

Wheat lipid transfer protein is a major allergen associated with baker's asthma

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Background: Baker's asthma is a frequent occupational allergic disorder mainly caused by inhalation of cereal flours. Lipid transfer proteins (LTPs) constitute a family of plant food panallergens, but their role as inhalant and wheat allergens is still unclear.

Objective: We sought to explore the involvement of wheat LTPs in baker's asthma caused by wheat flour sensitization.

Methods: Forty patients with occupational asthma caused by wheat flour inhalation were studied. Wheat LTP, Tri a 14, was purified by using a 2-step chromatographic protocol and characterized by N-terminal amino acid sequencing and 3-dimensional modeling. Its reactivity was confirmed by means of IgE immunodetection, ELISA and ELISA-inhibition assays, and skin prick tests.

Results: Specific IgE to Tri a 14 was found in 60% of 40 individual sera from patients with baker's asthma, and the purified allergen elicited positive skin prick test reactions in 62% of 24 of these patients. Tri a 14 and peach LTP, Pru p 3, showed a sequence identity of 45%, but the low cross-reactivity between both allergens detected in several individual sera reflected great differences in their 3-dimensional IgE-binding regions.

Conclusions: Wheat LTP is a major inhalant allergen associated with baker's asthma caused by wheat flour sensitization. Poor cross-reactivity with its peach homolog was found in some patients.

Clinical implications: LTPs can be considered relevant inhalant allergens linked to respiratory disorders. LTP from wheat (Tri a 14) can be used as a helpful tool for the diagnosis of baker's asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Baker's asthma, lipid transfer protein, wheat, cross-reactivity, inhalant allergen, IgE epitopes, 3-dimensional modeling, electrostatic potential

Abbreviations used

LTP: Lipid transfer protein
PB: Poisson-Boltzmann
PDB: Protein Data Bank
SPT: Skin prick test

Baker's asthma is one of the most common forms of occupational asthma, affecting up to 15% of bakers, millers, and pastry factory workers.^{1,2} This disorder is mainly caused by an IgE-mediated allergic response to inhalation of cereal flours. The major causative allergens of the baker's asthma condition are proteins derived from wheat and rye flours, although baking additives, such as fungal α -amylase, are also important.^{1,3} Salt-soluble proteins (albumins plus globulins), particularly members of the α -amylase/trypsin inhibitor family,^{4,5} thioredoxins,⁶ peroxidase,⁷ and other soluble enzymes,⁸ show the strongest IgE reactivities in wheat flour. In addition, prolamins (typical seed storage proteins not extractable by salt solutions) have also been claimed as potential allergens.⁹ However, the large variability of IgE-binding patterns of wheat proteins among individual sera from patients with baker's asthma,⁸ together with the great differences in the concentrations of potential allergens observed in commercial wheat extracts used for diagnosis,¹⁰ highlight the necessity to standardize and improve the diagnostic tools for baker's asthma.

Plant nonspecific lipid transfer proteins (LTPs) constitute a panallergen family of 9-kD (90-95 amino acid residues) basic polypeptides.^{11,12} Several members of this protein family have been identified as relevant allergens in plant foods, mainly Rosaceae fruits, and pollens, being particularly important in the Mediterranean area. The high resistance of LTPs to both heat treatment and digestive proteolytic attack has been related to the capability of these allergens to induce severe symptoms in many patients and has led to the proposition of LTPs as a model of true food allergens.^{11,12} LTPs are probably sensitizers through the oral route, but preliminary data suggest an alternative role as inhalant allergens, linked in some cases to plant food and pollen cross-reactions.^{11,13,14}

Data on the involvement of LTPs in allergic reactions to cereal flours or cereal-derived products are still scarce.

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This allergen family has been related to beer allergy,¹⁵ asthma produced by inhalation of rice seed dust,¹⁴ and cutaneous contact reactions to spelt wheat.¹⁶ The identification of maize LTP, Zea m 14, as a major allergen responsible for food-induced allergic symptoms is the best-established case of a cereal LTP linked to an IgE-mediated disease.¹⁷ We report here the identification of wheat LTP, designated Tri a 14 according to the International Union of Immunological Societies Allergen Nomenclature Sub-Committee, as a major inhalant allergen associated to baker's asthma caused by wheat flour. Its limited cross-reactivity with the peach LTP Pru p 3 is also illustrated.

