

Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100°C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results

Elide A. Pastorello, MD,^a Carlo Pompei, PhD,^b Valerio Pravettoni, MD,^a
Laura Farioli, BSc,^c Ambra Marianna Calamari, MD,^a Joseph Scibilia, MD,^d
Anna Maria Robino, MD,^a Amedeo Conti, PhD,^e Stefania Iametti, PhD,^f
Donatella Fortunato, BSc,^e Simona Bonomi, BSc,^a and Claudio Ortolani, MD^d
Milan and Turin, Italy

Background: In a previous study a 9-kd lipid-transfer protein (LTP) was identified as the major allergen of raw maize in a population of 22 anaphylactic patients. However, the stability of this protein in cooked maize is unknown.

Objective: We investigated the allergenicity of 5 maize hybrids and its modification after different thermal treatments by using sera from anaphylactic patients and patients with positive double-blind, placebo-controlled food challenges.

Methods: Five maize hybrids were extracted by using different methods, obtaining the water-soluble, zein, total zein, glutelin, and total protein fractions. The IgE-binding capacity of the different extracts, both raw and after thermal treatment, was investigated by means of SDS-PAGE immunoblotting. A 9-kd heat-stable allergen was purified by means of HPLC and sequenced. Changes in its secondary structure during and after heating from 25°C to 100°C were monitored by means of circular dichroism. **Results:** All raw maize hybrids showed similar protein and IgE-binding profiles. The SDS-PAGE of all the heat-treated hybrids demonstrated a decreased number of stained bands in respect to the raw samples. The IgE immunoblotting demonstrated that the major allergen of the water-soluble, total zein, total protein, and glutelin fractions was a 9-kd protein identified by means of amino acid sequence as an LTP and a subtilisin-chymotrypsin inhibitor (in total zein fraction). The IgE-

binding capacity of this 9-kd protein remained unchanged after thermal treatments, even though circular dichroism demonstrated an altered secondary structure.

Conclusions: Maize LTP maintains its IgE-binding capacity after heat treatment, thus being the most eligible candidate for a causative role in severe anaphylactic reactions to both raw and cooked maize. (*J Allergy Clin Immunol* 2003;112:775-83.)

Key words: Food anaphylaxis; maize allergy; lipid-transfer protein; thermal treatment; double-blind, placebo-controlled food challenge

The modern approach to food allergy must always take into account the modifications of the IgE-binding properties of a food after normal cooking procedures. Roasting, for example, can cause the Maillard reaction, which increases the allergenicity of some foods, such as peanuts.¹ More often, heating totally eliminates the allergenic potential of a food, as described for the chitinase-containing foods² involved in latex-fruit syndrome, in which the sensitization is exclusively directed toward the abovementioned class of heat-labile proteins.

On the contrary, when several allergens are involved in hypersensitivity to a single food, the final effect of cooking on its allergenicity depends on the stability of the various allergens recognized by the IgE of each allergic patient.

Recently, we demonstrated that the allergenic potential of a peach nectar was not modified by heat treatment at 121°C when the allergen recognized by the patients was the peach major allergen, a lipid-transfer protein (LTP) highly resistant to high temperatures.³ However, heating reduced IgE binding to all the other peach allergens.

LTP heating resistance has been demonstrated for other LTPs in foods, such as the LTP in barley, which survives malting and brewing processes.⁴

Recently, we described anaphylactic reactions to maize in a group of 22 subjects and identified the 9-kd LTP of maize as its major allergen.⁵ In Italy maize is traditionally consumed cooked as polenta, but nowadays different

From ^aAllergy Center, 1st Division of General Medicine Ospedale Maggiore IRCCS, Department of Internal Medicine University of Milan; ^bthe Department of Food Science and Microbiology, University of Milan; ^cUOOML, CEMOC, I.C.P., Milan; ^dBizzozzero Division, Niguarda Ca' Granda Hospital, Milan; ^eNational Research Council, ISPA, Turin; and ^fthe Department of Agri-Food Molecular Sciences, University of Milan.

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Reprint requests: Elide Anna Pastorello, MD, 1st Department of Internal Medicine-Padiglione Granelli, Ospedale Maggiore di Milano, IRCCS, Via Francesco Sforza 35, 20122 Milan, Italy.

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
LTP: Lipid-transfer protein
MW: Molecular weight
OAS: Oral allergy syndrome

forms of cooked maize are also frequently consumed, such as breakfast cereals, baked goods, and snacks.

We therefore set up a study to investigate modifications of IgE-binding properties of maize after different thermal treatments and to test the allergenic potential of different maize hybrids.

