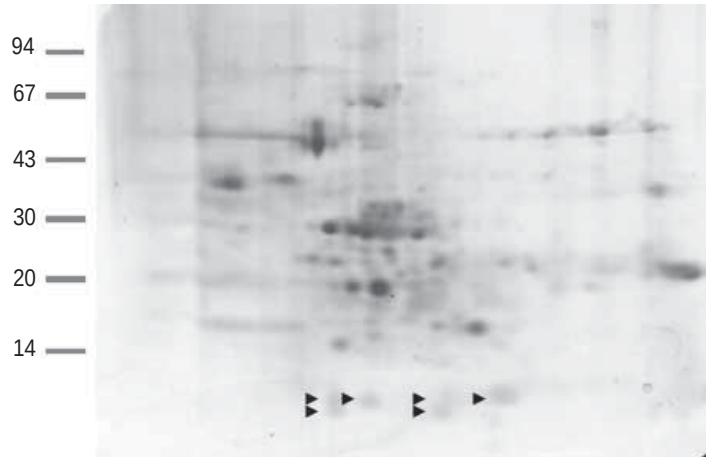

Figure 2. 2-D gel of raw tomato extract stained with Coomassie colloidal blue (Invitrogen, Milan, Italy). The arrows indicate protein spots at about 9 kDa.

bromophenol blue, 0.375 M Tris-HCl pH 8.8, 5 mM TBP) for 10 min and alkylated with equilibration buffer 2 (6M urea, 20% glycerol, 2% SDS, 0.002% bromophenol blue, 0.375 M Tris-HCl pH 8.8, 2.5% [w/v] iodoacetamide) for 10 min. The strips were transferred onto 4%-20% gradient gels (4%-20% Tris-glycine ZOOM gel; Invitrogen) and SDS-polyacrylamide gel electrophoresis (PAGE) was performed at 125 V. Gels were stained with Coomassie colloidal blue (Invitrogen) or blotted and incubated with the patient's serum as described above. A 2-D gel of raw tomato extract is shown in Figure 2.

Unfortunately, although some spots are clearly present at about 9 kDa (Figure 2, arrows), the IgE reactivity that had been previously observed on 1-D SDS-PAGE was no longer detectable on immunoblot with the patient's serum. This negative result was confirmed in several consecutive experiments.

Thus, N-terminal sequencing was attempted directly on the SDS-PAGE of raw tomato extract containing the IgE-binding protein. The band was excised from the SDS-PAGE gel, passively eluted by a slightly modified Zieske technique and microsequenced on a Procise 492 protein sequencer (Applied Biosystems, Foster City, California, USA), as described elsewhere [7]. Unfortunately, the IgE-reactive band contained several N-terminal sequences, thus making identification of the relevant allergen impossible.

Discussion

Our in vitro studies confirmed the clinical history by showing that this patient was sensitized to a heat-labile and pepsin-resistant tomato protein. The reasons why this patient had only gastrointestinal symptoms and did not experience systemic symptoms after the absorption of the allergen in the gut remain unclear. The presence of severe gastrointestinal symptoms including abdominal pain, cramps, and diarrhea seems to confirm that the allergen resisted pepsin digestion and reached the gastrointestinal tract in an unmodified form. Unfortunately, the harsh treatments applied to isolate the allergen may have prevented it from being detected in 2-DE, and the presence of several N-terminal sequences made identification impossible.

Several tomato allergens have been described to date, including Lyc e 1 (profilin, 14 kDa), Lyc e 2 (ß-fructofuranosidase, 50 kDa), Lyc e 3 (LTP, 6 kDa), Lyc e chitinase (31 kDa), Lyc e glucanase (55 kDa), and Lyc e peroxidase (44 kDa). The clinical relevance of each of these allergens is not clear, with the exception of profilin, which may cause a typical oral allergy syndrome in patients with multiple pollen allergy [8,9]. In our patient, the absence of IgE reactivity to profilin, a highly cross-reacting plant panallergen, was clearly suggested by the lack of IgE reactivity to all the pollen extracts and by the lack of reactivity to purified natural palm profilin on SPT. Despite having a similar molecular weight, there are several reasons why the allergen recognized by our patient did not correspond to tomato LTP [10]. Like profilins, LTPs are largely cross-reacting plant panallergens [11]. Our patient did not report any clinical allergy or show any skin reactivity to other plant-derived foods—she scored negative on SPT with commercial peach extract, a marker of LTP hypersensitivity [12], and did not react to recombinant peach LTP on ELISA. Moreover, she reacted to a heat-labile allergen, whereas LTPs are known for their extreme heat-stability [13]. Finally, the completely different molecular weight seems to exclude possible reactivity to other tomato allergens described to date. Thus, the allergen involved in this case of tomato allergy seems to be a novel one, although additional studies are needed to complete the characterization we undertook here. This study provides further confirmation that the clinical expression of food allergy strongly depends on the chemical and physical features of the causative allergens.

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