METHODS

Patients and sera

Forty consecutive patients with baker's asthma caused by wheat flour were recruited at the Servicio de Alergia, Fundacion Jimenez Diaz (Madrid, Spain; numbers 1 to 20 in Table I) and Seccion de Alergia, Hospital Rio Hortega (Valladolid, Spain; numbers 21 to 40 in Table I). The criteria for selection were as follows: First, there needed to be a convincing clinical history of occupational asthma provoked by inhalation of wheat flour in the workplace. The baker's asthma condition was further confirmed by means of specific inhalation challenge tests in all selected patients, using methods previously described.^{4,18} Second, a positive skin prick test (SPT) response to a homemade wheat extract was necessary (see below). Finally, a specific IgE level to wheat of greater than 0.35 kU/L (mean, 16.3 kU/L; range, 0.51–>100 kU/L), as determined by using the Pharmacia CAP System FEIA (Phadia, Uppsala, Sweden), was required. Demographic and clinical data of the patients studied are summarized in Table I. Five patients (numbers 2, 7, 9, 10, and 28 in Table I) were also sensitized to peach fruit, showing oral allergy syndrome after ingestion. None of the patients referred symptoms after eating wheat-derived products. Written informed consent was obtained from all patients, and the study was approved by the corresponding ethics committees of both Hospitals (Fundacion Jimenez Diaz, Madrid, and Rio Hortega, Valladolid, Spain).

A pool of sera from these patients (nos. 1, 2, 4–6, 10, 15–18, and 20–26 in Table I) was used for IgE immunodetection assays, and individual sera were tested in specific IgE determination and ELISA-inhibition experiments.

Wheat extracts

Whole seeds of bread wheat (*Triticum aestivum*, cultivar Astral) were ground, defatted with cold acetone (2×1 ; 1:5 [wt/vol] for 1 hour at 4°C), and, after drying, extracted with 0.1 mol/L Tris-HCl [pH 7.5] and 10 mmol/L EDTA (1:5 [wt/vol] for 1 hour at 4°C). After centrifugation (9000g for 30 minutes at 4°C), the supernatant was dialyzed against H₂O (cut-off point, 3.5 kD) and freeze-dried. This crude extract was used for both ELISAs and SPTs.

Additionally, grains were milled in a Laboratory Mill CD1 (Chopin Technologies, Villeneuve-la-Garenne Cedex, France) to separate flour and bran (final yield, 52.0% and 33.6%, respectively), which were independently extracted by using the methods described above. The protein concentration in all extracts was quantified according to the Bradford method.¹⁹

Isolation and characterization of Tri a 14

The wheat bran extract was fractionated by means of cation exchange chromatography on an Acell Plus CM Waters SepPak cartridge (Waters, Milford, Mass) by using 10 mmol/L ammonium

acetate (pH 6.8) as the equilibration buffer. The retained material, enriched in Tri a 14, was then eluted with 50 mmol/L ammonium acetate (pH 6.8), dialyzed, and freeze-dried. This Tri a 14-enriched fraction was further separated by means of reverse-phase HPLC on a Nucleosil 300-C4 column (8×250 mm; particle size, 5 μ m; Scharlau Science, Barcelona, Spain), eluting with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid (10% to 100% in 150 minutes; 1 mL/min). Chromatographic fractions containing wheat LTP Tri a 14 were located by means of immunodetection after SDS-PAGE with both anti-peach LTP Pru p 3 antibodies and a serum pool from patients with baker's asthma. These fractions were then pooled and freeze-dried.

Peach Pru p 3 allergen was purified as previously described.²⁰ The isolated allergens were quantified by using a commercial bichinchoninic acid test (Pierce, Cheshire, United Kingdom).

SDS-PAGE was performed on Bio-Rad Miniprotein III System gels (15% polyacrylamide), according to the method of Laemmli.²¹ Bands corresponding to Tri a 14 in bran and flour extracts were quantified by using the GelDoc 2000 System and the Quantity One software (BioRad, Richmond, Calif).