METHODS**Patients**

We used the sera of 3 groups of outpatients referred to the allergy units of the departments involved. Group 1 comprised 22 patients with maize allergy investigated in our first maize study.⁵ Sera from all 22 patients were pooled and used in immunoblot experiments. Groups 2 and 3 comprised 7 newly recruited patients. Group 2 (nos. 1-4 in Table I) had severe anaphylactic reactions after the ingestion of cooked maize flour products (ie, polenta) and was not provoked because of the severity of symptoms. Group 3 (nos. 5-7 in Table I) had a history of systemic reactions to maize and had positive double-blind, placebo-controlled food challenge (DBPCFC) results.

Serum-specific IgE antibodies to foods, including maize grain and pollens, were detected by using the CAP System (Pharmacia & Upjohn).

DBPCFCs

DBPCFCs were carried out according to the standardized procedure, as stated in the protocol of FAREDAT project QLK4-CT2001-00301. An informed consent form was signed by the patients. Both the administrator and the patient were blinded to the test material; active and placebo meals were randomized for each patient and administered on the same day, with a 3-hour interval between challenges. The active meal, prepared by the dieticians of the "Niguarda Cà Granda" Hospital, contained raw maize flour, water, sugar, potato flour, saffron, lemon juice, and vanilla flavor. The placebo meal had the same ingredients, but maize was replaced with wheat flour. A teaspoon (about 0.5 g) of the test meal was administered as the starting dose; the dose was increased by a factor of 1.5 or 2 every 20 minutes until the entire meal was eaten or until symptoms appeared.

The maximum cumulative dose of maize administered in DBPCFCs was 25 g, with a maximum single dose of approximately 10 g. Subjective symptoms were registered, and the challenge continued until objective symptoms appeared. Four subjects without maize allergy were challenged as negative control subjects.

Thermal treatments of the different maize hybrids

We used 5 samples of field maize hybrids named Alicia, Lolita, Proxima, Tevere, and Aramis, which were provided by the Experimental Institute for Cereal Culture (S. Angelo Lodigiano). All these hybrids are widely grown in Italy and, except for Proxima, in the United States. These samples were finely ground, and the flour was sifted. Six different heat treatments were carried out on each hybrid. For each heat treatment, a set of 5 enameled tin cans (74 × 104 mm) was prepared as follows. In each can 70 g of flour was vigorously stirred into 340 mL of tap water previously heated to 80°C to 90°C.

The cans were then mechanically sealed. For each set, a thermocouple was inserted into the center of one can to record the time-temperature profile. Each set was retorted at 100°C for different lengths of time to carry out 6 different cooking processes.

The C_0 value (cooking effect = equivalent time in minutes at 100°C), analogous to the F_0 value (sterilizing effect),⁶ was calculated to compare the thermal effect of the different heat treatments by using the following equation:

$$C_0 = \int_0^t \frac{dt}{10 \frac{T^* - T}{z}}$$

where t is the treatment time, T^* is 100°C, T is the actual temperature, and z is 25°C. The results are shown in Table II.

Allergen extract

Each hybrid, raw and postthermal treatments, underwent different extraction procedures. The water-soluble proteins were extracted by using an ammonium hydrogen carbonate buffer, as previously described.⁵ Because the thermally treated samples had a lower protein concentration than the raw samples, a Centriprep 3 concentrator with a 3000-d molecular weight (MW) cutoff point (Amicon Inc) was used to reach a value ranging from 0.86 to 4.91 mg/mL. The total zein and total protein fractions were extracted as described by Pasini et al⁷ by using 50% (vol/vol) propan-2-ol with 2.5% (vol/vol) 2-mercaptoethanol as the reducing agent for zeins and a TRIS-HCl buffer with 6 mol/L urea, 2.5% (wt/vol) SDS, and 2.5% (vol/vol) 2-mercaptoethanol for total protein fraction. The total zein fraction had an average protein concentration of approximately 9 mg/mL for the raw preparation and 4.5 mg/mL for the cooked preparation and a total protein of approximately 19.5 mg/mL for the raw preparation and 14 mg/mL for the cooked preparation. As a comparative method only for raw maize, the zein and glutelin fractions were also extracted by using the Osborne method, as modified by Weiss et al⁸ by using 75% (vol/vol) ethanol without any reducing agent for zeins and an SDS-dithiothreitol buffer for glutelins. The protein concentration was approximately 3.5 and 16 mg/mL for the zein and glutelin fraction, respectively.

All the protein contents were determined by using the method of Lowry et al.⁹

SDS-PAGE and immunoblotting

SDS-PAGE and IgE immunoblotting were carried out as previously described.¹⁰ The extracts were loaded at a protein content ranging from 0.034 to 0.19 mg/cm of gel for the water-soluble fraction, 0.36 to 0.18 mg/cm of gel for the total zein fraction, and 0.78 to 0.56 mg/cm of gel for the total protein fraction. The IgE binding of the water-soluble fractions of all the hybrids at each thermal treatment were investigated by using group 1 pooled serum and group 2 and 3 single patients' sera. The sera of the 7 new patients were used to evaluate the IgE-binding activity of the total zein and total protein fractions (the method of Pasini et al⁷) of Alicia hybrid raw and at the maximum thermal treatment. The zein and glutelin fractions, obtained by using the method of Weiss et al,⁸ of Alicia raw hybrid were studied by using 4 single sera (from patients 1, 2, 4, and 5). A serum pool from 5 nonallergic individuals was used as the negative control.