N-terminal amino acid sequencing was carried out by using standard methods with an Applied Biosystems 477A gas-phase sequencer (Foster City, Calif).

Immunodetection assays

Samples (15 μ g of protein of wheat extracts and 3 μ g of purified allergens) were separated by means of SDS-PAGE and then electrotransferred to polyvinylidene difluoride membranes. After blocking, membranes were incubated overnight with a pool of 17 sera from patients with baker's asthma (1:5 dilution), washed, and then treated with mouse anti-human IgE mAb HE-2 ascitic fluid (1:3000 dilution for 1 hour).²² After washing, a rabbit anti-mouse IgG peroxidase-conjugated antibody (1:5000 dilution for 1 hour; DAKO, Glostrup, Denmark) was added. Detection of IgE-binding components was achieved by means of enhanced chemiluminescence, according to the manufacturer's instructions (Amersham Biosciences, Little Chalfont, United Kingdom). A pool of 3 sera (1:5 dilution) from bakers nonallergic to wheat (negative IgE and SPT responses and no symptoms) was tested as a negative control.

Alternatively, replica gels were immunodetected with rabbit polyclonal antibodies to the peach LTP Pru p 3 (1:1000 dilution) and then treated with goat anti-rabbit IgG peroxidase-conjugated antibody (1:100000 dilution; Sigma, Steinheim, Germany) and revealed as described above.

Specific IgE determination and ELISA-inhibition assays

ELISAs to determine specific IgE of 40 individual sera (1:6 dilution) from patients with baker's asthma to the wheat extract (15 μ g/mL) or to the purified allergens Tri a 14 or Pru p 3 (3 μ g/mL) were carried out as previously described.²³ BSA (3 μ g/mL; $n = 21$; mean [OD] $\pm$ SD = 0.073 ± 0.031) and a serum pool from 3 bakers nonallergic to wheat (negative IgE and SPT responses; $n = 6$; mean [OD] $\pm$ SD to Tri a 14 = 0.120 ± 0.043 ; mean [OD] $\pm$ SD to Pru p 3 = 0.050 ± 0.010) were used as negative controls. Specific IgE levels of greater than 0.25 OD units (mean [OD] + $3 \times$ SD to Tri a 14 using the negative control serum pool: $0.120 + 3 \times 0.043 = 0.249$) were considered positive. All tests were performed in duplicate.

ELISA-inhibition assays were performed by using the same method, except that the individual sera were preincubated with 3 μ g/mL of the corresponding inhibitor (Tri a 14 or Pru p 3) during 3 hours at 25°C. All tests were performed in duplicate.

TABLE I. Demographic and clinical data of patients with baker's asthma and specific IgE levels and SPT responses to purified Tri a 14 and Pru p 3

Patient no.	Age (y)/sex	Duration of exposure (y)	Duration of symptoms (y)	Specific IgE to wheat (CAP, kU/L)	Specific IgE to* (ELISA, OD unit):		SPT* (mm)		Symptoms with peach	Sensitization to other noncereal plant foods
					Tri a 14	Pru p 3	Tri a 14	Pru p 3		
1	35/M	20	5	74.2	0.89	—	3	4	No	Walnut, kiwi
2	29/M	12	10	18.0	0.81	0.64	—	—	Yes (OAS)	Melon, kiwi
3	36/M	8	7.4	1.6	—	—	—	—	No	Peanut
4	39/M	20	2	25.8	0.60	0.46	3	—	No	No
5	35/M	12	6	24.3	0.64	—	ND	ND	No	No
6	28/M	14	2	2.5	0.43	—	—	—	No	No
7	27/M	2.6	0.8	7.8	—	—	ND	ND	Yes (OAS)	Kiwi, melon, walnut, chestnut, sunflower
8	60/M	46	22	3.4	—	—	ND	ND	No	No
9	22/M	5	0.4	3.0	0.26	—	ND	ND	Yes (OAS, CU)	Kiwi
10	26/M	5	3	>100	—	0.59	ND	ND	Yes (OAS)	Kiwi, melon, avocado, banana, chestnut
11	54/M	25	18	2.4	—	—	ND	ND	No	No
12	27/M	10	3	5.0	—	—	ND	ND	No	No
13	42/F	12	3	3.7	—	0.37	4	4	No	No
14	54/M	38	29	1.6	—	—	—	—	No	No
15	26/M	16	4	74.6	0.62	—	3	—	No	No
16	31/M	7	3	0.8	0.58	0.45	ND	ND	No	No
17	43/M	19	6	13.8	0.37	—	3	—	No	No
18	34/F	2	1	2.0	—	—	—	ND	No	No
19	41/F	1	0.5	1.7	—	—	ND	ND	No	No
20	21/F	3	1	19.0	0.63	0.40	3	4	No	Kiwi, melon, avocado, pineapple, mango
21	25/F	5	2	9.4	0.42	—	ND	ND	No	No
22	35/M	15	10	10.9	0.59	0.57	ND	ND	No	No
23	26/M	5	3	8.3	0.66	0.38	ND	ND	No	No
24	46/M	20	18	5.4	0.50	0.30	ND	ND	No	No
25	47/M	31	25	12.94	0.71	0.30	ND	ND	No	No
26	39/M	18	12	11.0	0.31	0.82	ND	ND	No	No
27	55/M	23	19	1.5	—	—	ND	ND	No	No
28	27/M	5	4	37.1	0.39	0.31	6	5	Yes (OAS)	Apple
29	55/M	22	18	1.4	0.27	—	4	—	No	Apple
30	52/M	32	29	1.7	0.26	—	3	4	No	Kiwi, melon
31	30/M	20	8	0.9	—	—	—	—	No	No
32	31/F	12	7	7.3	—	—	—	—	No	Peanut, lentil, hazelnut
33	36/M	15	10	6.0	0.67	0.36	6	5	No	Peanut
34	38/M	20	16	0.5	0.26	—	—	4	No	No
35	43/F	18	15	3.7	0.42	—	3	—	No	Lentil
36	17/M	1.5	1	7.3	0.33	1.42	5	10	No	Almond, lentil
37	24/M	4	2	4.9	—	0.73	—	8	No	No
38	30/F	10	5	7.7	—	—	6	—	No	No
39	45/F	25	21	>100	0.93	0.40	4	5	No	No
40	20/F	2	1	27.9	—	0.34	4	5	No	No

—, OD less than 0.25 units or mean wheal diameter less than 3 mm (see Methods section); ND, not determined; OAS, oral allergy syndrome; CU, contact urticaria.

*Values are presented as means (n = 2).

SPTs

SPTs with the wheat crude extract (2 mg/mL protein in a 1:1 [vol/vol] PBS buffer/glycerol solution) and with purified Tri a 14 and Pru p 3 (20 µg/mL) were carried out according to standard procedures. Histamine dihydrochloride (10 mg/mL) and PBS buffer/glycerol

solution served as positive and negative controls, respectively. Six bakers without symptoms, as well as negative IgE and SPT responses to wheat, were tested as a control group. SPT responses to the wheat crude extract and purified Tri a 14 and Pru p 3 were negative in all these subjects.

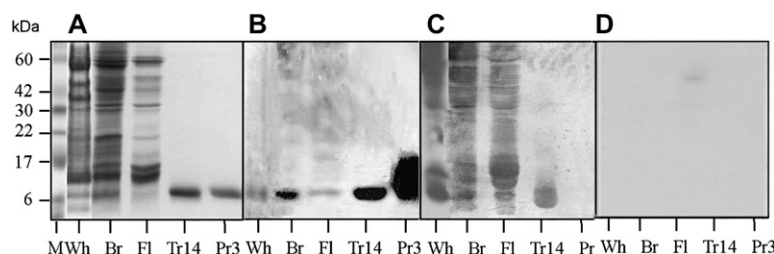


FIG 1. Protein staining (A) and immunodetection with anti-peach LTP Pru p 3 polyclonal antibodies (B) or IgE of a serum pool from patients with baker's asthma (C) or a negative control serum pool (see the Methods section; D) of the following SDS-PAGE-separated samples: extract from whole seed (Wh), bran (Br), and flour (Fl) of bread wheat and purified allergens Tri a 14 (Tr14) and Pru p 3 (Pr3). M, Molecular weight markers.