HPLC

The 9-kD protein of maize was present, and IgE-binding in all the hybrids, even after the maximum thermal treatment and different extraction methods. We then purified it by means of HPLC (AKTA Purifier, Pharmacia Biotech) from the water-soluble extract of raw Alicia hybrid (as described elsewhere⁵) and, for comparison, from

TABLE I. Patient characteristics

Patient no.	Age (y)	Sex	Maize symptoms at history	Causative cooked maize derivatives	Maize CAP System (kU/L)	Sensitization to other foods	Pollens causing symptoms	DBPCFC
1	19	M	Laryngeal edema, urticaria, dyspnea, dry cough	Popcorn, maize-containing chips	47.3	Peach, cherry, almond, hazelnut, peanut, strawberry	Birch, grass	NT
2	23	F	Laryngeal edema	Polenta	2.54	Peach, walnut, kiwi, rice	Birch, grass	NT
3	38	F	Itching, angioedema Asthma Laryngeal edema, severe anaphylaxis	Popcorn Maize cooking steam Polenta	1.11	Peach, cherry, apple	Birch, grass	NT
4	49	F	Laryngeal edema, dyspnea	Polenta	68.30	Peach, grape, tomato, chestnut, potato	Birch, grasses, pellitory	NT
5	49	F	Urticaria OAS, dyspnea	Roasted corn Polenta	68.30	Peach, potatoes, tomatoes	Birch, grasses	OAS, urticaria nausea, gastric burning, headache
6	31	M	Urticaria Urticaria, angioedema	Cooked maize Polenta	2.83	Peanut, strawberry	Grasses	Strong genital itching
7	35	F	Rhinitis, cough, diarrhea	Maize derivatives	7.51	Apple, tomato, kiwi, rice, peanut	Birch, grasses, mugwort	OAS, throat tightness, genital itching

TABLE II. Cooking effect (C₀ = equivalent time [in minutes] at 100°C) of the different thermal treatments applied

Treatment no.	Maize hybrid				
	Alicia	Lolita	Proxima	Tevere	Aramis
1	0.9	0.6	2.4	2.4	1.3
2	1.4	1.3	4.6	4.6	5.0
3	9.3	9.3	16.4	6.9	9.4
4	36.6	25.2	41.1	36.7	26.6
5	53.67	53.9	63.1	72.4	73.9
6	161.1	158.5	151.5	168.2	169.0

cooked Alicia. The latter required, compared with raw maize, an additional gel filtration passage and a passage on a reverse-phase C5 column (0.2 × 25 cm, Phenomenex, Jupiter 5μ, 300 Å) with 0.1% trifluoroacetic acid buffer, applying a linear gradient of 10% to 45% acetonitrile for 50 minutes.

The IgE-binding properties of the 9-kd protein purified from raw and cooked maize were verified by means of immunoblotting.

Immunoblotting inhibition

This experiment was done to verify whether the band identified as an LTP in the different fractions was actually the allergenic protein. Briefly, 500 μL of the pooled sera of patients 1, 2, 6, and 7 were incubated with 500 μL of different amounts of the 9-kd raw maize purified protein (1000, 500, 50, and 5 μg). The inhibited sera were matched with the blotted SDS-PAGE of the raw water-soluble fraction, of the total zein fraction, and of the total protein fraction (both raw and cooked; the method of Pasini et al⁷) and of the raw glutelin fraction (the method of Weiss et al⁸). IgE binding was detected as described above.

N-terminal sequence

The intact 9-kd protein purified from cooked maize was sequenced on an Applied Biosystem Procise 492 sequencer in pulse-liquid mode (Applied Biosystems).