Duplicates of each sample were tested. A mean wheal diameter of 3 mm or larger (15 minutes after puncture) compared with that produced by the negative control was considered a positive response.

Homology modeling, protein surfaces, and electrostatic potentials

The 3-dimensional structure of Tri a 14 was modeled by using the Swiss-Model Protein Modelling Server,²⁴ taking as template the model 1 of the nuclear magnetic resonance structure of wheat LTP, Protein Data Bank (PDB) code 1GH1²⁵ (95.5% of sequence identity with Tri a 14). Secondary structure was identified with the DSSP program.²⁶ Solvent-excluded molecular surfaces of the model structure of Tri a 14, as well as the crystal structure of Pru p 3 (PDB code 2B5S²⁷), were obtained with PyMOL 0.99 (pymol.sourceforge.net).

Electrostatic Poisson-Boltzmann (PB) potentials were computed with the APBS 0.4.0 software,²⁸ assigning AMBER99²⁹ charges and radii to all the atoms, including hydrogens, which were added and optimized with PDB2PQR.³⁰ The linearized PB equation was solved in sequential-focusing multigrid calculations in a mesh of $129 \times 97 \times 97$ points at 298.15 K and 0.150 M ionic concentration. Dielectric constants were 2 for the protein and 78.54 for water. The output mesh of 1213761 points was processed in scalar OpenDX format with PyMOL 0.99 to render isocontour maps. Potential values are given in units of kT per unit charge (k, Boltzmann's constant; T, temperature).

RESULTS

Salt-soluble proteins (albumins and globulins) from whole seed, bran, and flour of bread wheat were extracted with Tris-HCl buffer, separated by means of SDS-PAGE under reducing conditions, and IgE immunodetected with a serum pool from patients with baker's asthma. A complex pattern showing protein bands from 5 to 70 kd was observed by means of protein staining and IgE immunodetection (Fig 1, A and C). No band was recognized by a pool of sera from 3 bakers nonallergic to wheat (negative control; Fig 1, D). All 3 extracts contained a component of approximately 9 kd, designated Tri a 14, which was recognized by anti-peach LTP Pru p 3 polyclonal antibodies and IgE of the serum pool (Fig 1). Densitometric analysis of the corresponding band indicated a differential distribution of the 9-kd component between wheat flour and bran, with approximately 4.5-fold higher content in the latter. Tri a 14 was therefore isolated from the wheat bran extract by means of a 2-step chromatographic procedure (data not shown).

The purified protein behaved as a single band of the expected apparent molecular weight in SDS-PAGE and reacted with both anti-Pru p 3 antibodies and IgE of the serum pool from patients with baker's asthma (Fig 1). N-terminal amino acid sequencing of Tri a 14 rendered the 24-residue sequence IDXGHVDSLVRPXLSTYVQGG PGPS, confirming the LTP nature of the isolated wheat allergen. Furthermore, this N-terminal sequence fully matched that deduced from the nucleotide sequence of the cDNA clone Ta Ltp9.1a³¹ (accession no. AJ852536), which encodes an abundant LTP of hexaploid wheat seed. The full amino acid sequence of the mature wheat LTP (Tri a 14) encoded by this cDNA clone showed 45% identity with that of the peach LTP allergen Pru p 3 (Fig 2). Interestingly, differences in several amino acid residues included in the 3 sequential epitopes previously located in peach Pru p 3³² were found between both allergens (Fig 2). Moreover, peach Pru p 3 was not recognized by IgE of the serum pool from patients with baker's asthma (Fig 1, C) when using reducing conditions in the SDS-PAGE separation method.

The relevance of Tri a 14 as a major allergen involved in baker's asthma was supported by results obtained in both *in vitro* and *in vivo* assays. Specific IgE to the purified wheat allergen was detected in 24 (60%) of 40 individual sera from patients with baker's asthma (Table I), whereas 92% and 42% of these sera displayed specific IgE to the crude wheat extract and purified peach Pru p 3, respectively. Additionally, SPTs with purified Tri a 14 and Pru p 3 were performed in 24 of these patients, who agreed to return for *in vivo* testing. Positive responses to Tri a 14 and Pru p 3 were observed in 62% and 45% of the 24 patients tested, respectively (Table I).

To explore the potential cross-reactivity between the wheat and peach LTP allergens, ELISA-inhibition experiments were carried out testing both purified allergens as solid phase, as well as inhibitors, and 14 individual sera from patients with baker's asthma. The results are summarized in Fig 3. Although high inhibition levels were achieved by both allergens in some sera (for instance, sera 17 and 23 in Fig 3, A, or sera 16 and 22 in Fig 3, B), a very limited, or even null, cross-reactivity was found in other cases (for instance, sera 4 and 25), suggesting substantial differences in the IgE-binding regions of Tri a 14 and Pru p 3. This possibility was further confirmed by

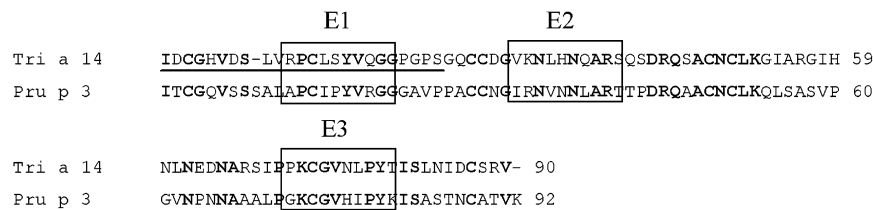


FIG 2. Alignment of the amino acid sequences of wheat Tri a 14 (deduced from the cDNA clone Ta Ltp 9.1b; accession no. AJ784902) and peach Pru p 3.²⁰ The experimental N-terminal sequence determined for Tri a 14 is underlined. Sequential IgE epitopes previously located in the Pru p 3 sequence³² are **boxed**.

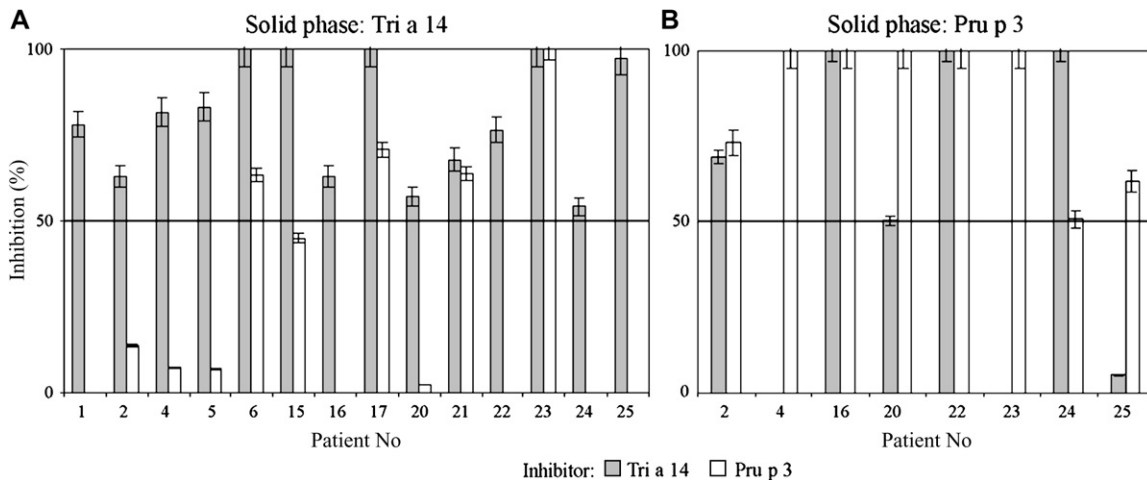


FIG 3. ELISA-inhibition assays with purified Tri a 14 (**A**) and Pru p 3 (**B**) in the solid phase and as inhibitors. Only those sera shown in Fig 3, A, that display significant specific IgE levels to Pru p 3 (Table I) are included in Fig 3, B. Means (n = 2) and SDs (bars) are represented.