In-gel reduction and digestion

The corresponding bands were cut from the SDS-PAGE gel of the total zein and total protein fractions (the method of Pasini et al⁷) of raw and cooked maize reduced with 100 mmol/L dithiothreitol (56°C for 1 hour) and alkylated by addition of 200 mmol/L iodoacetamide (in the dark at room temperature for 45 minutes) to verify whether the 9-kd protein was an LTP. The proteins were digested as already described.¹¹

Mass spectrometry

For matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, 0.5 μL of each peptide mixture from the in-gel digestion was applied to a target disk, as previously described.¹² Spectra were obtained by using a Bruker Reflex III MALDI-TOF spectrometer. ProFound (<http://prowl.rockefeller.edu/cgi-bin/ProFound>) and MS-Fit (<http://falcon.ludwig.ucl.ac.uk/MS-Fit.html>) software were used to interpret the mass spectrometry spectra of protein digests by means of the peptide mass fingerprinting method.¹³

Spectroscopy and temperature-ramp experiments

The spectroscopic measurements were performed on pure raw

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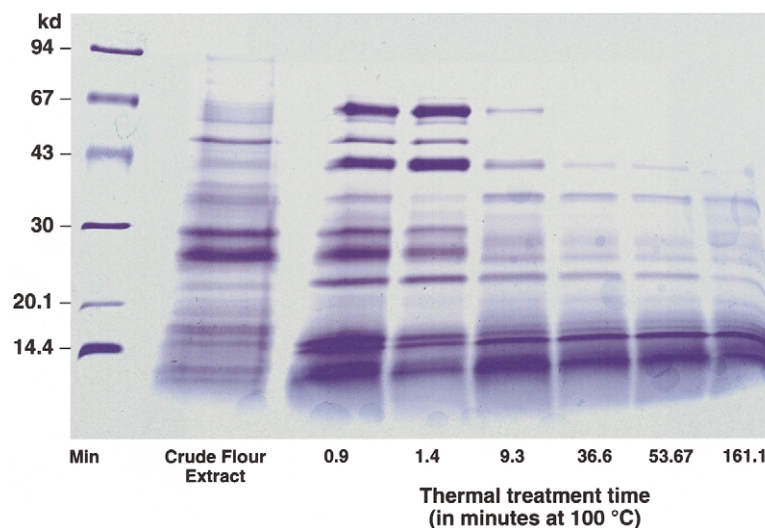


FIG 1. SDS-PAGE profile of the water-soluble fraction of Alicia hybrid, both raw and after different lengths of thermal treatment at 100°C.

maize LTP in PBS buffer (0.2 mg/mL concentration). Far-UV circular dichroism spectra were taken by using 1-mm path-length cells in a Jasco J-810 instrument and analyzed by using Jasco software.

Time-progressive heating of protein solutions was performed in computer-controlled, Peltier-driven cell holders fitting the spectropolarimeter. Typically, circular dichroism signals were monitored continuously while applying the following temperature gradient: 25°C for 5 minutes, from 25°C to 100°C for 75 or 375 minutes (heating rate, 1°C or 0.2°C/min), and 100°C for 5 minutes. The sample was then cooled to 25°C over a 15-minute period (cooling rate, 5°C/min) to assess the extent of reversibility of spectral modifications, and spectra were recorded again at 25°C on the heated and cooled protein solution.

RESULTS

Patients

Group 1 patient data have already been published.⁵ Group 2 and 3 patient demographic and clinical data are shown in Table I.

The double-blind results of group 3 patients showed that all of them had symptoms after the last dose: patient 5 had very severe urticaria, oral allergy syndrome (OAS), gastric burning, nausea and headache; patient 6 presented with strong genital itching, which was considered positive for the negativity of the placebo; and patient 7 had OAS, throat tightness, and genital itching.

SDS-PAGE and immunoblotting

The SDS-PAGE profiles of the 5 raw maize hybrids were similar both in the number and MW of the bands (data not shown). We observed a progressive decrease in the number and intensity of the higher MW bands from the raw to the maximally heated samples. This might be due to the higher water content of polenta and to a protein aggregation. On the contrary, the lower MW bands showed a weak decrease in intensity. Results are shown for the water-soluble fraction in Fig 1.

SDS-PAGE results of albumin-globulin, total zein, and total protein fractions obtained by using the Pasini extraction method⁷ from raw Alicia hybrid are shown in Fig 2, and the results of the same fractions obtained from maximally heated Alicia are shown in Fig 3. All the other hybrids had the same behavior (data not shown), and therefore further experiments were done on Alicia alone.

The IgE immunoblotting showed that the allergenic profile of the water-soluble fractions of the 5 raw maize hybrids were similar (data not shown). The most relevant allergens were at MWs of 94, 56, 37, 30, 28, 16, and 9 kD.

All the tested hybrids showed a similar decrease, from the raw to the maximum heated sample, in the IgE binding to the higher MW bands (Fig 4; data shown for Proxima, Tevere, Alicia), which was strictly related to the cooking time. The 9-kD band remained almost unchanged, showing only a slight decrease in radio staining. A similar decrease in IgE binding to Alicia hybrid after maximum thermal treatment was observed with sera of group 2 and 3 patients (Fig 5), who maintained IgE reactivity almost exclusively to the 9-kD protein.