comparing the surfaces of the 3-dimensional structures of both allergens, mainly those areas defined by the 3 IgE epitopes previously located in peach Pru p 3.³² The surface regions of epitopes E1, E2, and E3 covered relatively similar areas on the surfaces of Pru p 3 and Tri a 14 (Fig 4, B), which can explain their common reactivity in some patients. However, the electrostatic nature of both protein surfaces was rather different as a direct consequence of their distinct proportion of charged amino acids: 8 basic and 1 acidic in Pru p 3 and 12 basic and 7 acidic in Tri a 14. As is clearly shown in Fig 4, C, the spatial extent of the PB electrostatic potential, which provides a picture of the electric field arising from the protein, showed a completely different distribution, especially at the epitope regions. A great part of the Pru p 3 surface lacked any electrostatic activity, and the rest exhibited a dominant electropositive potential, whereas on the contrary, the whole surface of Tri a 14 displayed a marked field with large electronegative domains. This difference should be invoked to rationalize the different reactivity observed in some patients.

DISCUSSION

Baker's asthma is among the most important occupational allergic respiratory diseases.^{1,2} However, major

wheat allergens involved in this condition have not been fully elucidated. Thus individual members of the α -amylase inhibitor and thioredoxin families that have been described as relevant allergens in wheat flour⁴⁻⁶ show prevalences of IgE reactivity of less than 50% in patients with baker's asthma. Furthermore, specific IgE to WTAI-CM3 and WTAI-CM16, 2 well-characterized subunits of the trypsin/ α -amylase inhibitor family,⁵ was found in less than 35% of a subgroup of sera from bakers reported herein (sera 1-20 in Table I; A. Palacin et al, unpublished data).

LTPs are abundant proteins in cereal grains,³³ but only those of maize and beer have been identified as relevant food (or beverage) allergens.^{15,17} In fact, whether the apparent low allergenicity of wheat, rye, and barley LTPs is caused by food processing or the proteins themselves are weakly allergenic has been a matter of debate.¹² In contrast, the role of LTPs as major fruit (peach, apple, cherry, and grape), vegetable (asparagus and lettuce), and nut (hazelnut and chestnut) allergens in the Mediterranean population, frequently associated with severe systemic symptoms, is well established.^{11,34}

The isolation and characterization of Tri a 14 has allowed us to characterize this wheat LTP as a major allergen in baker's asthma caused by wheat flour. The purified allergen most probably corresponds to the product

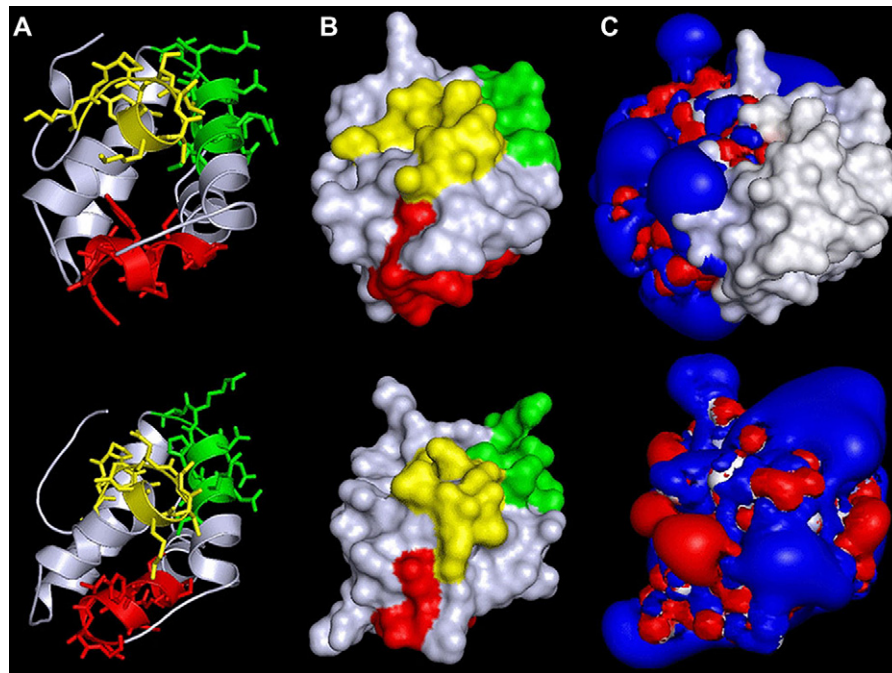


FIG 4. Epitopes previously defined in Pru p 3³² (top) shown also for Tri a 14 (bottom): E1, residues 11 to 20 (red); E2, residues 31 to 40 (green); and E3, residues 71 to 80 (yellow). **A**, Ribbon diagram; **B**, molecular surface; **C**, Isocontours -1.0 (red) and $+1.0$ (blue) of the PB potential superimposed on the surfaces in Fig 4, B.