The immunoblotting of the total zein and total protein fractions, obtained by using the method of Pasini et al,⁷ of Alicia hybrid (both raw and after maximum thermal treatment) with the sera of patients 1 to 7 showed 2 different allergenic patterns: patients 3, 4, and 5 reacted to proteins with MWs ranging from 9 to 94 kD, whereas patients 1, 2, 6, and 7 reacted only to the 9-kD component (data not shown). The sera with a similar IgE pattern were pooled: patients 3, 4, and 5 for pool 1 and patients 1, 2, 6, and 7 for pool 2. Immunoblotting results are shown in Fig 6. In the total zein fraction serum pool 1 evidenced an IgE-binding activity against proteins between 20 and 30 kD and between 38 and 60 kD, which are probably dimers or trimers formed from aggregation of A-zein and B-zein monomers. Serum pool 2, exclu-

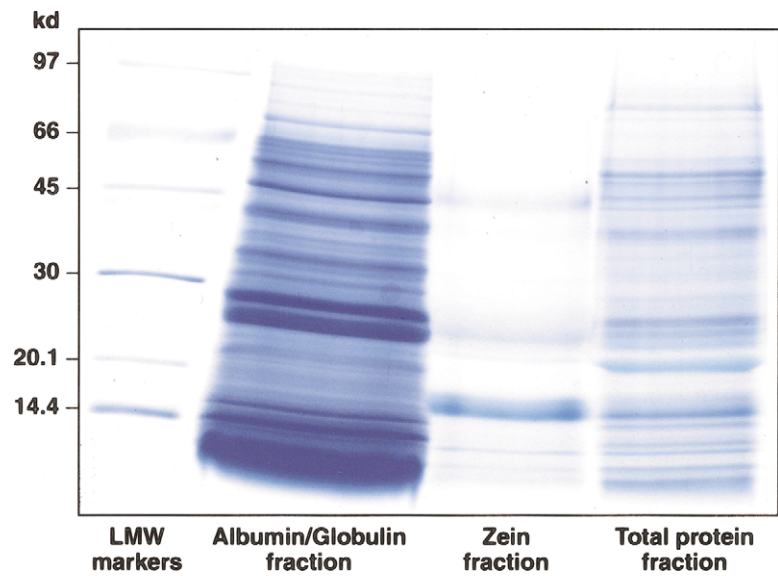


FIG 2. SDS-PAGE profile of the 3 fractions of Alicia raw hybrid obtained by using the method of Pasini et al.⁷

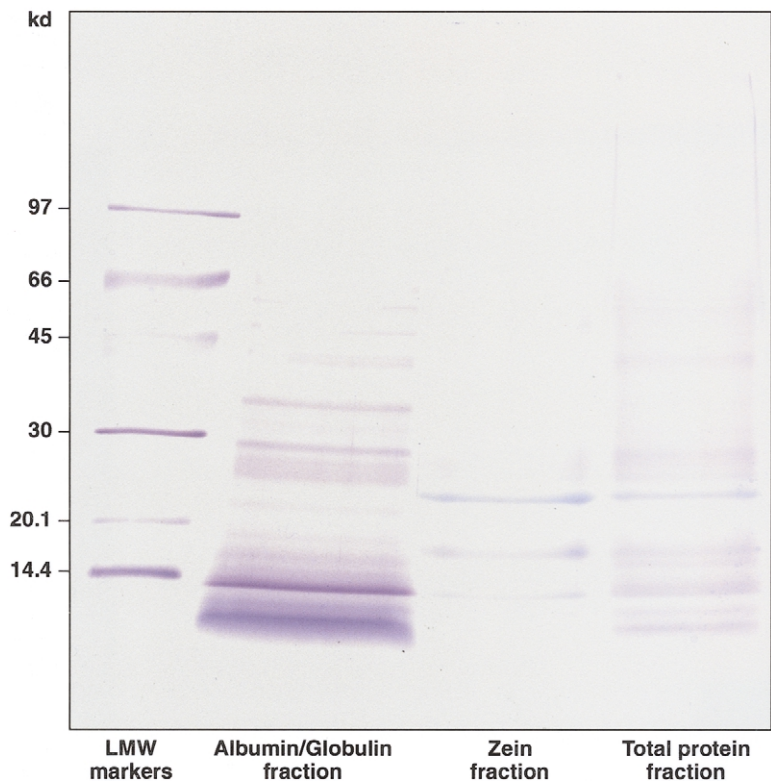


FIG 3. SDS-PAGE profile of the 3 fractions of Alicia hybrid after the maximum thermal treatment obtained by using the method of Pasini et al.⁷

sively reacting to the 9-kd band in the water-soluble fraction, also recognized only this protein in the total zein and total protein fractions. Thus the only allergen recognized by the 2 pools in all the fractions obtained by using the method of Pasini et al⁷ was the 9-kd protein. After the

maximum thermal treatment, the IgE-binding capacity was generally reduced.

The IgE immunoblotting of the zein and glutelin fractions of Alicia raw hybrid obtained by using the method of Weiss et al⁸ made with the sera of patients 1, 2, 4, and 5 showed that

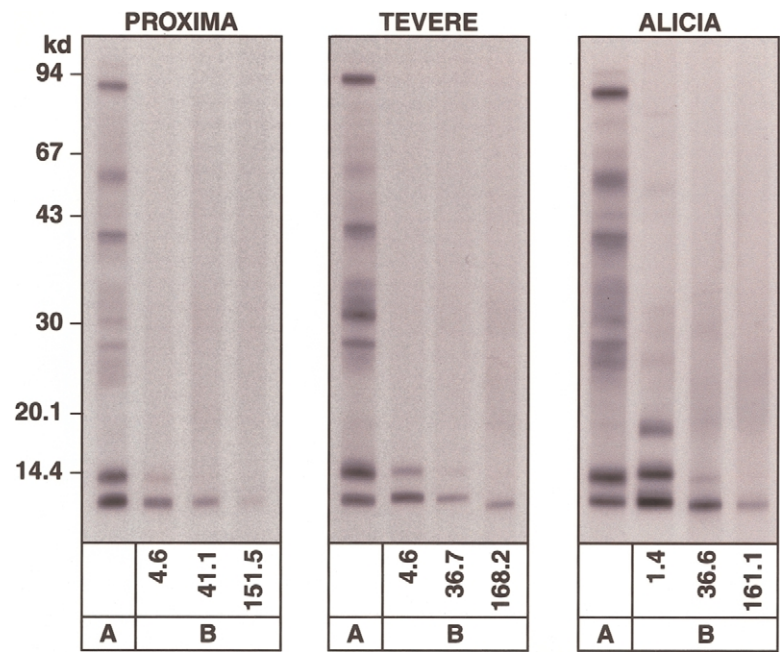


FIG 4. IgE immunoblotting of the water-soluble fraction of 3 maize hybrids, both raw (*lane A*) and after different lengths of thermal treatment (*lanes B*), with the pooled sera from patients of group 1 with maize allergy.

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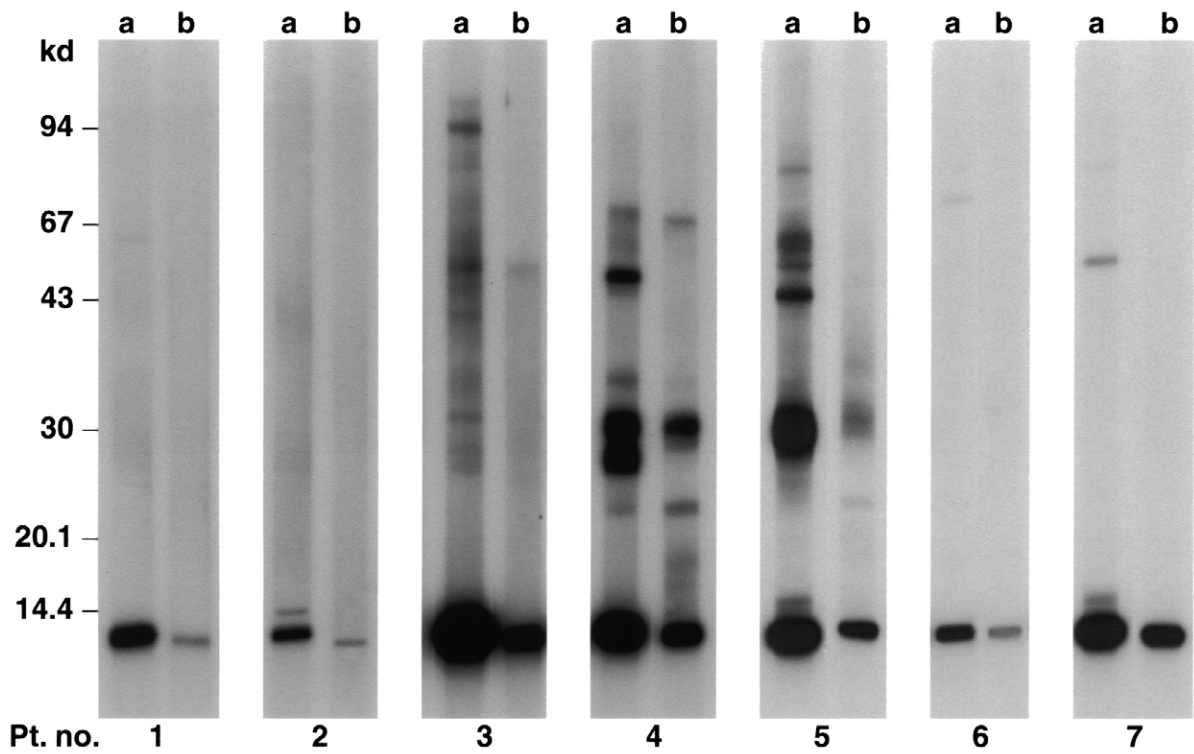


FIG 5. IgE immunoblotting of the water-soluble fraction of Alicia hybrid, both raw (*lane a*) and after the maximum thermal treatment (*lane b*), with the sera of anaphylactic patients (patients 1, 2, 3, and 4) and patients with positive DBPCFC results (patients 5, 6, and 7).