of a previously described cDNA clone (TaLtp9.1a)³¹ highly expressed in mature seeds of bread wheat. Tri a 14 displays an N-terminal amino acid sequence typical of the LTP family, a deduced molecular size of 9606 d, an isoelectric point of 8.20, and a moderate similarity (45% identity) to peach Pru p 3, which is used as a model LTP food allergen. The isolated allergen preferentially accumulates in wheat bran, which is fully in line with the location of LTPs in outer epidermal cell layers of the exposed plant surfaces.^{11,34}

Tri a 14 is strongly recognized by serum IgE from bakers with asthma sensitized to wheat. Moreover, the high proportion (60%) of these sera that present specific IgE to the purified allergen, together with its potency to elicit positive SPT reactions (62% of the tested patients), support its characterization as a major allergen related to baker's asthma caused by wheat flour. However, other bran and flour wheat allergens also contributed to the baker's asthma condition shown by the group of patients here studied, as clearly indicated by the results presented in Fig 1, C, and Table I.

None of the 40 patients with baker's asthma here studied described symptoms after eating wheat-derived products. In addition, around 60% of the subgroup of these patients who showed specific IgE to Tri a 14, Pru p 3, or both ($n = 28$) exhibited no sensitization to noncereals plant foods (Table I). Together, both the clinical characteristics of baker's asthma and the high *in vitro* and *in vivo* reactivity of Tri a 14 clearly suggest that members of the LTP family can sensitize through the respiratory route, being linked to systemic symptoms, such as asthma. Their

potential role as inhalant allergens has been previously suggested in 3 patients with rhinoconjunctivitis and asthma caused by rice powder inhalation¹⁴ and, more extensively, in subjects with allergy to mugwort and plane pollens.^{13,35} However, sensitization to Tri a 14 through ingestion induced by cross-reactivity between the LTP wheat allergen and its homologs from fruits, vegetables, or nuts (see Table I) cannot be ruled out, at least in some patients. The cross-inhibition between Tri a 14 and peach Pru p 3 found in the same individual sera by means of ELISA-inhibition assays points to this sensitization route. Furthermore, sensitization by means of ingestion has been reported in the case of maize and barley (beer) LTPs.^{15,17}

Finally, the limited IgE cross-reactivity between Tri a 14 and peach Pru p 3 should be emphasized. Two different lines of experimental evidence point to this fact. First is the lack of recognition of Pru p 3, but not of the wheat LTP allergen, by IgE of a serum pool in immunodetection assays after SDS-PAGE separation under reducing conditions. Second is the low or null cross-inhibition between both allergens found in ELISA-inhibition assays with several individual sera. It seems that, in addition to having common IgE epitopes, Tri a 14 and Pru p 3 display relevant specific IgE-binding regions different for each allergen. The 3-dimensional modeling of both LTP proteins reveals great differences in the PB electrostatic potential, particularly in those regions previously mapped as IgE epitopes of Pru p 3,³² and hence a differential interaction with IgE antibodies must be expected for both allergens. Moreover, this could explain in part the low association usually found between sensitization to wheat

flour inhalation and peach fruit ingestion exhibited by patients with baker's asthma. Nevertheless, cross-reactivity with LTP allergens from fruits and vegetables cannot be completely disregarded as the cause of sensitization to Tri a 14 in some patients with baker's asthma, as has been mentioned above.

In conclusion, wheat LTP Tri a 14 has been identified as a major allergen linked to baker's asthma caused by wheat flour, showing a limited IgE cross-reactivity with the model LTP food allergen from peach fruit. Hence its inclusion in a panel of purified allergens for component-resolved diagnosis of baker's asthma should be taken into consideration.

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