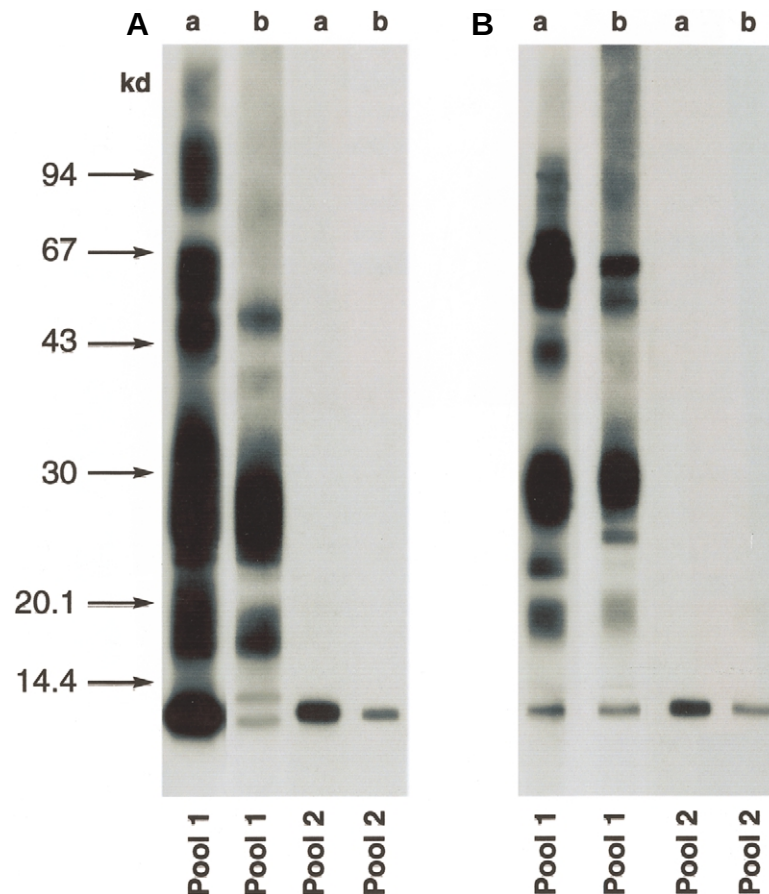


FIG 6. IgE immunoblotting of the total zein (A) and total protein (B) fractions of Alicia hybrid, obtained by using the method of Pasini et al,⁷ both raw (lane a) and after the maximum thermal treatment (lane b), with serum pool 1 and 2.

the IgE antibodies of all the sera recognized the 9-kD protein in the glutelin fraction but not in the zein fraction, which in this method is extracted without any reducing agent (data not shown). As a consequence and as expected, only A-zeins were extracted and recognized by IgE antibodies.

All the immunoblotting performed with the nonallergic patients' sera did not evidence any IgE binding.

HPLC

The 9-kD LTPs were pure and suitable for amino acid sequencing. The IgE-binding activity of LTP purified from cooked maize was fairly reduced compared with that of LTP purified from raw maize (data not shown).

Immunoblotting inhibition

The pure LTP completely inhibited the IgE binding to the 9-kD component of the water-soluble, total zein, glutelin, and total protein fractions. The 9-kD band generally reappeared only at an inhibitor content of lower than 5 µg (data not shown).

N-terminal sequence

The N-terminal sequence of the 9-kD protein purified from cooked maize was Ala-Ile-Ser-Cys-Gly-Gln-Val-

Ala-Ser. This corresponded to the sequence of the raw maize 9-kD LTP.

In-gel reduction and digestion and mass spectrometry

The maize LTP (SWISS PROT accession number, P19656) was reliably identified in the total protein fraction of raw and cooked maize, matching 4 peptides each (54% of molecule coverage). A maize subtilisin-chymotrypsin inhibitor (National Center for Biotechnology Information accession no. 2118425) was identified in the total zein fraction of raw maize by using the same method, matching 4 peptides (72% of molecule coverage). This is in agreement with the results of Fountoulakis et al,¹⁴ who reported that proteins with MWs of 10 to 20 kD are usually identified with a smaller number of peptides.

Effects of temperature on the structural features of maize LTP

The room-temperature Far-UV circular dichroism spectra of untreated and isolated maize LTP clearly indicate the prevalence of helical structures, as evident from its crystallographic structure (data not shown).¹⁵ However, obser-

vation of the distinctive circular dichroism features of helical structures required diluted solutions (<0.2 mg/mL) of the protein. The signature transitions at 220, 205, and 190 nm are not observed at higher protein concentration. This suggests concentration-dependent aggregation of the protein in the PBS buffer used in these studies. The signature ellipticities of the native diluted protein are irreversibly lost on heating. Temperature-induced loss of structure is progressive. Significant changes in the ellipticity at 220 nm start at 70°C and are completed at 100°C. Progress of heat denaturation was kinetically limited, as indicated by its sensitivity to heating rate. Heating from 25°C to 100°C at rates faster than 0.5°C/min resulted in incomplete loss of the signature ellipticity of the protein, even when reaching 100°C. At a heating rate of 0.2°C/min, it was possible to observe complete denaturation at 100°C, with an estimated denaturation midpoint temperature of 83.5°C.

DISCUSSION

In this study we demonstrated that there are no differences between the various worldwide-cultivated hybrids of maize as to protein profile and allergenic properties. We then confirmed our previous observation that the most relevant maize allergen is a 9-kd LTP, adding here the finding that it is relatively heat stable.

By following controlled heating conditions, such as temperature actually reached and the kinetics of heating, we demonstrated that 160 minutes of thermal treatment at 100°C almost totally eliminated the IgE-binding activity of the higher MW bands but did not affect that of LTP. This makes its role in maize-induced food allergic reactions even more important because maize is consumed cooked.

This observation appears of particular interest also from an immunologic point of view because we found that heating irreversibly alters the secondary structure of purified maize LTP. Given that IgE binding to LTP in the heated samples is still strong, we can argue that the working epitopes on LTP are linear rather than conformational because the primary structure is usually unaltered by physical treatment. Yet another possibility is that the loss of high-order structure of LTP when cooking maize flour might be restricted or prevented by the relatively low water content of the food or by the presence of other polymers in the flour (proteins and starch). We should also add that we found that faster heating does not alter maize LTP, thus increasing the allergenic potential of polenta.

The heat resistance of LTP is an important requirement in determining the severe reactions we described here, which are common for the reactivity to LTP-containing foods. Similarly, we recently described OAS from hazelnut in patients sensitized to Cor a 1.01 but anaphylactic reactions in patients sensitized to hazelnut LTP, which was resistant to roasting.¹⁶

The clinical role of LTP is further emphasized by our demonstration that maize LTP is extractable not only in the water-soluble fraction but also in total protein and glutelin fractions, where LTP is still evident after exposure of maize to the maximum thermal treatment. The

identification of LTP in raw and cooked total protein fractions and the demonstration of the IgE immunoblotting inhibition by pure LTP are very strong arguments in favor of the assumption that LTP is the most important allergen also in these fractions. The acid environment present in the stomach could thus theoretically further increase the extraction of LTP already present in the water-soluble fraction. The demonstration that LTP is extractable also in these fractions is not surprising if we consider that, as was recently very clearly defined by Shewry and Halford,¹⁷ LTP belongs to the prolamin superfamily. One of the characteristics of this superfamily is the high number of disulfide bridges, which are typically reduced by the methods applied for the extraction of polymeric glutelins.

Other thermostable allergens recognized by serum pool 1 in the total protein fraction were MWs of 30, 23, and 50 kd (Fig 6). The latter allergen could correspond to that recently identified by Pasini et al.⁷ None of our patients included in serum pool 2 reacted to these allergens but only to LTP. Even though we cannot exclude that other proteins are relevant allergens in maize, we can conclude that LTP is the only protein recognized by IgE of all the patients evaluated in the present study.

We did not identify LTP in the total zein fraction, but the 9-kd band recognized by IgE immunoblotting was a subtilisin-chymotrypsin inhibitor. Because this and LTP belong to a so-called α -amylase inhibitor family, this could explain the high cross-reactivity rate demonstrated by immunoblotting inhibition, probably because of an overlapping structure. It is interesting to note that barley LTP was initially designated as a "putative protease/amylase inhibitor."¹⁸ The same situation might be true for this maize protein.

In a previous study⁵ we demonstrated that maize LTP did not show any cross-reactivity with grass pollen allergens, thus confirming that the sensitization to LTP is through the intestinal route and not mediated by means of sensitization to homologous pollen allergens. A very important point is that LTP sensitization is typical of Southern European populations, giving rise to sensitization to multiple vegetable foods that might or might not be botanically related, such as Rosaceae fruits, grape,¹⁹ and hazelnut.¹⁶ This clinical situation might thus be well defined by the term *LTP syndrome*.

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